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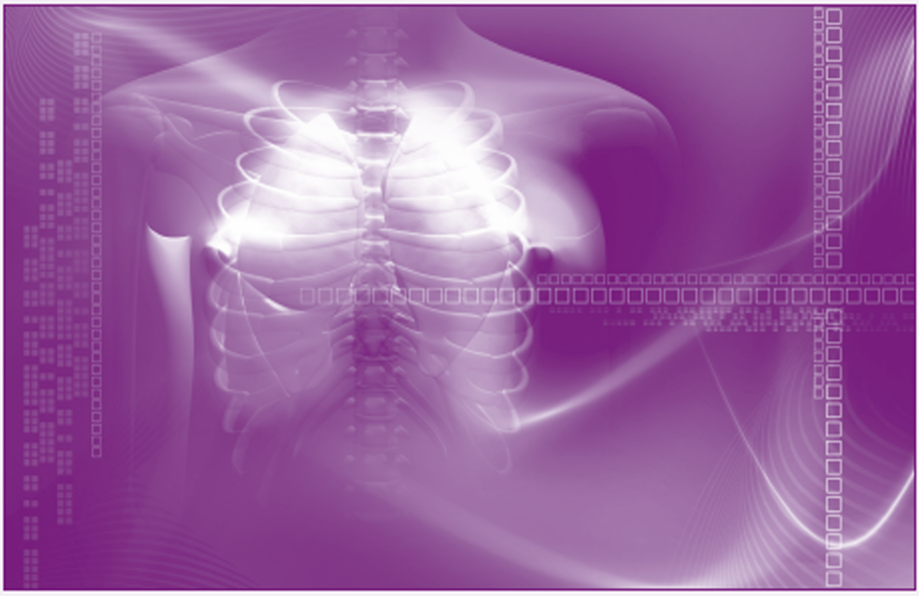
Executive Editor: Claude Lenfant

Pharmacology and Therapeutics of Airway Disease

Second Edition



edited by
Kian Fan Chung
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Pharmacology and Therapeutics of Airway Disease

LUNG BIOLOGY IN HEALTH AND DISEASE

Executive Editor

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Pharmacology and Therapeutics of Airway Disease

Second Edition

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Introduction

In 1993, volume 67, *Pharmacology of the Respiratory Tract*, appeared as part of this series of monographs Lung Biology in Health and Disease. In the preface, the editors, K. Fan Chung and Peter J. Barnes, indicated that the volume combined two concepts and stated "one deals with basic mechanisms of cell activation and pathophysiology, and the other with several functional aspects of specific cell types found in the normal and abnormal respiratory tract."

There is no doubt that volume 67 opened the window to a new world of research on the treatment of airway dysfunctions and pathological alterations. This expose of new knowledge significantly departed from what had been investigated for decades before, relative to the treatment of asthma, chronic bronchitis, and emphysema.

New hypotheses presented in volume 67 stimulated many investigations adding knowledge at a pace never seen before in research of the respiratory tract with regard to asthma and chronic obstructive pulmonary disease, or COPD—the term that is now widely used; this series of monographs became an important vehicle for the transfer of new advances. In fact, 31 monographs about asthma, 12 about COPD, and 13 that cover both conditions have appeared since 1993—and more are in preparation!

This new volume, also edited by K. Fan Chung and Peter J. Barnes, has a different title from that of their first monograph, *Pharmacology and Therapeutics of Airway Disease*. This is because a new array of therapeutic approaches often derived from very basic research advances can be described and are utilized clinically. Many alter the natural history of asthma and COPD, and more importantly, have considerably improved the quality of life for patients who are diagnosed with these airways diseases.

In the preface of this new volume, the editors state, "This volume is addressed to respiratory physicians, respiratory investigators, and respiratory allied professionals," the readers who have the most opportunity to provide help to the patients. Thus, it is a privilege to introduce this new volume. As the executive editor of this series of monographs, I thank the editors and the authors for the opportunity to present it to our readership.

Claude Lenfant, MD
Vancouver, Washington, U.S.A.

Preface

Since the publication of the first edition, there have been many significant advances in the pharmacology and therapeutics of the respiratory tract. This has been evident by the introduction of a number of new entities in the treatment of many respiratory tract diseases. In parallel, there have been advances in the assessment and evaluation of new drugs, and in understanding of the pathophysiology of respiratory tract diseases and of the methods of measuring airway and clinical responses such that potential targets for therapy have been identified and are being investigated, together with new therapeutic uses of already-available drugs.

Airway diseases comprise asthma and chronic obstructive pulmonary disease (COPD), which cause much chronic respiratory disability and death in the world. They are characterized by intermittent and/or chronic airflow obstruction, which in severe asthma and COPD can be poorly responsive to available treatments. The morbidity and mortality caused by these diseases are increasing, in particular COPD, which is now the sixth most common cause of death worldwide but predicted to become the third commonest cause within the next 10 years. There is therefore a pressing need to obtain even more effective treatments than that we currently have.

This volume is addressed to the respiratory physician, respiratory investigators, and respiratory allied professions. It not only provides the concepts upon which many treatments are used for these conditions, but also emphasizes the way in which these treatments work, and how new treatments can be discovered and tested in different patient groups. The book focuses on the state-of-the-art pharmacologic and therapeutic approaches in controlling pathophysiological processes in the airways, and reviews normal and abnormal physiologic, biochemical, and molecular aspects of the respiratory tract. It explores the basic mechanisms of inflammatory cell activation and pathophysiology in disease, and therapeutic ways of controlling inflammation, ways of reversing or preventing airflow obstruction, and symptomatic treatments for advanced disease.

We hope that this volume will be useful to those who deal with the many patients who suffer from airway disease. We would like to thank all the contributors to this volume and the editors at Informa Healthcare for their encouragement and help during the inception of this book.

Kian Fan Chung
Peter J. Barnes

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Principles of Airway Pharmacology and Therapeutics

PETER J. BARNES
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I. Introduction

Airway pharmacology is concerned with the action of drugs on target cells of the airways and improving our understanding of the mechanism of action of drugs used to treat airway diseases. This should lead to advances in drug development, so that treatment may be more specific and maximizes the beneficial effects. Drugs may also be used as specific probes to analyze pathophysiological processes in airway disease. This chapter concerns the general pharmacological principles of drug action in the airways, with particular emphasis on the application of pharmacology to understanding obstructive airway diseases and their therapy.

II. Receptors

Most hormones, neurotransmitters, mediators, cytokines, and growth factors produce their effects by interacting with specific protein recognition sites or receptors on target cells. Because receptors are specific, they allow a cell to recognize only selected signals from the myriad of molecules that come into contact with the cell. They play an important role in disease, since their function may be altered, resulting in abnormal cellular responsiveness. Many drugs used in the treatment of airway diseases stimulate (agonists) or block (antagonists) specific receptors.

There have been major advances in elucidating the function, regulation, and structure of receptors, made possible by the development of radioligand binding, in which highly potent radiolabeled agonists or antagonists are used to characterize and directly quantify and map receptors. Many receptors have now been cloned, making it possible to deduce their amino acid sequence and structure and to determine the critical parts of the receptor protein that are involved in ligand binding and interaction with intracellular second messengers. Receptor cloning and production of pure receptor proteins have also made it possible to produce specific antibodies for use in immunocytochemical studies. Furthermore, advances in molecular biology have made it possible to study the regulation of receptor genes. Some receptors termed orphan receptors have been cloned, but their endogenous ligands have not been identified. This has led to the discovery of new endogenous ligands and identification of novel drug targets. The cloning of the human genome has revealed many orphan receptors.

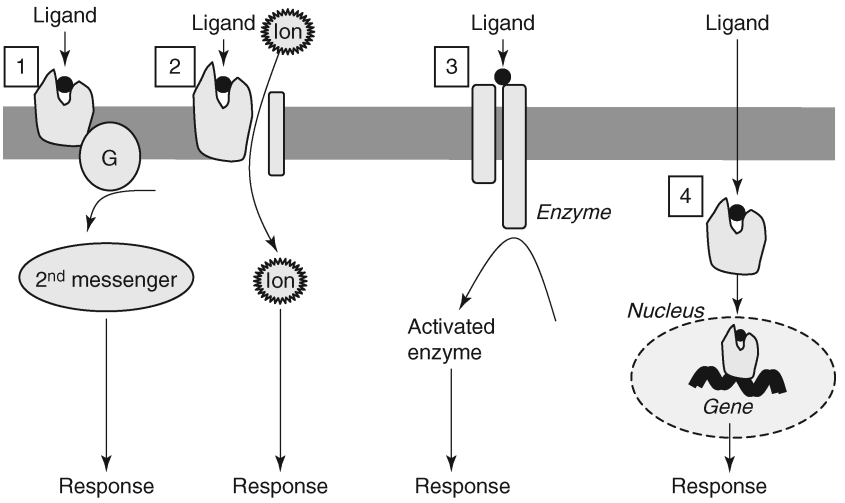

Figure 1 Several classes of receptor are recognized. (1) G protein–coupled receptors, which activate second messengers to induce a response; (2) receptor-operated ion channels; (3) enzyme-linked receptors; and (4) intracellular receptors, which translocate to the nucleus to regulate gene expression.

A. Receptor Classification

Most receptors are proteins located within the cell membrane, which interact with specific ligands outside the cell, leading to a conformational change, which results in activation of a second messenger system within the cell and subsequently to the typical cellular response. Cell surface receptors include (Fig. 1)

- guanine nucleotide–binding protein (G protein)–coupled receptors (GPCRs, e.g., β-adrenergic receptors, chemokine receptors),
- ion channel–linked receptors (e.g., nicotinic receptors),
- enzyme-linked receptors [e.g., platelet-derived growth factor (PDGF) receptors],
- cytokine and growth factor receptors, which usually have at least two subunits (e.g., interleukin-5 receptors), and
- intracellular receptors, such as steroid and thyroid receptors, where the ligand diffuses into the cell and usually binds to cytosolic receptors, which translocate to the nucleus and interact with recognition binding sites on DNA to regulate the transcription of target genes.

Molecular cloning techniques have made it possible to recognize several families of receptor that share a common structure and to trace the evolutionary lineage of receptors within receptor families.

B. G Protein–Coupled Receptors

Many different receptors interact with G proteins, which act as a coupling mechanism linking receptor activation to intracellular signal transduction pathways. All of these

receptors have structural similarities and constitute a large supergene family. Over 1000 GPCRs, making up >1% of the human genome, have now been cloned and sequenced (1,2). Each receptor is a single polypeptide chain, ranging in size from ~400 to >1000 amino acids, with seven hydrophobic sequences that cross the cell membrane. Many drugs used in the treatment of airway diseases interact with GPCRs. In addition, many unknown (orphan) GPCRs have been discovered, which are now targets for the development of novel drugs (3). For example, polymorphisms of the orphan G protein–coupled receptor for asthma susceptibility (GPRA) was discovered when polymorphisms of its gene were linked to increased asthma susceptibility and airway hyperresponsiveness (4). The endogenous agonist of this receptor has now been identified as neuropeptide S, which has been linked to anxiety and arousal, but the function of neuropeptide S in asthma has not yet been defined (5).

Rhodopsin as a Model Receptor

The first and most carefully characterized GPCR was rhodopsin in light-sensitive rods of the retina, which is coupled to a unique G protein called transducin, and this has served as a useful structural model for other receptors in this superfamily. Analysis of the amino acid sequence of rhodopsin revealed seven hydrophobic (lipophilic) stretches of 20 to 25 amino acids, which are linked by hydrophilic regions of variable length. The most likely spatial arrangement of the receptor in the cell surface membrane is for the seven hydrophobic sections (each of which is in the form of an α-helix) to span the cell membrane. The intervening hydrophilic sections are exposed alternately, intracellularly and extracellularly, with the amino (N)-terminal exposed to the outside and the carboxy (C)-terminal within the cytoplasm. The extracellular regions of rhodopsin recognize the specific ligand (retinal), and the intracellular regions interact with transducin. More recently, the three-dimensional (3D) structures of other GPCRs, including the β_2-adrenergic receptor, have been characterized, and these generally conform to the rhodopsin model (6).

