G proteins, or GTP-binding proteins, are a family of proteins that act as links in signaling chains across cell membranes in all eukaryotic cells. These pathways usually include a receptor molecule, a G protein as the signal transducer, and an effector enzyme or ion channel that can be activated or inhibited by the G protein. A large variety of stimuli reaching the cell surface, including numerous neurohumoral and olfactory signals and light, can be transmitted by these specific signaling mechanisms. It is remarkable that this diversity of signals is processed by structurally similar transducing mechanisms, including the activation of a GTP-binding protein. From sequence and structure analyses, we know that receptors that transduce a signal through the activation of a G protein share a common structure consisting of seven transmembrane-spanning domains, intracellular and extracellular loops, an extracellular amino terminus, and a cytoplasmic carboxy terminus. The cytoplasmic loops, particularly the third cytoplasmic loop, and the carboxy terminus appear to be involved in G protein coupling. G proteins also share a common structure, which is heterotrimeric, consisting of an α/β/γ-subunit; the α-subunit is thought to be responsible for the specific action by directly interacting with the effector. In addition, the α-subunit binds and subsequently hydrolyzes GTP, the important events that first activate and then deactivate the G protein.

Since the first evidence of a GTP-dependent step in the activation of adenyl cyclase in 1971 and the following purification and characterization of a GTP-binding protein as a regulator of adenyl cyclase, now called Gₛ, at least 15 G protein α-subunits have been discovered, and this number is still increasing. An inhibitory G protein, termed Gᵢ, has been shown to inhibit adenyl cyclase activity. At least three isoforms of Gᵢ exist and are expressed in a tissue-dependent manner; their individual functions have not been fully clarified. A heterotrimeric G protein called transducin is located in the outer segments of retinal rods and transduces the signal from the light-activated rhodopsin to the cyclic GMP (cGMP)–phosphodiesterase. There are other G proteins that activate membrane-bound phospholipase C. G proteins play a major role in the modulation of ion channels, including a K⁺ channel, which can be activated by a Gₛ, and voltage-dependent Ca²⁺ channels and Na⁺ channels, which can be positively or negatively regulated by Gₛ, respectively. A G protein termed Gₛ is expressed in high quantities in brain. It may be involved in K⁺ channel opening, regulation of opioid-mediated Ca²⁺ channels, and stimulation of phospholipase C. Important signal transduction in olfaction is mediated by a G protein called Gₛ whose α-subunit shows an 88% homology with the α-subunit of Gₛ. An additional large number of other GTP-binding proteins exist, some of them resembling small-molecular-weight species like the ras proteins. In the larger-molecular-weight range, complementary DNA (cDNA) cloning has demonstrated the existence of several more proteins that are homologous to the described α-subunits found in heterotrimeric G proteins. Their specific functions have yet to be determined.

Some G proteins appear to be able to interact with more than one receptor as well as with more than one cellular effector, indicating that a diversity of signals may result in the same cellular response and, conversely, that a single stimulus can trigger several cellular reactions. The resulting effect probably depends on the differential expression of the G proteins and effector proteins in various cell types as well as the stoichiometry of the components of the signaling pathways, which is known to vary among tissues and cell types.

**Activation and Deactivation Cycle of a G Protein**

Despite the diversity of function, the mechanism leading to the activation and deactivation of a G protein is common among the different G proteins (Figure 1). In the inactive state, the α-subunit of a G protein carries a GDP molecule attached to its nucleotide-binding site. Upon interaction with a specific activated receptor, which causes a conformational change, the GDP is replaced by GTP, which increases the affinity of the α-subunit for the effector. This results in a conformational change of the effector, leading to a cellular reaction. After a short period, the effector releases the activated G protein from its binding, and the α-subunit exchanges the GTP for GDP again. This change results in the release of the α-subunit, which is then free to bind to another effector and thereby repeat the cycle. This cycle is repeated until the concentration of the activated receptor is reduced. These cycles can result in a sustained cellular reaction that is regulated by the concentration of the activated receptor.
The interaction of G-proteins with receptors is a complex and dynamic process involving multiple steps. G-proteins, which consist of α-, βγ-, and sometimes δ-subunits, play a crucial role in signal transduction. The activation cycle of a G protein involves several key steps:

1. **Binding of Hormone (H) to a G Protein-Coupled Receptor (R):** The hormone (H) binds to the receptor (R) on the cell membrane, changing the receptor's conformation and enabling it to interact with the G protein.