Structure

All GPCRs share the common feature of seven similar hydrophobic transmembrane segments (7TM). There is also some sequence homology of the intracellular loops (which interact with various species of G protein) but less similarity in the extracellular domains. For example, there is a 50% homology between rat β_2-adrenergic and muscarinic M_2 receptors. There is also close homology between the same receptor in different species. Thus, there is 95% homology between rat and pig heart M_2 receptors. These similarities demonstrate that G receptor–linked receptors form part of a supergene family that may have a common evolutionary origin.

Members of the G protein receptor supergene family are generally 400 to 500 amino acids in length, and the receptor cDNA sequence consists of 2000 to 4000 nucleotide bases (2–4 kb) (Fig. 2) (2). The molecular weight of the cloned receptors predicted from the cDNA sequence is 40 to 60 kDa, which is usually less than the molecular mass of the native receptor, when assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. This discrepancy is explained as due to *glycosylation* of the native receptor. For example, β_2 receptors contain two sites for glycosylation on asparagine (Asn/N) residues near the N-terminus, and it is estimated that N-glycosylation accounts for 25% to 30% of the molecular mass of the native receptor. Receptor

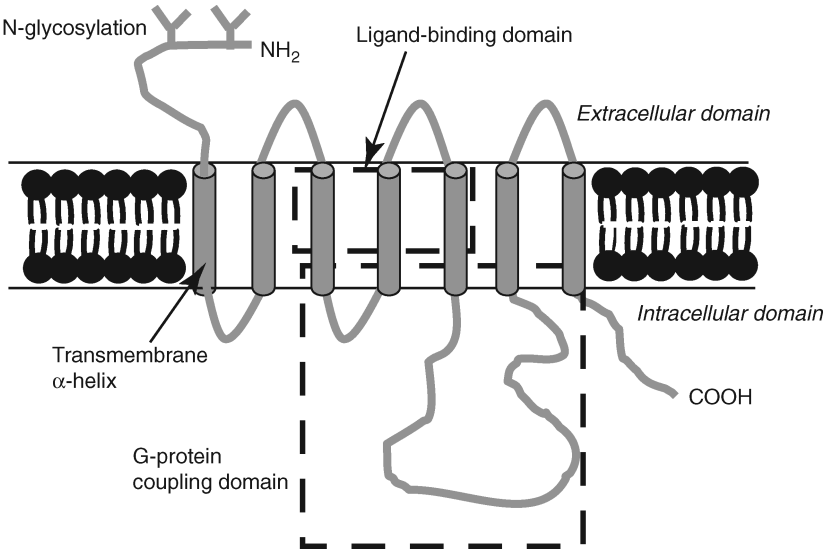

Figure 2 Structure of a G protein–coupled receptor. The peptide chain is folded seven times across the cell membrane. The hydrophobic segments that cross the cell membrane are in the form of an α-helix. Small ligands interact deep within the cell membrane between the α-helices, whereas peptide ligands interact with the extracellular parts of the receptor. Intracellular loops (especially the third intracellular loop) are important in interaction with the G protein. Most of these receptors are glycosylated at extracellular loops.

glycosylation does not affect receptor affinity for ligand or coupling to G proteins, but may be important for the trafficking of the receptor through the cell during down-regulation, or for keeping the receptor correctly orientated in the lipid bilayer.

Another feature of these receptors is palmitoylation, when cysteine residues covalently bind palmitic acid via a thioester bond, thus anchoring the receptor chain to the cell membrane. This confers 3D stability to the receptor, and disruption of this bond in β receptors (by mutation of Cys341) alters both binding characteristics and coupling to G proteins and may affect desensitization of the receptor (7).

Deletion mutagenesis (deleting sections of the peptide sequence) and site-directed mutagenesis (substitution of single amino acids in the polypeptide chain) have established that the ligand-binding domain is well conserved between members of the same family. In the case of β-adrenoceptors, there is good evidence for a ligand-binding cleft between the transmembrane-spanning domains within the cell membrane (7). Critical amino acids for the interaction of endogenous adrenergic agonists (norepinephrine and epinephrine) are asparagine (Asp) in the third transmembrane loop (TM3, Asp113) and serines in TM5 (Ser204, Ser207), which interact with the hydroxy groups on the catechol ring (Fig. 3).

The binding site for antagonists differs from those of naturally occurring ligands, and for antagonist binding to β receptors, 7TM appears to be critical. Binding of substance P

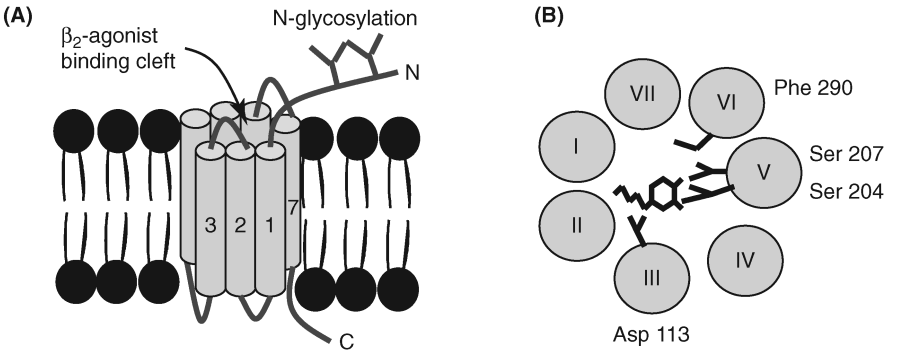

Figure 3 Ligand binding domain of the β_2-adrenergic receptor, showing the clustering of the seven transmembrane domains to form a binding cleft (**A**) and the interaction between the catecholamine and critical amino acids in the transmembrane domains (**B**).

to the NK_1 receptor occurs to extracellular domains of the receptor, whereas antagonist binding of the nonpeptide NK_1 antagonist CP96,345 binds to a transmembrane domain (His197) (8).

One special type of GPCR is the proteinase-activated receptor (PAR), exemplified by receptors for thrombin and tryptase (9). There are currently four PARs recognized; thrombin activates PAR1, 3, and 4, whereas trypsin, mast cell tryptase, and other serine proteases activate PAR2. Activators of these receptors are enzymes that cleave a site on the extracellular domain of the receptor, thus revealing an active site that then binds to and activates the remaining receptor protein (Fig. 4). PAR may play an important role in lung diseases, leading to a search for specific antagonists (9).

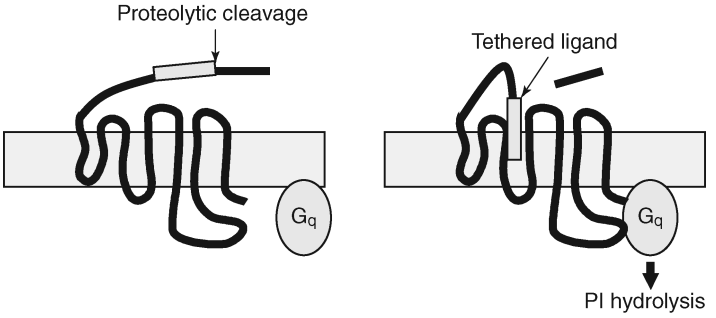

Figure 4 PARs are G protein–coupled receptors that are activated by proteinases, such as thrombin (PAR1, 2, 4) and tryptase (PAR2). Cleavage occurs at the N-terminal end of the receptor to reveal a tethered ligand sequence that then inserts into the binding cleft of the receptor, resulting in coupling of a G protein (usually G_q) to effector mechanisms such as PI hydrolysis. *Abbreviations*: PARS, proteinase-activated receptors; PI, phosphoinositide.

C. Receptor Dimerization

Most GPCR undergo physical association not only with themselves (homodimerization) but also with other GPCR (heterodimerization), and this may influence receptor function and interaction with signaling pathways, providing a novel mechanism of receptor interactions that may be relevant to airway diseases (10–12). Recognition of GPCR heterodimers might also lead to the development of novel drugs that only recognize the heterodimers and therefore have a greater selectivity. For example, heterodimerization between prostaglandin E (EP_1) receptors and β_2 receptors in airway smooth muscle cells results in uncoupling of β_2 receptors and a diminished bronchodilator response to β_2-agonists (13). β_2 Receptors are also known to dimerize with β_1, β_3, opioid, and angiotensin AT_1 receptors. Heterodimerization of chemokine receptors may be important in determining cell recruitment and may account for why selective antagonist of one receptor may inhibit responses to other chemokines that signal through different receptors (14). For example, CXCR1/CXCR2 and CCR2/CCR4 heterodimers have been identified and appear to be induced when the chemokine ligands for both receptors are present together.

D. G Proteins

G proteins link activation of 7TM receptors to enzymes or ion channels that then mediate the characteristic response (15). All G proteins have GTPase intrinsic activity and catalyze the conversion of guanosine triphosphate (GTP) to guanosine diphosphate (GDP). G proteins are made up of three separate units and are termed heterotrimeric G proteins; the α-subunit interacts with the receptor, binds GTP, and interacts with the effector enzyme, such as adenylyl cyclase and phospholipase C (PLC). The β- and γ-subunits are hydrophobic and are associated as a $\beta\gamma$ complex within the cytoplasmic surface of the cell membrane. G proteins are freely diffusible within the cell membrane, and the pool of G proteins may interact with several receptors. In the resting state, the G protein exists as a $\alpha\beta\gamma$ heterotrimer with GDP occupying the binding site on the α-subunit. When a receptor is occupied by an agonist, a conformational change occurs and the intracellular loops of the receptor protein acquire a high affinity for $\alpha\beta\gamma$, resulting in the dissociation of GDP and its replacement with GTP, which in turn causes α-GTP to dissociate from the $\beta\gamma$-subunits (Fig. 5). α-GTP is the active form of the G protein and diffuses to associate with effector molecules, such as enzymes and ion channels. This process is terminated by hydrolysis of GTP to GDP via the GTPase activity of the α-subunit. The resulting α-GDP dissociates from the effector molecule and reassociates with $\beta\gamma$, in readiness for activation again (16).

In addition to the classical heterotrimeric G proteins with $\alpha\beta\gamma$-subunits, there are several other small G proteins with GTPase activity, such as the Rho, Rab, and Rac subfamilies, which are not activated directly by GPCR but play a key role in regulating actin and cytoskeletal organization (17). Rho activates specific Rho kinases, leading to a cascade of interacting signals within the cell. For example, Rho GTPases are involved in contractile responses in airway smooth muscle and in fibrosis so that Rho kinase inhibitors might have therapeutic potential in asthma (18). Rab GTPases are involved in secretion and may therefore play a role in mucus secretion and in mast cell degranulation (19).

Several receptors, such as β receptors and vasoactive intestinal polypeptide (VIP)-receptors, stimulate adenylyl cyclase via a stimulatory G protein, G_s, whereas other

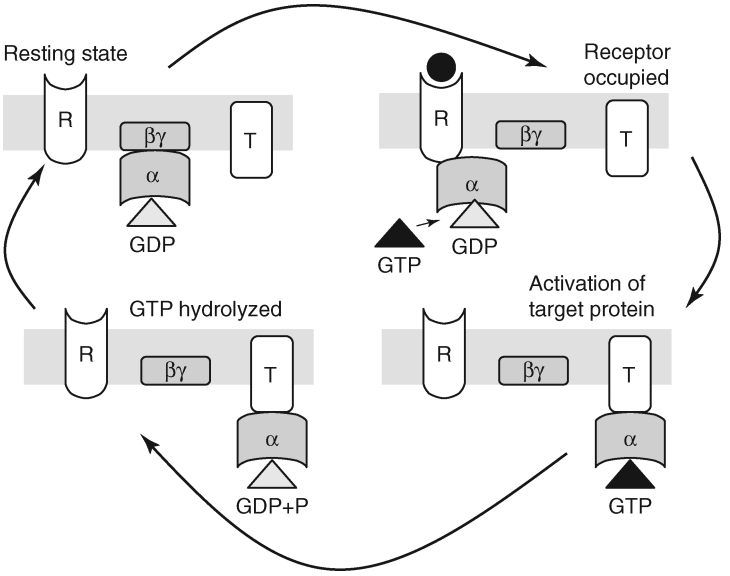

Figure 5 Transducing function of G proteins. G proteins couple receptor activation to a target membrane protein, such as an enzyme (e.g., adenylyl cyclase) or an ion channel (T). Each G protein is made up of three subunits (α, β, and γ). In the inactive state, GDP binds to the α-subunit, but when a receptor (R) is activated it interacts with the α-subunit, displacing GDP for GTP, and resulting in the association of the α-subunit with the effector system. GTP is then hydrolyzed by the intrinsic GTPase activity of the α-subunit, resulting in activation of T. This then allows the α-subunit to associate with the βγ-subunits that remain fixed in the hydrophobic cell membrane. The α-subunit is therefore believed to act as a "shuttle" coupling receptor activation to stimulation of the target protein. *Abbreviations*: GDP, guanine diphosphate; GTP, guanine triphosphate

receptors, such as muscarinic M_2 receptors, inhibit adenylyl cyclase via G_i (Fig. 6) (15). G_s may be stimulated directly by cholera toxin, whereas Gi is inhibited by *pertussis* toxin, and these toxins have proved to be useful in elucidating the involvement of a particular G protein in a specific receptor-mediated response. Other G proteins are now recognized that couple receptors that activate phosphoinositide hydrolysis (G_0, G_q) and particular ion channels in the cell membrane (e.g., G_k, which is coupled to potassium channels). G proteins may play a very important role in the regulation of cell responsiveness, and there is evidence that receptors may become uncoupled from G proteins under certain conditions. For example, in fatal asthma there is evidence for a reduced responsiveness of airway smooth muscle to β-agonists, yet the number and affinity of β receptors on airway smooth muscle is not reduced and the response to other smooth muscle relaxants is not impaired, suggesting that the receptors have become *uncoupled* from G_s in severe asthma (20).

While receptors affect the function of G proteins, G proteins also influence the interaction of ligands with their receptors. Thus, when coupled to an inactive G protein, the receptor exists in a state of high affinity for the agonist. Agonist binding releases G_α

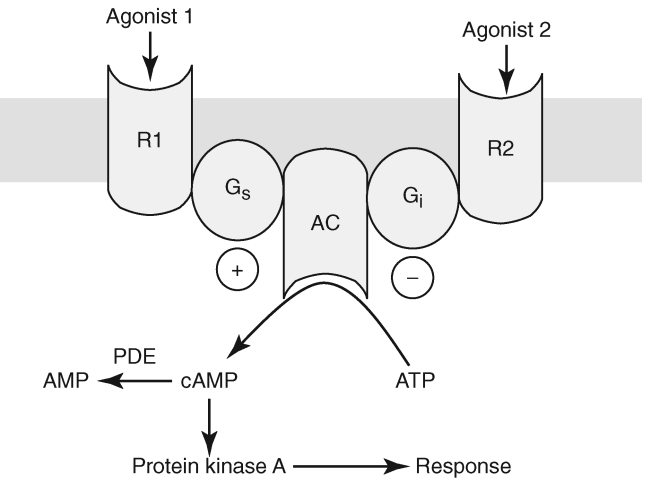

Figure 6 G proteins and adenylyl cyclase. Receptors (R_1 and R_2) are coupled to G proteins that stimulate (G_s) or inhibit (G_i) adenylyl cyclase (AC), resulting in increased or decreased formation of the second messenger, cAMP from ATP. cAMP is degraded to AMP via PDE. cAMP activates protein kinase A, which phosphorylates target proteins leading to a response. *Abbreviations*: cAMP, cyclic adenosine 3′,5′-monophosphate; ATP, adenosine triphosphate; AMP, adenosine monophosphate; PDE, phosphodiesterases.

from contact with the receptor, resulting in a reduction in agonist affinity, referred to as the "guanine nucleotide shift."

E. Second Messengers

The ligand that activates a receptor is described as the first messenger and leads, via activation of a G protein, to the typical cellular response via a second messenger, such as a change in intracellular calcium ion (Ca^{2+}) concentration or cyclic 3′,5′ adenosine monophosphate (cAMP) concentration. While the number of surface receptors that may respond on any particular cell is very large, only a very limited number of signal transduction and second messenger systems have been described. Thus, the surface receptors determine cellular responsiveness and sensitivity, rather than the intracellular mechanisms that are activated by the receptor-ligand interaction. Great progress has been made in understanding the intracellular mechanisms involved in receptor-mediated effects, through the development of techniques, such as intracellular dye indicators, which reflect intracellular concentrations of ions (e.g., fura-2 that detects intracellular Ca^{2+} concentrations), by more sensitive biochemical assays, and by the development of patch-clamping techniques.

Adenylyl Cyclase

Many receptors produce their effects by interaction with the membrane-bound enzyme adenylyl cyclase to either increase or decrease production of cyclic 3′,5′ adenosine monophosphate (cAMP) (Fig. 6). At least nine closely related forms of adenylyl cyclase

have now been differentiated, and there is increasing evidence that these isoforms may be differentially regulated (21). Thus, protein kinase C (PKC) phosphorylates and activates certain isoforms (types 1, 2, and 3), which may be a mechanism for the interaction of different receptors, whereas it has no effect on other isoforms (4–6). The formation of cAMP leads to the characteristic cellular response via the activation of a specific protein kinase, protein kinase A (PKA), by dissociating a regulatory (inhibitory) subunit. PKA then phosphorylates serine and threonine residues on specific proteins such as regulatory proteins, ion channels, and enzymes within the cell, which lead to the characteristic response. For example, in airway smooth muscle cells PKA phosphorylates large-conductance calcium-activated potassium (K^+) channels, which open leading to K^+ efflux from the cell, hyperpolarization and relaxation (22). PKA also phosphorylates and therefore inactivates myosin light-chain kinase, resulting in a direct relaxant effect on the contractile machinery.

It is now increasingly recognized that cAMP also actives signaling mechanisms other than PKA, such as ion channels and the ubiquitous guanine nucleotide exchange factors Epac 1 and 2 (23). For example, cAMP-induced inhibition of IL-5 in human T lymphocytes is independent of PKA (24). On the other hand, β_2-agonists inhibit the release of certain cytokines from airway smooth muscle cells via an effect on PKA, since these responses are completely inhibited by transfection with the selective endogenous PKA inhibitor called PKA inhibitor-α.(25)

Phosphodiesterases

cAMP is hydrolyzed within cells by a family of enzymes termed phosphodiesterases (PDEs). At least 12 PDE families have now been distinguished on the basis of substrate specificity, inhibition by selective inhibitors, and molecular cloning (26). In airway smooth muscle, PDE3 and PDE4 isoenzymes are involved in cAMP-mediated relaxation, whereas in inflammatory cells (mast cells, eosinophils, neutrophils, macrophages, T lymphocytes) and airway epithelium, PDE4 predominates. This has led to the development of several selective PDE4 inhibitors to treat asthma and chronic obstructive pulmonary disease (COPD), although side effects after oral administration are a major problem that has limited development (27). Each PDE has several subtypes encoded by different genes, and each subtype of PDE has several splice variants, so multiple forms of PDE exist in the cell. This may allow precise control of intracellular cyclic nucleotide concentrations. For example, there are four distinct PDE4s, PDE4A, PDE4B, PDE4C, and PDE4D, each of which has several splice variants that are differentially expressed and regulated (28). This may be relevant in drug design as inhibition of one subtype may mediate the desired effect and another may be responsible for side effects. For example, PDE4B appears to mediate the anti-inflammatory effect of PDE4 inhibitors, whereas PDE4D mediates the nausea and vomiting that are often dose-limiting (29,30).

Phosphatidylinositol Hydrolysis

Another signaling system involves breakdown of a membrane phospholipid, phosphatidylinositol (PI), which results in increased intracellular Ca^{2+} concentration. Over 100 receptors are coupled via G_q or G_i to the membrane-associated enzyme phosphoinositidase or PLC, which converts phosphoinositide(4,5)bisphosphate (PIP_2) to inositol

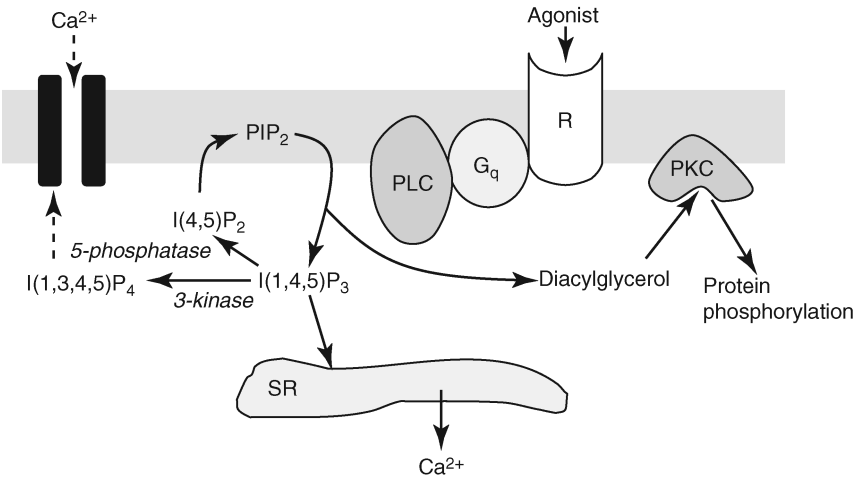

Figure 7 Phosphoinositide hydrolysis. Occupation of a surface receptor leads to activation of the enzyme PLC via a G protein (G_q). PLC converts phosphoinositide 4,5,-bisphosphate (PIP_2) to inositol (1,4,5) trisphosphate and diacylglycerol. $I(1,4,5)P_3$ binds to receptors on sarcoplasmic/endoplasmic reticulum (SR) to release Ca^{2+}, resulting in a rise in intracellular Ca^{2+} concentration and cell activation. $I(1,4,5)P_3$ is dephosphorylated to $I(4,5)P_2$, inositol phosphate, and inositol, which is then reincorporated into membrane phophoinositides. In some cells it is further phosphorylated to $I(1,3,4,5)P_4$, which may be involved with calcium entry and refilling of intracellular stores. Diacylglycerol activates PKC, which phosphorylates various regulatory proteins in the cell. *Abbreviations*: PLC, phospholipase C; PKC, protein kinase C.

(1,4,5)trisphosphate (IP_3) and 1,2-sn-diacylglycerol (DAG) (Fig. 7). Four main groups of PLC have now been identified (PLC-β, PLC-γ, PLC-δ, PLC-ε), each of which has subclasses (PLC-β1, PLC-β2, PLC-β3, etc.) based on amino acid structure of different cloned genes (31). These isoenzymes are differentially coupled to different receptors and are subject to differential regulation.

IP_3 binds to a specific receptor on endoplasmic/sarcoplasmic reticulum, which leads to the release of Ca^{2+} from intracellular stores. Thus, PI hydrolysis links occupation of a surface receptor to intracellular Ca^{2+} release. Most of the mediators that contract airway smooth muscle act on receptors that activate PI hydrolysis in airway smooth muscle (32). IP_3 is broken down into the inactive IP_2 by IP_3 kinase and subsequently to inositol, which is reincorporated into phophoinositides in the cell membrane. IP_3 may also be phosphorylated by IP_3 kinase to IP_4, which may be involved in opening receptor-operated calcium channels and the refilling of intracellular stores. Calcium release in response to agonists or IP_3 occurs in a series of oscillations, which is probably mediated via calcium-induced calcium release and the opening of calcium channels on the cell membrane, and the frequency of oscillation may be important in the type of cell activation that ensues (33).