2. **Exchange of Nucleotides:** In the active state, Ga interacts with effector molecule(s). A GTPase activity hydrolyzes bound GTP releasing free phosphate (P₃) or βγ-GDP, and Ga exhibits high-affinity interaction with α-subunit.

3. **Hydrolysis of GTP:** After binding GTP, Ga forms Ga·GTP and undergoes a conformational change, enhancing its ability to interact with other proteins. Ga·GTP can then hydrolyze to form Ga·GDP and γ-phosphate, which is released from the G protein.

4. **Activation of the Effector:** The G protein promotes the activation of effector molecules, which can be enzymes, ion channels, or other regulatory proteins.

5. **GTPase Activity:** Ga·GDP is released, and Ga·GDP-GTP exchange occurs, allowing Ga to cycle back to the inactive state. This cycle is essential for the continuous signal transduction pathway.

The G protein cycle is crucial for understanding how receptors and G proteins work together to transduce signals into cellular responses. The interplay between different subunits, nucleotide states, and effector interactions determines the specificity and duration of the signal.

**Example Diagram:**

- **Figure 1:** Activation cycle of a G protein. Binding of a hormone (H) to a G protein–coupled receptor (R) catalyzes exchange of nucleotides GDP and GTP, and βγ-subunits are released from α-subunit. In the active state, Ga interacts with effector molecule. A GTPase activity hydrolyzes bound GTP releasing free phosphate (P₃), and βγ-subunits reassociate with α-subunit.

**Concepts:**
- G protein coupling
- Hormone-receptor interaction
- Nucleotide exchange
- Signal transduction pathways

Understanding these processes is essential for the development of targeted therapies and understanding disease mechanisms.
ble of interacting with their respective receptor, suggesting an important role of the carboxy terminus in the interaction between the G protein and the receptor. Further evidence for this was found by the analysis of a defect in a mutant murine lymphoma cell line, the S49 unc ("uncoupled") mutant. In these cells, adenyl cyclase cannot be stimulated by hormonal agonists (although \( \beta \)-adrenergic and prostaglandin E1 receptors are present), but it activates normally when \( G \) is stimulated with aluminum fluoride or nonhydrolyzable GTP analogues. A point mutation in the primary structure of \( G_\alpha \) has been found in the unc mutant replacing an arginine residue located six amino acids from the carboxy terminus by a proline. This structural change is most likely responsible for the receptor–G protein uncoupling.

The \( \alpha \)-subunits of \( G_\alpha, G_\beta, G_\delta \), and transducin share a homology that is highest in certain regions that are thought to be involved in guanine nucleotide binding. These same homologous regions are also found in bacterial elongation factor Tu and in the ras oncogenes, small polypeptides with GTP-binding activity. Elongation factor Tu has been crystallized and three-dimensionally characterized, which led to the proposal of a general G protein model with a domain for effector binding, another for receptor interaction, and a region for guanine nucleotide binding. The sequence homology of putative guanine nucleotide–binding domains has also been successfully used in cloning of the cDNA of new GTP-binding proteins using the polymerase chain reaction method and specific primers that anneal to these conserved regions. These domains are located at residues 39–56, 222–228, 245–253, and 287–299 in \( G_\alpha \) and in corresponding regions of other G protein \( \alpha \)-subunits.

Mutations affecting amino acids that are located within these domains and have functional implications have been described specifically for \( G_\alpha \). The so-called S49 unc mutant expresses a \( G_\alpha \) that is unable to couple to the receptors, as described earlier. Another mutant form of \( G_\alpha \), again found in an S49 cell line, called H21a, can interact with the receptor but is not capable of stimulating adenyl cyclase by a stimulatory \( G_\beta \) and an inhibitory pathway \( G_\gamma \) as found in cardiocytes. Multiple stimulatory \( (R) \) hormone receptors (described in text) can interact with \( G_\beta \), which upon activation (indicated by *) and release of \( \beta \)-subunits, stimulates adenyl cyclase \((AC)\). In addition, \( G_\delta \) can positively regulate \( Ca^{2+} \) channels and may inhibit \( Na^+ \) channels, both by direct interaction and by a cAMP-dependent mechanism via protein kinase A \((PKA)\). Inhibitory \( G \) protein can be activated by a number of inhibitory \((R)\) receptors and inhibits adenyl cyclase. In addition, it activates inward rectifying \( K^+ \) channels and can interact with phospholipase C \((PLC)\), which generates diacylglycerol \((DAG)\) and 1,4,5-inositol triphosphate \((1,4,5-IP_3)\) as second messengers. \( \beta \)-subunits are shared by \( G_\beta \) and \( G_\gamma \), can inhibit the \( \alpha \)-subunits, and may inhibit adenyl cyclase directly. Depending on the cell-specific expression of stimulatory and inhibitory receptors and the different effectors, one stimulus can trigger a complex cellular response.
cyclase. This is due to a point mutation (glycine 226 to alanine) that is located in a region that appears to be responsible for a conformational change during the binding of GTP.30 This GTP-induced rearrangement is thought to allow the G protein to discriminate between GDP and GTP. Other crucial residues within the postulated GTP-binding domains are based on mutational analysis of the ras proto-oncogene, a homologous 21-kd GTP-binding protein.19 Mutations of codon 12 or codon 61, corresponding to glycine 49 and glutamine 227 in G, are found in ras oncogenes of malignant tumors and transforming retroviral ras genes, resulting in a reduced GTPase activity. Site-directed mutagenesis of the corresponding residues in G, results in a reduction of the GTPase activity as well37,38 and thus recapitulates the findings with ras. The mutated G, is now constitutively active. Additional evidence to support the hypothesis of a general G protein structure has been obtained from the crystallographic structures of elongation factor Tu and ras and confirms the role of the proposed GTP-binding sites in activation and deactivation of the protein.19,34 Certain functions can clearly be assigned to defined regions of the molecule and even to specific amino acid residues. These various approaches to examining structure-function relations have begun to clarify the intramolecular and intermolecular mechanisms occurring during the activation of a G protein. Site-directed mutagenesis combined with radiographic crystallographic and nuclear magnetic resonance techniques will surely allow further insight into the structure-function relation of this class of molecules.

The lowest degree of sequence homology among different G proteins is found in three regions—at the very N terminus, in a region encompassing amino acids 80–150, and in a region approximately 50 amino acids from the carboxy terminus. The latter region is probably responsible for the specificity of receptor interaction, whereas the region in the middle of the protein may encompass the specific effector-binding domain. The involvement of additional regions in effector coupling, including the amino and carboxy termini, has been suggested in experiments in which chimeric G, molecules have been expressed in cultured cell lines.39,40 The amino terminus may also encompass the domain responsible for interaction with βγ complex.41,42

Even the isoforms of G, show an increased diversity in these regions, while sharing almost complete homology in the others.8,43 This heterogeneity located at certain "hot spots" further supports the hypothesis that these regions are responsible for the specific function of the G protein and thus reveal a close structure-function relation.

G Proteins in the Heart and Their Functions

Many G proteins have been found in the heart that serve to transduce signals that regulate myocardial functions, including contractility, chronotropy, and dromotropy.2 The β-adrenergic receptor—adenyl cyclase system has been of particular interest in the heart because by production of the second-messenger cyclic AMP (cAMP) and the subsequent activation of protein kinase A, it activates the most potent stimulus to enhance contractile function. Similarly, the stimulatory G protein, Gs, plays an important role during the activation of sarcolemmal adenyl cyclase.