The formation of DAG activates PKC by causing it to translocate to the cell membrane and by dramatically increasing its sensitivity to Ca^{2+}. Activated PKC is then capable of phosphorylating various cell membrane–associated proteins, including

receptors, G proteins, and regulatory proteins. Several isoenzymes of PKC are now recognized and play a critical role in the regulation of inflammatory and structural cells of the airways, although the role of individual isoenzymes in regulating cell function is not yet clear as selective inhibitors have been difficult to find (34). PKC may be activated directly by tumor-promoting phorbol esters, such as phorbol myristate acetate (PMA). In some species, PMA and other phorbol esters cause prolonged contractile responses in airway smooth muscle, but in other species, bronchodilatation is observed. It has been suggested that PKC may be important for the prolonged contractile responses seen in asthmatic airways. Several PKC isoenzymes have been identified in human airway smooth muscle (35). PKC inhibitors, such as staurosporine (which is not very selective) and Ro 31-8220, may be useful in elucidating the role of PKC but have no selectivity for different isoforms.

Guanylyl Cyclase

It was previously believed that, while relaxation of smooth muscle is brought about by receptors that activate cAMP, contraction is due to the production of another cyclic nucleotide, cyclic 3′,5′ guanosine monophosphate (cGMP), formed by the activation of guanylyl cyclase (36). This is now known to be incorrect and the increase in cGMP is secondary to a rise in intracellular Ca^{2+} concentration. Indeed cGMP causes relaxation of smooth muscle and is the major mechanism of vasodilatation after nitrovasodilators (such as sodium nitroprusside) and dilators (such as acetylcholine), which release nitric oxide (NO) from endothelial cells. cGMP is also involved in the relaxant response of airway smooth muscle to nitrovasodilators and to atrial natriuretic peptide (ANP), which is a potent bronchodilator in vitro (37). Guanylyl cyclase exists in particulate form, binds natriuretic peptides, and also exists as a soluble form that binds NO (38). cGMP is broken down by PDEs, and in particular the PDE5 isoenzyme (39). PDE5 inhibitors, such as sildenafil, have potent vasodilator effects on pulmonary vessels and weak bronchodilator effects.

Ion Channel–Coupled Signaling

G proteins may also couple receptors to ion channels, including K^+, Na^+, and Ca^{2+} channels. Thus, certain muscarinic receptors are coupled via G proteins (G_0) to K^+ channels and Ca^{2+} channels. In airway smooth muscle, β_2 receptors are directly coupled via G_s to the opening of large-conductance K^+ (maxi-K) channels, and the same channels are inhibited by M_2 receptors via G_i (22). These G proteins allow rapid cell activation by GPCR, in contrast to the slower responses mediated by changes in cyclic nucleotides.

F. Cytokine Receptors

The effects of cytokines are mediated via specific surface receptors, many of which have now been cloned. There is a very complex network of cytokines and chemokines (small chemotactic cytokines) that orchestrate the inflammation in asthma, COPD, and interstitial lung disease, and all of their complex effects are mediated by surface receptors (40).

Chemokine Receptors

Chemokines, such as CXCL8 (IL-8), CCL5 (RANTES), and CCL11 (eotaxin), bind to receptors that are coupled to G proteins, and their receptors have the typical 7TM GPCR

structure (41,42). Many chemokines have overlapping activities and interact with common receptors on target leukocytes. Over 20 chemokine receptors have now been characterized and they appear to be differentially expressed on different inflammatory cells, thus explaining the differential chemotactic effects of these cytokines. For example eosinophils express CCR3 that is activated by CCL5, CCL13 (MCP-4), CCL11, CCL24 (eotaxin-2), and CCL26 (eotaxin-3), thus accounting for the selective chemotactic effects of these chemokines on eosinophil migration. Because of the 7TM structure of chemokine receptor, small molecule inhibitors are feasible. This has led to the development of several chemokine antagonists for the treatment of asthma and COPD (43,44).

Cytokine Receptor Superfamilies

Most cytokine receptors have a primary structure that is quite different from the 7TM spanning segments associated with GPCR. Many cytokine receptors have at least two subunits that interact to activate signal transduction pathways within the cell (45). For example IL-5, IL-4, and IL-13 receptors have an α- and β-chain. The tumor necrosis factor α (TNF-α) receptor is a 55-kDa peptide (p55) that has a single transmembrane-spanning helical segment, an extracellular domain that binds TNF-α, and an intracellular domain (46). The intracellular domain leads to activation of several kinases and ceramide, which subsequently lead to the activation of transcription factors, such as nuclear factor–kappaB (NF-κB) and activator protein 1 (AP-1). A second TNF receptor (p75) has also been cloned, but differs markedly in sequence and may be linked to different intracellular pathways (46). There are now almost 30 receptors and 20 cytokines in the TNF superfamily, which have complex and interacting signal transduction pathways (47).

Molecular cloning has now revealed that although cytokines may be structurally diverse, their receptors may be grouped into various families that share structural homology (48). The immunoglobulin superfamily of receptors includes the receptors for IL-1 and PDGF, T-cell antigen receptors, and certain cell-surface adhesion molecules. The hematopoietin receptor superfamily includes receptors for IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, interferons, granulocyte-macrophage colony-stimulating factor (GM-CSF), growth factor, and erythropoietin receptors. The receptor proteins are orientated with an extracellular N-terminal domain and a single hydrophobic transmembrane-spanning segment. There is striking homology in the extracellular ligand–binding domain with four conserved cysteine residues. There is very close homology between the receptors for IL-3, IL-5, and GM-CSF, all of which stimulate growth of eosinophils. Molecular cloning has demonstrated that each of these receptors consists of α- and β-chains and share a common β-chain, which may explain why they have overlapping biological activities.

The second messenger system used by cytokines are highly complex, involving many interacting pathways that allow for the possibility of signal splitting, so that the same activating signal may result in the activation of several parallel pathways. Which signal pathways predominate is determined by other signals impinging on the cell. Most cytokines activate a group of transcription factors and result in prolonged cellular activation and gene transcription, in contrast to the relatively rapid and transient signaling of most GPCR.

G. Enzyme-Linked Receptors

Some receptors contain an enzyme domain within their structure so that when the enzyme is activated by a ligand the enzyme becomes activated, leading to signal transduction through the formation of a specific substrate within the cell. The best characterized of these enzyme-linked receptors is receptor tyrosine kinases (RTKs) that have intrinsic protein tyrosine kinase activity.

Receptor Tyrosine Kinases

Activation of RTKs results in phosphorylation of tyrosine residues on certain target proteins that are usually associated with cell growth and chronic activation of cells. More than 50 different RTKs belonging to at least 14 distinct families have now been identified (49). These receptors include epithelial growth factor (EGF), PDGF, vascular endothelial growth factor (VEGF), and insulin receptors (Fig. 8). Small molecule inhibitors, such as gefitinib and erlotinib, which block EGF receptors, have been developed for treating lung cancer (50).

RTKs share a similar general structure, consisting of a large extracellular N-terminal portion that contains the ligand recognition domain, a single short membrane-spanning region (α-helix), and a cytoplasmic C-terminal portion (~250 amino acids) that contains the tyrosine kinase activity and autophosphorylation sites. The extracellular

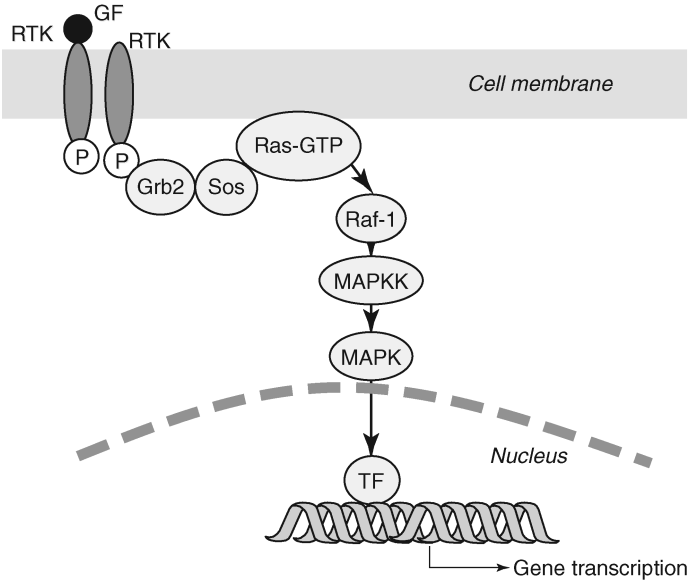

Figure 8 Activation of RTK by GF results in a cascade of enzyme activation, involving the adaptor proteins Grb2, Sos, and Ras-GTP, leading to the activation of MAPK, which then activate TFs to regulate expression of genes involved in cell proliferation. *Abbreviations*: RTK, receptor tyrosine kinase; GF, growth factors; MAPK, mitogen-activated protein kinases; TFs, transcription factors; MAPKK, MAPK kinase.

domain usually contains cysteine-rich regions and/or immunoglobulin-like motifs, with a large number of disulfide bonds forming a highly specific tertiary structure that is needed to establish ligand-binding specificity. All RTK (with the exception of the insulin receptor family) undergo a transition from a monomeric to a dimeric state (either homodimers or heterodimers) following binding of their specific ligands. Another characteristic of RTK is that they undergo internalization into two types of intracellular vesicles: pitted vesicles coated with the protein clathrin and smooth vesicles lacking clathrin. There is a spontaneous internalization, but this is rapidly accelerated when the receptor is occupied by a ligand. A proportion of the receptors is degraded, whereas others are recycled to the cell surface.

Signal Transduction

RTKs phosphorylate intracellular molecules containing Src homology 2 and 3 (SH2 and SH3) domains. These SH2 and SH3 domains are short sequences of about 100 and 50 to 60 amino acids, respectively, which function to specify the interaction with a target protein (51). The SH2 motifs recognize phosphotyrosine residues and are responsible for interactions with autophosphorylated RTK, the specificity of which depends on the amino acid sequences surrounding both the tyrosine autophosphorylation site on the RTK and the substrate's SH2 domain. RTK substrate proteins containing SH2 and SH3 motifs may contain enzymatic activity.

Most RTK stimulate the mitogen-activated protein kinase (MAPK) pathways through a complex multistep signaling cascade initiated by translocation of the adaptor protein Grb2 to the cytoplasmic membrane. This results in the activation of Ras proteins, small molecular weight GTPases, in turn activate Raf, which is a serine/threonine kinase that activates the MAPK pathway (52). There is increasing evidence that RTK may interact with several cell signaling pathways to elicit their effects, and it now seems likely that specific pathways are selected under certain conditions. For example, PDGF, which may exist in the dimeric forms AA, BB, or AB, may interact with different receptor dimers ($\alpha\alpha$, $\alpha\beta$, $\beta\beta$), resulting in activation of different signal transduction pathways.

MAP Kinase Pathways

RTK and other extracellular signals activate several MAP kinase cascades, resulting in a cascade of kinase activation that result in activation of transcription through the transcription factors Jun, Elk-1, and ATF2 (53,54).

Receptor Serine/Threonine Kinases

Similar to RTK, there are some receptors that are linked to serine/threonine kinase activity. The best-known example is transforming growth factor β (TGF-β), which exists in three mammalian isoforms encoded by separate genes, all of which may have complex and divergent effects on cell activity (55). TGF-β receptors signal through regulatory Smad pathways within the cell, including Smad2 and Smad3, together with coactivator Smads, whereas inhibitory Smads, such as Smad6 and Smad7, act as a feedback inhibitory mechanism. Some of the effects of TGF-β are mediated via inhibition of cyclins that regulate the cell cycle, and this may account for the diverse effects of TGF-β depending on the stage of the cell cycle (56).

Receptor Protein Tyrosine Phosphatases

Little is known about the third type of enzyme-linked receptors, which have a high level of intrinsic enzyme activity (57). Occupation by a ligand may turn this enzyme activity off, thus resulting in cell suppression. These receptors appear to be important in cell differentiation and include CD45 (also known as leukocyte common antigen) that is involved in T-lymphocyte signaling (58).