G, The Stimulatory Protein of Adenylyl Cyclase

The physiological stimulation of adenylyl cyclase activity by hormones requires Gs. This protein is present in most eukaryotic cells, including atrial and ventricular myocytes, and has been purified to homogeneity.5 The cDNA of its α-subunit has been cloned from several tissues.1 G, couples a number of membrane receptors to the adenylyl cyclase catalytic unit, including those for β-adrenergic agents, dopamine, adenosine, histamine, prostacyclin, and peptide hormones such as adrenocorticotropic, luteinizing hormone, follicle-stimulating hormone, glucagon, secretin, vasoactive intestinal peptide, and antidiuretic hormone. In addition, G, also directly activates Ca2+ channels, most importantly, cardiac Ca2+ channels, through its α-subunit.13 Additional evidence for a direct stimulation of Ca2+ channels by G, or its activated α-subunit exists in several tissues such as skeletal muscle T tubules,11,14 cardiac ventricular myocytes in culture, and guinea pig or bovine sarcolemma.13 Ca2+ influx in adrenal cells is stimulated by adrenocorticotropic hormone (which causes an activation of Gs) independently of changes in cAMP levels45 and subsequent phosphorylation of the channel. However, Ca2+ channels have been known for some time to be regulated by phosphorylation and dephosphorylation cycles involving cAMP-dependent protein kinase.40 Because G, also mediates cAMP production and subsequent activation of its protein kinase, G, acts by two distinct pathways to activate Ca2+ channels—by stimulation of adenylyl cyclase and production of a second messenger and by a more rapid direct mechanism. The various isoforms of G, appear to activate Ca2+ channels of skeletal muscle T tubules in a qualitatively and quantitatively similar manner as shown with purified G,α-isoforms produced in Escherichia coli using recombinant DNA technology.44 In addition, G, appears to mediate a direct (i.e., cAMP-independent) inhibition of Na+ channels in neonatal ventricular myocytes from rat.14 Because all of the isoforms have also been shown to stimulate adenylyl cyclase, G, is located in a pivotal position among several signal transduction pathways in the cell membrane. However, despite this apparent functional similarity, detailed quantitative analysis of isoform-specific activities of G, reveals some differences among the isoforms (see below).

Four isoforms of G, are known to exist. They are encoded by a single gene47 and are generated by alternative splicing of the precursor RNA transcript.48 These four proteins migrate on sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) as two major bands having apparent molecular weights ranging from 42 to 45 and from 46.
to 52 kd, respectively. They differ by 15 amino acid residues, beginning at residue 73, which are encoded by exon 3. In addition, an alternative acceptor splice site at exon 4 can be used to generate isoforms, including an additional serine residue. More recently, a variant Gα messenger RNA (mRNA) isoform was identified in canine heart that is transcribed from an alternative promoter-leading exon element.49 Because this alternative first exon does not encode for a peptide sequence, the resulting protein lacks the first 59 amino acids found in all other Gα isoforms. The function of the putative protein product encoded by this variant mRNA is unknown. The four conventional isoforms are differentially expressed in tissues.1,50 In the adult canine ventricle, Gα is represented mainly by its 45-kd form, unlike other tissues, such as the liver, in which both 45- and 52-kd forms are detectable at similar levels.1,31,51 The large and short isoforms appear to differ in the rate with which they exchange GTP for GDP,24,52 the long form performing this function more rapidly. This could have implications for the activation of adenylyl cyclase in tissues expressing predominantly one or the other isoform. Assuming that the activity of adenylyl cyclase is dependent on the relative amount of the GTP-bound form of Gα and that the rate of GTP–GDP exchange will affect this, one might hypothesize that adenylyl cyclase would be activated to a greater extent in tissues expressing predominantly the long form of Gα. Compared with basal activity,53 the receptor–G protein–stimulated activity of myocardial adenylyl cyclase has been shown to be rather low compared with that of hepatic membranes.54 Besides possible differences in β-receptor content and catalytic unit activity, this could be explained by the differential expression of the Gα isoforms. Similar data have been obtained with purified components of the adenylyl cyclase system using myocardial adenylyl cyclase and liver Gs (mixed forms) versus turkey erythrocyte Gs (only small form).55 The functional consequences of tissue differences in Gα isoform expression, however, remain poorly defined. Actual isoform expression that would have allowed a comparison of the stimulatory activity of the different isoforms on myocardial function has not been accomplished in cardiocytes in culture or in the intact heart. Further experiments addressing such basic concerns as the role of receptor–G protein stoichiometry and Gα isoform content in modulating cardiac responsiveness are required to answer these questions.