H. Ion Channel Receptors

Although several receptors are linked via G proteins to ion channels, such as Ca^{2+} and K^+ channels as discussed above, other receptors are ion channels themselves. Ion channel receptors are oligomeric proteins containing about 20 transmembrane segments arranged around a central aqueous channel. Binding of the ligand and channel opening occur very rapidly (within milliseconds). This is in contrast to the *slow* receptors, such as GPCR, which involve a series of catalytic steps. The best-characterized example is the nicotinic acetylcholine receptor (nAChR), which is made up of four subunits that form a cation channel. When activated by Ach, the channel opens to allow the passage of Na^+ ions (59). This type of receptor, which can respond rapidly as no intracellular mechanisms are involved, is known as a fast receptor and is usually involved in synaptic transmission (Fig. 9). nAChR are involved in ganglionic transmission in parasympathetic ganglia within the airways and are blocked by hexamethonium, which therefore blocks ganglionic transmission and cholinergic reflex bronchoconstriction. nAChR are widely distributed and $\alpha 4\beta 2$ nAChR in the brain are responsible for nicotine addiction. Varenicline is a partial antagonist of $\alpha 4\beta 2$ nAChR, acting to block the addictive effects of nicotine and its withdrawal symptoms (60). Other examples include glutamate and γ-aminobutyric acid ($GABA_A$) receptors.

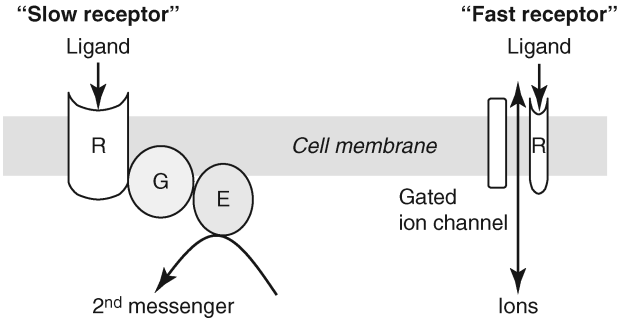

Figure 9 Fast and slow receptors. Some receptors are ion channels and consist of several subunits grouped round an aqueous channel through which ions (e.g., Na^+, K^+, Ca^{2+}, Cl^-) enter or leave the cell when agonists bind to one or more of the subunits. Such receptors function very rapidly (in milliseconds) and are involved in neurotransmission (e.g., nicotinic receptors in airway ganglia). Other receptors, which are coupled via G proteins (G), produce their effects more slowly, since a sequence of enzymatic events is necessary before a response occurs. *Abbreviations*: E, enzyme; R, receptor.

I. Intracellular Receptors

Several ligands cross the cell membrane to interact with intracellular (cytosolic) receptors rather than surface receptors. There is a family of steroid receptors that recognize different endogenous steroids such as glucocorticoids, mineralocorticoids, androgens, and estrogens. Indeed, steroid receptors belong to a supergene family that also includes thyroid hormone, retinoic acid (vitamin A), and vitamin D receptors (61). The exploration of cDNA libraries for related sequences has led to the discovery of over 40 "orphan" nuclear receptors whose ligands are now being identified (62). One new class of nuclear receptor so identified is the peroxisome proliferator-activated receptor (PPAR), with α, γ, and β/δ subtypes, for which selective ligands have now been identified. PPAR may be involved in metabolism and inflammation and may be endogenously activated by lipid mediators (63). For example, PPARγ agonists, such as rosiglitazone, appear to have anti-inflammatory effects on lung inflammation and are also potentially anti-fibrotic, so they have the potential for treating small airway fibrosis in COPD as well as interstitial lung diseases. Several other orphan receptors, including liver X receptors (LXR), farnesoid X receptors (FXR), pregnane X receptors (PXR) and retinoic acid X receptors (RXR) appear to play a role in the inflammatory process and may lead to the discovery of novel anti-inflammatory treatments (64).

Intracellular receptors share a common general structure with a central DNA-binding domain, characterized by the presence of two "zinc fingers," which are loops stabilized by four cysteine/histidine residues around a zinc ion. These zinc fingers anchor the receptor to the double helix at specific hormone response elements in the promoter region of target genes. Ligands bind in the C-terminal domain, which also contains sequences important for binding of associated chaperone proteins (e.g., heat shock proteins) and a nuclear localization signal involved in transporting the receptor from the cytoplasm into the nucleus. The N-terminal domain is involved in transcriptional regulation (*trans*-activation) and in the interaction with other transcription factors.

Steroid Receptors

Several steroid receptors have now been cloned, and their structures have been shown to differ. However, there is some homology between these receptors since they all interact with nuclear DNA, where they act as modulators of the transcription of specific genes. For example, glucocorticoid receptors (GRs) are normally present in the cytosol in an inactive form bound to two molecules of a 90-kDa heat shock protein (hsp90), which cover the DNA binding domain. Binding of a corticosteroid to its receptor results in the dissociation of hsp90, and the occupied receptor then undergoes a conformational change that allows it to bind to DNA (65,66).

The DNA-binding domain of steroid receptors is rich in Cys residues. Formation of a complex with zinc is able to fold the peptide chain into a finger-shaped conformation and the zinc is coordinated by four Cys residues. GRs have two zinc fingers that are loops of approximately 15 residues, each of which is held in shape by four cysteine residues surrounding an atom of zinc. Zinc fingers are essential for the interaction with the DNA double helix. Steroid receptors recognize specific DNA sequences—in the case of GR, glucocorticoid response elements (GREs), which have the consensus sequence GGTAnnnTGTTCT. Dimers of GR occupied by steroid bind to the GRE on the DNA double helix and either *increase* (+GRE) or much less commonly *decrease* (−GRE) the

rate of transcription by influencing the promoter sequence in the target gene. Indeed repression of target genes may be the most important aspect of corticosteroid action in inflammatory diseases, such as asthma, since corticosteroids may inhibit the transcription of many cytokines that are involved in chronic inflammation. The major mechanism of gene repression is mediated via an interaction between the activated GR and transcription factors, such as NF-κB and AP-1 that are activated via inflammatory signals such as cytokines. NF-κB activates inflammatory genes by recruiting coactivator molecules that have histone acetyltransferase activity, leading to acetylation of core histones associated with inflammatory genes, which results in increased gene transcription. Activated GRs inhibit coactivator molecules and also recruit histone deacetylase-2 (HDAC2), which deacetylate the hyperacetylated histones, resulting in switching off of the activated inflammatory genes (66) (Fig. 10).

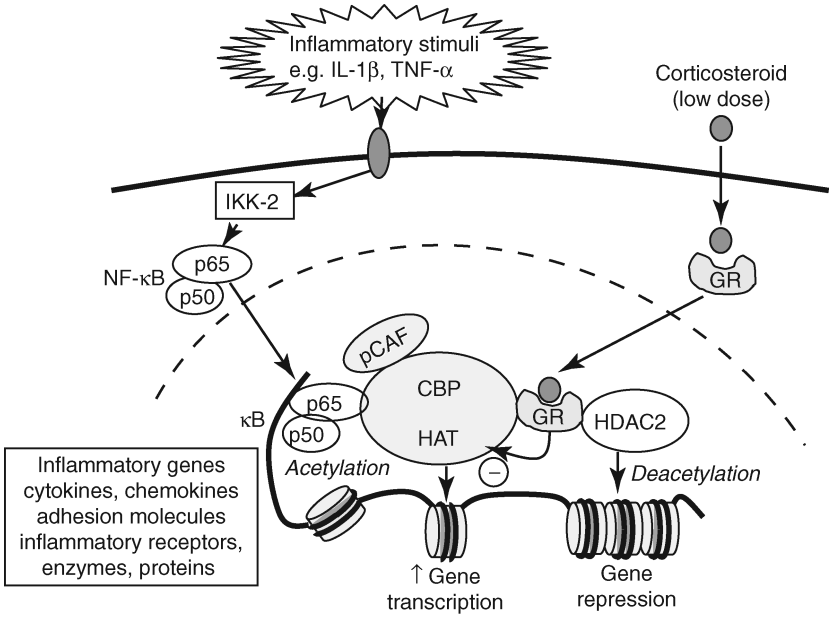

Figure 10 Corticosteroid suppression of activated inflammatory genes. Inflammatory genes are activated by inflammatory stimuli, such as IL-1β or TNF-α, resulting in activation of IKK2 (inhibitor of I-κB kinase-2), which activates the transcription factor NF-κB. A dimer of p50 and p65 NF-κB proteins translocates to the nucleus and binds to specific κB recognition sites and also to coactivators, such as CBP, which have intrinsic HAT activity. This results in acetylation of core histone H4, resulting in increased expression of genes encoding multiple inflammatory proteins. GRs after activation by glucocorticoids translocate to the nucleus and bind to coactivators to inhibit HAT activity directly and recruit HDAC2, which reverses histone acetylation leading to suppression of these activated inflammatory genes. *Abbreviations*: IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; NF-κB, nuclear factor kappa B; CBP, CREB-binding protein; HAT, histone acetyltransferase; GRs, glucocorticoid receptors; HDAC2, histone deacetylase-2.

J. Receptor Subtypes

The existence of receptor subtypes is often first indicated by differences in the potency of a series of agonists in different tissues. This could be due to differing proportions of coexistent receptor subtypes, or may indicate the existence of a novel receptor subtype. Molecular biology can resolve these possibilities since molecular techniques can clearly discriminate between different subtypes of receptor and show that they are encoded by different genes. Thus, the human β_1 receptor is clearly different from the β_2 receptor in its amino acid sequence, with a 54% homology, and the NK_1 receptor, which is selectively activated by substance P, has a 48% homology with the NK_2 receptor that is activated by the related tachykinin neurokinin A. A third tachykinin receptor, NK_3 receptor, which is selectively activated by neurokinin B, has also been cloned.

Using cross-hybridization in which a known receptor cDNA sequence is hybridized with a genomic library, it has also been possible to detect previously unknown subtypes of a receptor. For example, an atypical β receptor, which does not clearly fit into the β_1 or β_2 receptor subtypes, has been suspected in adipose tissue and some smooth muscle preparations. A distinct β_3 receptor was subsequently identified, cloned, sequenced, and expressed (67). The β_3 receptor is clearly different from either β_1 or β_2 receptors (about 50% amino acid sequence homology) and appear to be important in the regulation of metabolic rate, but has not been detected in lung homogenates (68).

Molecular biology has been particularly useful in advancing our understanding of muscarinic receptors. Five distinct muscarinic receptors have been cloned from rat and human tissues (69). The m1, m2, and m3 receptor genes correspond to the M_1, M_2, and M_3 receptors identified pharmacologically, whereas m4 and m5 receptors are previously unrecognized pharmacological subtypes that occur predominantly in the brain and for which no selective drugs have yet been developed. Interestingly m4 receptors have been demonstrated in rabbit lung using antibodies against the cloned m4 receptor, and their presence confirmed by cDNA probes for the m4 receptor. These m4 receptors are localized to vascular smooth muscle and alveolar walls, but have not been observed in lungs of other species, including humans (70). The reason for so many different subtypes of a receptor that recognize a single agonist is still not certain, but it seems likely that they are linked to different intracellular pathways and that the regulation of the intracellular portion of the amino acid sequence may be unique to each subtype. The m1, m2, and m5 receptors stimulate PI hydrolysis through a *pertussis*-insensitive G protein, whereas m2 and m4 receptors inhibit adenylyl cyclase via G_i. It is possible that the difference in protein structure may reflect regulation at a transcriptional level from DNA through different promoters, leading to variations in tissue or developmental expression, or to differences at a posttranslational level, allowing regulation by intracellular mechanisms, such as phosphorylation at critical sites on intracellular loops.

K. Receptor Interactions

Activation of one receptor may influence the function of a separate receptor via a number of interacting mechanisms. The opposing effects of receptors, which increase and decrease adenylyl cyclase activity via G_s and G_i, respectively, are well described. In airway smooth muscle, M_2 receptors inhibit adenylyl cyclase, whereas β_2 receptors stimulate this enzyme, so that there are opposing effects. This may explain why it is more difficult for β agonists to reverse contraction of airway smooth muscle induced by cholinergic agonists compared with histamine. Conversely, β-agonists may also

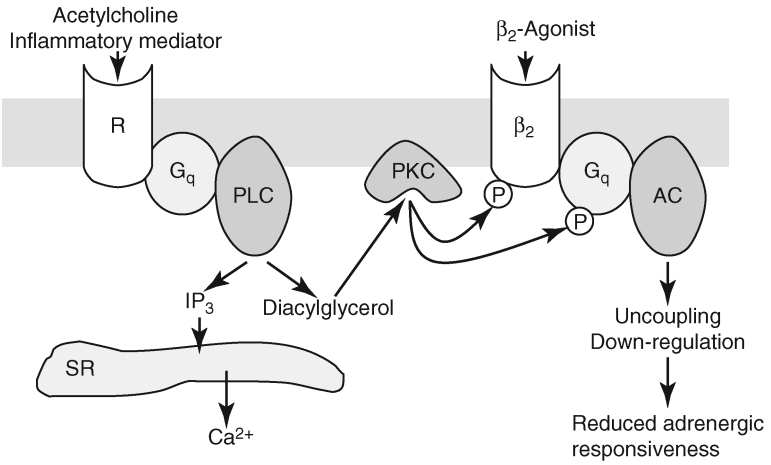

Figure 11 Receptor interactions ("cross talk"). The activation of one surface receptor may affect the function of a different receptor type via interaction of intracellular mechanisms. For example, activation of an inflammatory mediator or muscarinic receptor (R) on airway smooth muscle cells may stimulate phosphoinositide hydrolysis, with activation of PKC via diacylglycerol formation. This may result in phosphorylation (P) of other receptors, such as β_2 receptors (β_2) or a stimulatory G protein (G_s), resulting in downregulation and uncoupling of β_2 receptors, with reduced responsiveness to β_2-agonists. *Abbreviations*: PKC, protein kinase C; G_q, receptor-coupled G protein that activates phosphoinositide hydrolysis; PLC, phospholipase C; AC, adenylyl cyclase; IP_3, inositol (1,4,5) trisphosphate; SR, sarcoplasmic/endoplasmic reticulum; Ca^{2+}, calcium ions.

influence the expression of M_2 receptors (71). Several other interactions between receptors are recognized. Receptors that increase cAMP will oppose the effects of receptors that elevate intracellular Ca^{2+} via several mechanisms, including stimulation of Ca^{2+} sequestration and exchange (72).

PI hydrolysis leads to activation of PKC, which then phosphorylates receptors and G proteins, resulting in uncoupling and impaired receptor function (73) (Fig. 11). In airway smooth muscle, this may be an important interaction in inflammation since inflammatory mediators will stimulate PI hydrolysis in airway smooth muscle cells and via activation of PKC will phosphorylate G proteins, leading to uncoupling of β_2 receptors. This may explain the reduced bronchodilator response to β agonists in vitro observed in airways taken from patients with fatal asthma attacks. G protein receptor kinases (GRK) may be activated by GPCR occupation and then phosphorylate other receptors, leading to altered signaling in heterologous GPCR (74). Receptor interactions may also occur through receptor heterodimerization as discussed above.

An additional type of interaction may operate at the level of gene transcription. Cytokines may activate transcription factors that have an effect on a target gene, and steroid receptors may interact with the same gene with an opposing effect. There may also be a direct interaction between transcription factors within the cytoplasm. For example, activated GRs bind directly to the AP-1 complex and thereby prevent its interaction with the target gene. In human lung, for example, cytokines such as TNF-α

and phorbol esters, which activate PKC, lead to activation of AP-1 and NF-κB binding to DNA; this effect may be blocked by corticosteroids. cAMP may exert a profound modulatory effect on MAPK signaling pathways and may activate the transcription factor CREB (cAMP response element binding), which itself interacts with GRs and with AP-1 (75).

A good example of receptor interaction is provided by the interaction of β_2 receptors and GRs, which is relevant to the treatment of asthma and COPD, as combination inhalers containing a long-acting β_2-agonist and a corticosteroid are now commonly used as first-line therapy. Corticosteroids increase the expression of β_2 receptors in lungs and so enhance the effects of β_2-agonists and prevent receptor downregulation (76,77). In turn, β_2-agonists increase the action of GRs by facilitating nuclear translocation for the cytoplasm and increasing the binding of GRs to GRE, thus increasing the effects of corticosteroids.

III. Drug-Receptor Interactions

The binding of a drug to its receptor is a dynamic process and follows the laws of mass action. At equilibrium, there is a balance between the rate of association and the rate of dissociation of a drug. The concentration of drug giving half maximal activation is the EC_{50}, which describes the potency of the drug. The *affinity* of the drug describes the balance between association and dissociation and can be quantified as the dissociation constant, K_d, which is the logarithm of the concentration of drug needed to occupy 50% of the receptors. Drugs with a low K_d therefore have a high affinity for their receptor.

A. Radioligand Binding

Binding between a hormone or drug and its receptor may be studied directly by radioligand binding. A radiolabeled ligand (usually a high-affinity antagonist, such as [^{125}I] iodocyanopindolol for β receptors) is incubated with a receptor preparation (either a membrane preparation from the tissue of interest or, in the case of some ligands, with intact cells). The binding interaction between ligand and receptor obeys the law of mass action. As the concentration of ligand is increased, the proportion of ligand binding to receptors increases until saturation occurs when all the receptors are occupied. Nonspecific binding to nonreceptor sites is determined by parallel incubations with radioligand in the presence of an excess of unlabeled agonist or antagonist (e.g., 200 μM isoproterenol or 1 μM propranolol for β receptors). Specific binding (i.e., total − nonspecific binding) may be analyzed by a Scatchard plot, which will give a straight line if a single class of binding site is involved, the slope of which is related to binding affinity ($1/K_d$), and the intercept on the *x*-axis gives the maximum number of binding sites (B_{max})—a measure of receptor density. Radioligand binding studies can also be used to investigate selectivity of drugs for the receptor using competition between the competitor drug and a fixed concentration of radioligand. Receptors may be characterized in this way, using the rank order of potency of agonists or antagonists.

Binding studies can also be used to determine the distribution of receptors in tissues, using autoradiography. The radioligand is incubated with frozen sections of the tissue of interest using optimal conditions and using an excess of nonradiolabeled competitor to define nonspecific binding, as in membrane-binding studies. The distribution of specific binding is then used to map tissue localization of receptors.

B. Agonists and Antagonists

After binding to the receptor, the response is activated via second messenger systems described above. Different agonists may elicit variable degrees of response, which is described as *efficacy*. A drug that produces less than a maximal response (E_{max}) is known as a *partial agonist*. In airway smooth muscle, isoproterenol (isoprenaline) is a full agonist and produces a maximal response, whereas albuterol (salbutamol) and salmeterol act as partial agonists, giving less than 50% of the maximal relaxation seen with isoproterenol.

Antagonists have zero efficacy. They block the effects of an agonist by interfering with its binding to the receptor. When antagonists interact with agonists at a common receptor, the antagonism is competitive. This can be demonstrated by a rightward shift in the log concentration-response curve (Fig. 12). For true competitive antagonism (e.g., between a β_2-antagonist and β-agonist in airway smooth muscle), the shift is parallel. The amount of shift observed with each concentration of agonist can be used to calculate the affinity of the antagonist for the particular receptor.

Sometimes a drug interferes with an agonist effect in a noncompetitive manner by inhibiting any of the steps that lead to the typical agonist effect. This results in a nonparallel shift in the agonist dose-response curve and a reduced maximal response (Fig. 12). Studies with overexpressed GPCR or mutated receptors have demonstrated that there may be constitutive activation of the receptor in the absence of any agonist. Drugs that reduce this constitutive activation by binding to the receptor are known as *inverse agonists* (78). It is now clear that many drugs that were assumed to be antagonists of GPCR act as inverse agonists, and this may be explained by stabilization of the inactive state of the receptor (79). There may be constitutive overactivity of GPCR in disease, and this could account for phenomena such as bronchoconstriction after

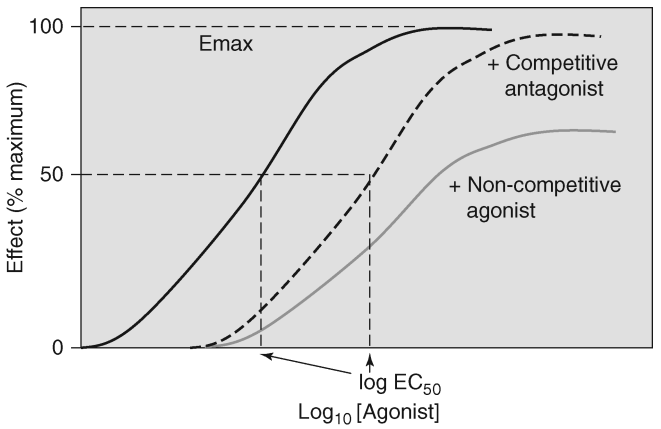

Figure 12 Dose-response curve to an agonist is affected by the presence of antagonists. A competitive antagonist causes a parallel rightward shift in the dose-response curve, with an increase in the concentration of drug that yields half-maximal activation (EC_{50}) but no change in the maximal response (E_{max}), whereas a noncompetitive antagonist shifts the dose response to the right in a nonparallel fashion and also decreases E_{max}.

β-adrenergic blockers in asthma but not in normal individuals as a result of the inverse agonism of certain β-blockers (78).

Another type of antagonism, which is relevant to lung diseases is *functional antagonism*, which describes an interaction between two agents that have opposite functional effects on the same cellular response. Thus, β-agonists act as functional antagonists in airway smooth muscle since they counteract the contractile effects of any spasmogen, including histamine, leukotriene D_4, thromboxane, bradykinin, and acetylcholine.

Two drugs may interact to produce effects that are more than additive. If two drugs given together produce an effect that is greater than the additive effect of the drugs given separately, this is known as *synergy*. *Potentiation* is when one drug given alone has no effect, but increases the response to a second drug. *Tolerance* refers to a diminishing response to a drug that is administered repeatedly, whereas *tachyphylaxis* usually describes tolerance of rapid onset, so that it may be seen after only one administration of the drug. *Desensitization* is a term that includes rapid and long-term loss of response.

The interaction between a ligand and its receptor has several characteristics. Binding is rapid reversible and is temperature dependent. There is stereoselectivity with the *levo*-isomer (*R*-isomer) usually binding more effectively than the *dextro*-isomer (*S*-isomer). Many ligands are a mixture of the active *R*-isomer with the inactive *S*-isomer, so that the racemic mixture has half of the activity. It is claimed that for some racemates the inactive isomer is actually detrimental. For example, *S*-albuterol (levalbuterol) has been reported to increase airway hyperactivity in animal models so that the *R*-albuterol is more effective than *RS*-albuterol (80). However, no clear advantage of *R*-albuterol over *RS*-albuterol has been demonstrated in clinical studies (81).

IV. Receptor Regulation

Receptors are subject to many regulatory factors that may operate at several sites (Fig. 13). Some factors influence the gene transcription of receptors, either increasing or decreasing transcription. Other factors influence the stability of mRNA and thus the amount of receptor protein that is formed. Translation of receptor protein may also be regulated. Once the receptor protein is inserted into the membrane, the receptor may be regulated by phosphorylation as a result of various receptor kinases, and receptor phosphorylation is one of the major means of receptor regulation (82). Some receptors are also tyrosine nitrated or ubiquitinated, resulting in abnormal function and increased degradation.

A. Desensitization

Tachyphylaxis or desensitization occurs with most receptors when exposed to an agonist. This phenomenon has been studied in some detail with β_2 receptors and involves several distinct processes that may operate simultaneously or sequentially (83).