The α-subunit of Gs is a substrate for ribosylation catalyzed by a toxin from Vibrio cholerae. This reaction covalently modifies an arginine residue (presumably Arg 201 in the large form and Arg 187 in the short form), which is located near the postulated GTP-binding region. The modification irreversibly activates Gs by preventing the hydrolysis of GTP, a mechanism that has also been described for transducin.56 The covalent incorporation of an ADP-ribose into the Gα molecule can be used to radioactively label Gα and visualize it by SDS-PAGE and autoradiography.51 This method of determining the amount of ribosylated substrate with a molecular weight of 45 or 52 kd has been used extensively as a quantitative determination of Gα stoichiometry in diseases, in disease models, and during cell differentiation.31,54,57–59 There are doubts, however, as to whether the ribosylation of Gα is a linear assay for Gs due to several technical problems, including ribosylation of proteins other than Gα and inefficient ribosylation causing an underestimation of Gα compared with antibody-based techniques.60 Thus, more quantitative and specific assays for Gα have been developed. Antibodies raised against different portions of the Gα protein have been proven valuable for its quantitation.48,54,60–62

A functional assay for Gs was developed using plasma membranes prepared from a cell line lacking Gα but expressing adenylyl cyclase.53 This mutant cell line, termed S49 cyc−, was selected from the wild-type murine lymphoma cell line S49.64 Plasma membranes from this cell line are reconstituted with activated Gs and the stimulated adenylyl cyclase activity is a function of the amount of reconstituted Gs activity. This assay was invaluable for the purification and functional characterization of the Gs protein and for the determination of the functional activity of gene products expressed from the cloned Gα cDNA.65 This procedure also provides a reliable assay for Gs functional activity and has been used to quantitate changes in Gs in various pathophysiological states in different species.31,61,62,66

Gs—The Inhibitory Protein of Adenylyl Cyclase

The inhibition of adenylyl cyclase by a distinct GTP-binding protein coupling to inhibitory receptors was definitively demonstrated in 1982.67 Included within this category are α2-adrenergic, dopaminergic, muscarinic, adenosine, serotonin, opioid, somatostatin, thrombin, bradykinin, neuromedin Y, and prostaglandin receptors.1 In the heart, Gs acts as an inhibitory regulatory component of adenylyl cyclase, predominantly when activated by muscarinic acetylcholine receptors but also when activated by α2-adrenergic, adenosine, somatostatin, and neuromedin Y receptors.1

A variety of functional studies have been performed to characterize the properties of this inhibitory class of G proteins to determine the mechanism through which they act. However, this remains a controversial issue. Gilman and associates7,22,68 have convincingly argued that dissociation of Gs into α and βγ by activation of an inhibitory receptor allows the excess βγ, by the law of mass action, to associate with and inhibit the activity of Gα. However, alternative hypotheses exist, particularly because it has been shown in the cyc− variant (which lacks Gs) that activation of Gs via an inhibitory receptor can depress forskolin-stimulated adenylyl cyclase activity in the absence of any interaction with functional Gs.68,69 However, in support of the hypothesis of Gilman and coworkers, no one has yet demonstrated that purified
Gα has any direct inhibitory effect on purified cyclase catalytic unit. In addition, data have also been presented to suggest that βγ may either directly or indirectly inhibit adenyl cyclase by interaction with calmodulin, independent of its effect on the activity of Gα.\textsuperscript{70}

As is the case for Gs, the interaction with adenyl cyclase is not the only possible activity of Gi. In sympathetic and parasympathetic ganglia, K⁺ channels are activated by muscarinic stimulation that is modulated via a G protein. In the heart, the activation of atrial K⁺ channels has been demonstrated to be mediated by Gs (also called Gt) after muscarinic receptor activation.\textsuperscript{11} This modulation results in a hyperpolarization of atrial muscle cells and pacemakers and acts synergistically with the cyclase inhibitory pathway to decrease the rate of pacemaker firing and slow the heart rate. It may also be an important mechanism counteracting the arrhythmogenic effect of sympathetic activation.