G Protein Receptor Kinases

In the short term, desensitization involves *phosphorylation*, which uncouples the GPCR from G_s, via the action of enzymes called GRK, of which seven are now identified (84). This has been studied in most detail for β receptors that are phosphorylated by GRK2, also known as β-adrenergic receptor-specific kinase (βARK). The site of this

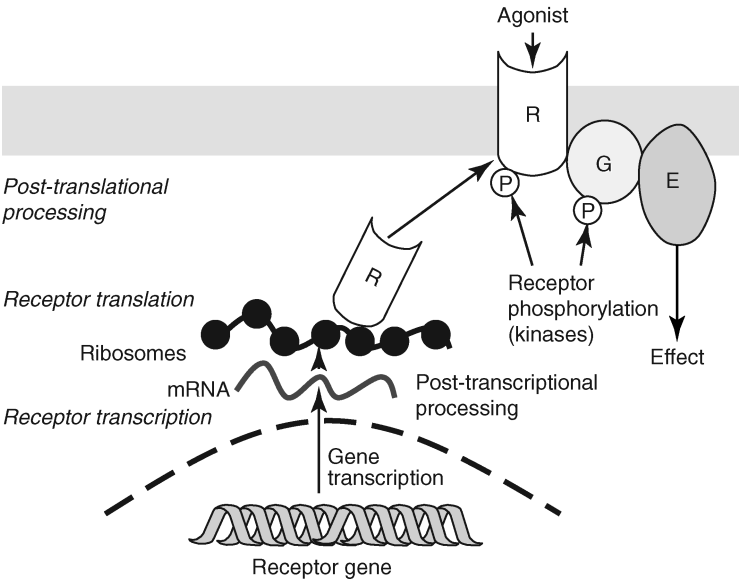

Figure 13 Regulation of receptor expression. The expression and function of surface receptors may be regulated in several ways, including effects on receptor synthesis (gene transcription, posttranscriptional processing/mRNA stability, protein translation and posttranslational processing). Once the receptor is inserted in the membrane it may be inactivated and uncoupled by phosphorylation via various kinases. *Abbreviations*: R, receptor; G, G protein; E, effect; P, phosphorylation.

phosphorylation appears to be on the Ser/Thr-rich region of the third intracellular loop and the C-terminal tail, since their replacement reduces the rate of desensitization. GRK2 is also involved in the phosphorylation of several other receptors, including muscarinic and tachykinin receptors. The expression of GRK2 varies between cells. There is low expression of GRK2 in airway smooth muscle cells that show resistance to desensitization, whereas expression of this enzyme is high in mast cells that are much more easily desensitized (85). GRK2 expression is increased in lungs after exposure to β_2-agonists, thus contributing to uncoupling of β_2 receptors (86) and is increased by corticosteroids, which therefore reverse uncoupling and restore responsiveness to β_2-agonists (87). GPCR are also regulated by other kinases, including PKA and PKC (82). For β_2 receptors, this appears to be via phosphorylation of GRK2, enhancing its ability to uncouple receptors rather than direct phosphorylation of β_2 receptors.

Arrestins

Another protein, β-arrestin, is also involved in uncoupling the phosphorylated β receptor from the G protein and in resensitization of receptors (Fig. 14). There are two β-arrestins and they appear to be universally expressed and involved in the coupling of many GPCRs. β-Arrestins determine whether β_2 receptors are degraded within the cell by

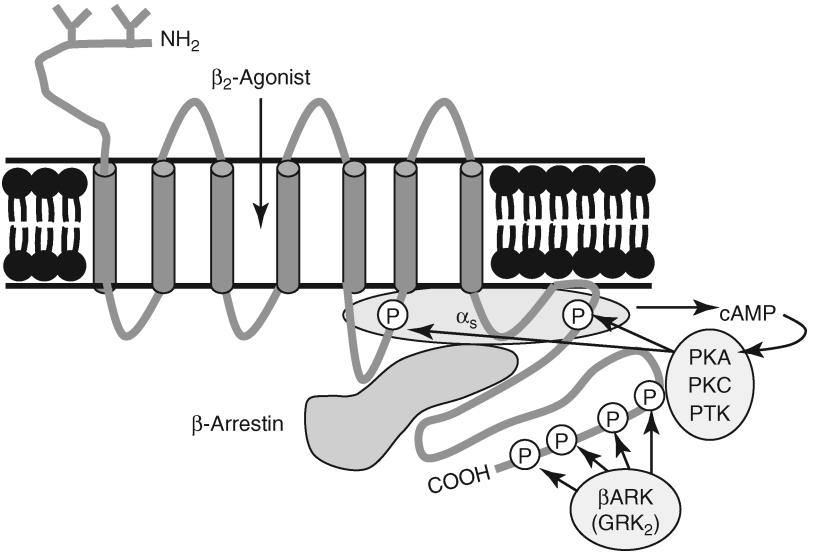

Figure 14 Mechanisms of short-term desensitization of β_2-adrenergic receptors. The β receptor is phosphorylated by βARK and other GRK on its C tail (COOH), resulting in increases in binding of β-arrestin, which leads to uncoupling of the receptor from the α-subunit of the stimulatory G protein (α_s) and internalization of the receptor. PKA, activated by an increase in cAMP phosphorylates the receptor at other sites on the third intracellular loop or may phosphorylate GRK. PKC and PTK may also phosphorylate the receptor, resulting in uncoupling. *Abbreviations*: βARK, beta-adrenergic receptor kinase; GRK, G protein receptor kinases; PKA, protein kinase A; cAMP, cyclic 3′,5′ adenosine monophosphate; PKC, protein kinase C; PTK, protein tyrosine kinases.

endocytosis or are recycled to the cell membrane (88) (Fig. 15). β-Arrestins terminate receptor function and also allow receptors to interact with signal transduction pathways [such as MAPK and PI3K (phosphoinositide-3-kinase)] independently and therefore allow the receptor to regulate different responses in the cell. Ligands that preferentially stimulate the interaction between receptor and β-arrestins have now been identified, and these drugs may have a different spectrum of activity to the classical drugs that activate receptor–G protein coupling. Ligands that are biased toward receptor–G protein interaction with less effect on β-arrestins do not desensitize the receptor and thus have greater and more prolonged effects.

Downregulation

Longer-term mechanisms of desensitization include downregulation of surface receptor number, a process that involves internalization (*sequestration*) of the receptor and its subsequent degradation. Downregulation of β_2 receptors results in a rapid decline in the steady-state level of β_2-receptor mRNA. This suggests that downregulation is achieved in part, either by inhibiting the gene transcription of receptors or by increased post-transcriptional processing of the mRNA in the cell. Using actinomycin D to inhibit

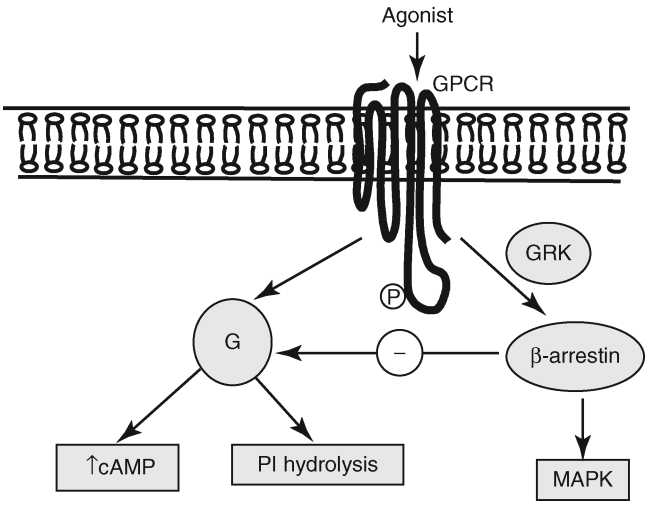

Figure 15 G protein–coupled receptors signal through G proteins and also via β-arrestins. When the receptor is occupied by its agonist, signal transduction pathways are activated, such as changes in cAMP or increased PI hydrolysis. Agonists also O-phosphorylate the receptor via GRK, which recruit β-arrestin that then stimulates other signaling pathways, such as MAPK, and also inhibits G protein–mediated signal transduction. *Abbreviations*: cAMP, cyclic 3′,5′ adenosine monophosphate; PI, phosphoinositide; GRK, G protein receptor kinases; MAPK, mitogen-activated protein kinases.

transcription, it has been found that β_2-receptor mRNA stability is markedly reduced in these cells after exposure to β-agonists (89). Furthermore, by isolating nuclei and performing a nuclear run-on transcription assay, it is apparent that β-agonist exposure does not directly alter receptor gene transcription. Longer-term exposure to β agonists may also result in inhibition of β-receptor gene transcription, mediated via the effects of a cAMP-specific transcription factor (CREB). Long-term exposure to β-agonists results in reduced transcription of β_2 receptors in the airways (90,91).

B. Corticosteroid Modulation

Certain G protein–linked receptors are also influenced by corticosteroids. Thus, pulmonary β receptors are increased in density by pretreatment with corticosteroids (92). Corticosteroids increase the steady-state level of β_2-receptor mRNA in cultured smooth muscle cells, thus indicating that steroids may increase β-receptor density by increasing the rate of gene transcription. The increase in mRNA occurs rapidly (within 1 hour), preceding the increase in β receptors, and then declines to a steady-state level about twice the normal. The cloned β-receptor gene contains three potential GRE, and incubation of human lung with corticosteroids results in a doubling of the rate of transcription (92). By contrast, corticosteroids decrease transcription of tachykinin NK_2 receptors (93).

C. Receptor Ontogeny

Another area in which molecular biology of receptors may be relevant is in studying the development of receptors and the factors that determine expression of particular receptor genes during development. In fetal lung, there is a marked increase in the expression of β_2 receptors in the perinatal period and this is critically dependent on glucocorticoids (94). There may be differential expression of receptor subtypes during development. For example, muscarinic receptor subtypes in the lung show differential changes around the perinatal period (95). Receptors also play an important role in lung development. For example, receptors for hepatocyte growth factor are expressed in airway epithelium during lung development and appear to mediate branching of the airways (96).

D. Pulmonary Disease

There are several pulmonary diseases in which altered expression of receptors may be relevant to understanding their pathophysiology. Molecular biology offers a new perspective in investigating these abnormalities of receptor expression by providing insights into whether the abnormality arises through altered transcription of the receptor gene or due to abnormalities in posttranscriptional or posttranslational processing. There is some evidence that β-adrenoceptor function may be impaired in airway smooth muscle of patients with fatal asthma (97). However, binding and autoradiographic studies have not demonstrated any reduction in β receptors in airway smooth muscle, suggesting that the reduced bronchodilator responses to β-agonists may be due to uncoupling of the receptor (98). Similarly no differences in muscarinic receptors have been detected in asthmatic lungs using binding approaches (98).

Relatively few studies have explored whether there are any differences in the expression of mediator receptors in asthmatic airways. There is some evidence for increased expression of platelet-activating factor (PAF) receptor mRNA in lungs of asthmatic patients, although whether this has functional significance is not known (99). Bradykinin B_1 and B_2 receptors are upregulated by inflammatory stimuli such as TNF-α, and this effect appears to be due to prolongation of mRNA half-life (100). There is also evidence for increased NK_1 receptor expression in the lungs of patients with asthma and COPD (101,102).

E. Transcriptional Control

Receptor genes, like any other genes, may be regulated by transcription factors, which may be activated within the cell under certain conditions, leading to increased or decreased receptor gene transcription, which may in turn alter the expression of receptors at the cell surface. Little is known about the transcription factors that regulate receptors, but these may be relevant to diseases, such as chronic inflammation. The transcription factor AP-1, a Fos-Jun heterodimer, may be activated via PKC. AP-1 increased transcription of several genes, including some receptor genes. For example, the gene coding for the NK_1 receptor has an AP-1 site, which leads to increased gene transcription and a GRE that conversely results in decreased transcription (103). Chronic cell stimulation, via activation of PKC, may therefore lead to an increase in NK_1 receptor gene expression, which could lead to increased neurogenic inflammation. An increased NK_1 receptor gene expression is present in asthmatic airways (101). By contrast, corticosteroids reduce NK_1-specific mRNA in human lung, probably via an inhibitory effect of GR on AP-1.

V. Ion Channels

Movement of ions across the cell membrane is important in determining the state of cell activation. Ions cross the cell membrane through protein-lined pores called channels, several of which have now been cloned and characterized electrophysiologically. Most channels are made up of distinct subunits that are grouped together in the cell membrane (for example, nAChR as discussed above). Whether the channel is open or closed depends on different factors for each channel, but may be determined by receptor activation, the polarization of the cell membrane, or the presence of particular ligands that interact directly with the channel.

A. Calcium Channels

Voltage-Gated Channels

Several types of Ca^{2+} channel have now been identified and characterized electrophysiologically by molecular cloning and by the use of antagonists and toxins. Voltage-gated channels mediate Ca^{2+} entry into cells in response to membrane depolarization. Electrophysiological studies reveal different Ca^{2+} currents designated L-, N-, P-, Q-, R-, and T-type. High-voltage-activated Ca^{2+} channels that have been characterized biochemically are complexes of a pore-forming α1-subunit of approximately 190 to 250 kDa, a transmembrane, disulfide-linked complex of α2-and δ-subunits, an intracellular β-subunit and in some cases a transmembrane γ-subunit. Ten α1-subunits, four $\alpha 2\delta$ complexes, four β-subunits, and two γ-subunits are now identified (104). The Cav1 family of α1-subunits conduct L-type Ca^{2+} currents, which initiate muscle contraction, endocrine secretion, and gene transcription, and are regulated by protein phosphorylation. The Cav2 family of α1-subunits conducts N-type, P/Q-type, and R-type Ca^{2+} currents, which initiate rapid synaptic transmission and are regulated primarily by direct interaction with G proteins and SNARE proteins and secondarily by protein phosphorylation. The Cav3 family of α1-subunits conducts T-type Ca^{2+} currents, which are activated and inactivated more rapidly and at more negative membrane potentials than other Ca^{2+} current types.

L-type channels (for long lasting) open in response to depolarization of the cell, resulting in influx of Ca^{2+} to increase intracellular Ca^{2+} concentration (Fig. 16); these channels are blocked by dihydropyridines, such as nifedipine, and by verapamil. Voltage-sensitive calcium channels are important in contractile responses of pulmonary vascular smooth muscle, but are less important in the contractile response of airway smooth muscle or in the activation of inflammatory cells. T-type calcium channels are also opened by depolarization but are insensitive to dihydropyridines. Electrophysiological studies have revealed the presence of both L- and T-channels in airway smooth muscle, although the L-channels are less sensitive to dihydropyridines than the L-channels in the myocardium (105). N-type channels, which are largely restricted to neurons, are also sensitive to depolarization and insensitive to dihydropyridines, but are blocked by ω-conotoxin.

Receptor-Operated Channels

Receptor-operated channels (ROCs) are channels that open in response to activation of certain receptors; these receptors are not well defined, but recently blockers have been developed. Contraction of airway smooth muscle in response to agonists such as acetylcholine and histamine is independent of external Ca^{2+} and is not associated with ^{45}Ca uptake, suggesting that calcium entry is not important for initiation of contractile

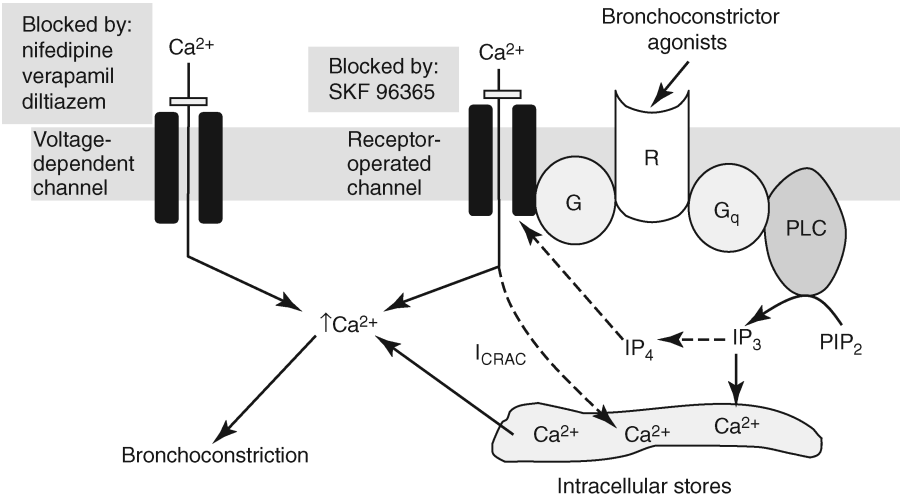

Figure 16 Calcium ion (Ca^{2+}) channels. There are at least two classes of calcium channel in the cell membrane. One is activated by depolarization (voltage-dependent channels) and the other via receptors (ROC). Ca^{2+} are also released from intracellular stores via phosphoinositide hydrolysis and the action of IP_3. Intracellular stores are refilled by calcium release-activated calcium current (I_{CRAC}). IP_4 generated from IP_3 may activate calcium entry via receptor-operated channels. *Abbreviations*: IP_3, inositol (1,4,5) trisphosphate; IP_4, inositol (1,3,4,5) tetrakis phosphate; G, G protein; PLC, phospholipase C; PIP_2, phosphoinositide 4,5,-bisphosphate.

responses. Entry of Ca^{2+} via ROCs may be important in refilling intracellular stores. ROCs are members of an increasing group of calcium channels called transient receptor potential (TRP) cation channels, which is a superfamily of at least over 30 channel proteins divided into six main groups. TRP channels are involved in regulation of inflammatory cells, structural cells, and sensory nerves and may be important novel targets for drug discovery (106). For example, transient receptor potential vanilloid (TRPV) channels on sensory nerves mediate to response to capsaicin and acids. TRPC3 and TRPC6 are expressed in human airway smooth muscle and may correspond to ROCs involved in Ca^{2+} entry (107).

A rise in intracellular Ca^{2+} concentration is associated with cell activation, but recovery depends on removal of Ca^{2+} by sequestration or by pumping out of the cell in exchange for Na^+. In airway smooth muscle, there is a pump that exchanges $3Na^+$ for each Ca^{2+} that is linked to the activity of Na^+–K^+ ATPase, which maintains the inwardly directed Na^+ gradient by exchanging intracellular Na^+ for extracellular K^+. In airway smooth muscle, there is also an active uptake of Ca^{2+} into intracellular stores, which may be stimulated by cAMP (32).

I_{CRAC}

When Ca^{2+} are released from intracellular stores via the action of IP_3, these stores are refilled by calcium entry via specific channels called CRAC, measured as a current called I_{CRAC} (Ca^{2+} release-activated Ca^{2+} current) that is activated by depletion of

intercellular stores (store-operated calcium entry) (108). This is an important mechanism in inflammatory cells, such as mast cells and T lymphocytes. Inhibitors of I_{CRAC} are therefore potential immunomodulators or anti-allergy drugs. TRPC1 is thought to function as a component of store-operated calcium channels (109).

In airway smooth muscle, several types of calcium channel have been identified and these may lead to contraction via different spasmogens. L-channels respond to depolarization and ROCs are activated by various spasmogens, but contraction initially is independent of Ca^{2+} entry and due to release of Ca^{2+} from internal stores in response to IP_3. These internal stores are then refilled via store-operated calcium channels. In addition, cyclic ADP-ribose also mobilizes calcium from the sarcoplasmic reticulum by acting on ryanodine receptors (32).

B. Potassium Channels

Recovery of cells after depolarization depends on the movement of potassium ions (K^+) out of the cell via K^+ channels in the cell membrane. This results in hyperpolarization of the cell with relaxation of smooth muscle and inhibition of cell activity. Conversely, blockade of K^+ channels with drugs such as tetraethylammonium and 4-aminopyridine results in increased excitability or hyperresponsiveness of cells. Over 50 different K^+ channel genes have been identified in humans, using selective toxins, patch-clamping techniques, and cloning (110). K^+ channels may be subdivided into four main classes:

1. *Voltage-gated channels* that open on depolarization of the membrane, which are rectifying channels that return the cell membrane to its previous polarized state. This is a diverse group of channels, some of which are blocked by α-dendrotoxin.
2. *Ca^{2+}-activated channels* open in response to elevation of intracellular Ca^{2+} concentration. Large-conductance (maxi-K or big) K^+ channels are found in smooth muscle and neurons and are blocked by the scorpion venoms charybdotoxin and iberiotoxin, as well as several small molecules (111). Small conductance channels, some of which are blocked by apamin are found in neurons.
3. *Receptor-coupled channels* are opened by certain receptors via a G protein, but no specific blockers have been found.
4. *ATP-sensitive channels* are opened by a fall in intracellular ATP concentration. These channels are found in smooth muscle and in the islet cells of the pancreas. They are blocked by sulfonylureas, such as glibenclamide, and are opened by drugs such as cromakalim (BRL 34915), its active enantiomer lemakalim (BRL 38227), RP 53891, and HOE 245.

K^+ channels play an important role in relaxation of airway smooth muscle (112,113). β-Agonist-induced bronchodilatation is markedly inhibited by charybdotoxin (114), indicating that opening a maxi-K channel is involved in the relaxant response. K^+ channel openers, such as cromakalim and levcromakalim, act on ATP-sensitive K^+ channels and are dilators of animal and human airways (115). K^+ channel openers therefore have potential as bronchodilators and vasodilators, although when given orally they may cause cardiovascular side effects due to systemic vasodilatation, which limit their usefulness in asthma therapy. K^+ channels are also involved in neurotransmitter release (112). Cromakalim inhibits cholinergic neurotransmission and the release of

neuropeptides from sensory nerves in airways (116). Modulation of neurotransmission is also mediated by opening of maxi-K channels, since charybdotoxin reverses the modulatory effect of many agonists on sensory and cholinergic nerves (117). K^+ channels are also involved in mucus secretion (118). K^+ channel openers therefore have several potential applications in the therapy of airway disease (119).

C. Sodium Channels

Na^+ channels are involved in depolarization and release of neurotransmitters. Drugs that block Na^+ channels, such as tetrodotoxin and local anesthetics, act as nerve blockers. However, tetrodotoxin has no direct effect on smooth muscle. Na+ channels in airway epithelium (ENaC) play an important role in regulating mucus hydration and mucociliary clearance and play a key role in alveolar fluid absorption. ENaC dysfunction is important in acute respiratory disease syndrome and in cystic fibrosis (120,121). Each channel is comprised of three subunits designated α, β, and γ and several new drugs that target these channels are in development of the treatment of lung disease.

D. Chloride Channels

Although less well characterized than other ion channels, it is increasingly recognized that chloride (Cl^-) channels play an important role in pulmonary physiology and pathology. The cystic fibrosis transmembrane regular (CFTR) that is functionally abnormal in cystic fibrosis plays a critical role in airway hydration and has been extensively investigated. Calcium-activated Cl^- channels (CLCA) play an important role in mucus secretion and are now targeted to treat mucus hypersecretion (122). Volume and voltage-dependent Cl^- channels appear to play a role in airway responses to indirect bronchoconstrictors and are blocked by furosemide and cromolyn (cromoglycate) sodium (123).

VI. Enzymes

Many drugs produce their therapeutic effect by inhibition of particular enzymes. Most commonly the drug molecule is a substrate analog that acts as a *competitive inhibitor*. The interaction between drug and enzyme obeys the law of mass action and may be analyzed in the same way as drug-receptor interactions. An example is L-N^G-nitro arginine, which acts as a competitive inhibitor of NO synthase by substituting for the natural substrate L-arginine. The enzyme blockade may be overcome by increasing the concentration of L-arginine, whereas D-arginine, which is not a substrate for this enzyme, has no effect. Many drugs act noncompetitively. An example is aspirin, which noncompetitively blocks cyclooxygenase by acetylating the active catalytic site of the enzyme. Another type of interaction involves a false substrate, where the drug undergoes chemical transformation by the enzyme to form a product that subverts the normal metabolic pathway. The best example of this is α-methyldopa, which mimics DOPA, causing norepinephrine to be replaced by methylnorepinephrine, which is inactive.

Enzymes are increasingly recognized as playing an important part in the pathophysiology of various diseases and are increasingly a target for drug therapy. Drugs that inhibit 5′-lipoxygenase, which generates leukotrienes, such as zileuton, are now used in the treatment of asthma. Drugs that inhibit neutrophil elastase may be useful in the future management of cystic fibrosis and COPD, whereas drugs that inhibit tryptase and chymase from mast cells may be potential treatments in asthma.