Three distinct isoforms of Gα are known to exist. Each has been expressed in a bacterial system as a recombinant protein.\textsuperscript{12} As observed with the interaction of the Gα isoforms and the slow Ca²⁺ channel, the three Gα isoforms were nearly equipotent in an in vitro system in activating K⁺ channels in experiments using inside-out membrane patches from single atrial cells of adult guinea pigs. Based on their cDNA sequences, the isoforms have been shown to be generated from three separate genes and share 87–94% homology at the amino acid level.\textsuperscript{8,43} Northern blotting has revealed that their specific mRNA content varies among different tissues and that not all isoforms are expressed in every tissue.\textsuperscript{8} Regarding the expression in the heart, Gα2 appears to be the predominant isoform found in ventricle,\textsuperscript{43} which expresses less Gα1 and only very little, if any, Gα1. Gα3 is most abundant in liver and human erythrocytes,\textsuperscript{71} and Gα1 has been found as the predominant form in brain.\textsuperscript{8} Although the functional specificity of the individual isoforms has not been identified and each of the isoforms is capable of carrying out the known functions of Gi, the tissue-specific pattern of expression suggests that functional diversity may nevertheless exist. Additional evidence in support of this concept arises from the fact that the lowest degree of homology among the isoforms occurs in regions that constitute the postulated receptor or effector domains.\textsuperscript{1,35,43}

In addition to cyclase inhibitory and K⁺ channel opening activity of Gα, some evidence suggests the involvement of Gi in muscarinic activation of phospholipase C.\textsuperscript{10} This enzyme catalyzes the breakdown of phosphatidylinositol biphosphate to inositol triphosphate (IP3) and diacylglycerol (DAG), which as second messengers can mobilize Ca²⁺ from intracellular stores and activate Ca²⁺-dependent protein kinase. Whether Gi is mediating the activation of phospholipase C in cardiac myocytes is not clear because pertussis toxin does not inhibit the receptor-stimulated synthesis of inositol phosphates in cardiac atria.\textsuperscript{72} However, others have demonstrated pertussis toxin sensitivity of this same pathway in cultured rat myocytes.\textsuperscript{73} This discrepancy could stem from the expression of different G proteins in cultured myocytes versus atrial cells. There is evidence, however, that activation of phospholipase C in many cell types occurs via one or more distinct G proteins termed Gp, which are clearly different from the Gt isoforms. These G proteins may also play important roles in cardiocytes, predominantly in α₁-adrenergic activation of phospholipase C.

When Gi is involved in signal transduction, the receptor-mediated effects, independent of the involved subtype of Gi, can be attenuated by treatment with pertussis toxin. In a manner similar to cholera toxin, this agent promotes the ADP-ribosylation of a cysteine residue near the carboxy terminus of Gα that is thought to encompass a region of the molecule important for receptor coupling (see above). This ribosylation also occurs in a third type of G protein, Gαi. Pertussis toxin has been useful both in functional studies for the evaluation of G protein–coupled receptors and for the visualization of the Gα/Gβγ-subunit when radioactive nicotinamide dinucleotide (NAD) is used. The Gα isoforms migrate on SDS-PAGE with apparent molecular weights of 40 and 41 kD; Gαi has an apparent molecular weight of 39 kD.

Although several studies have reported that the level of Gαi protein is severalfold greater than that of Gα in the heart,\textsuperscript{31,74} their mRNA levels are inversely related.\textsuperscript{43} Gαi mRNA is present in levels at least fourfold greater than that of Gα2 in canine ventricle. This interesting observation indicates that different mechanisms are involved in determining the steady-state levels of these α-proteins. This could involve differences in translational efficiency and/or protein and mRNA stability. Alterations in these processes in pathophysiologic states could therefore directly impact on the ratio of stimulatory to inhibitory α-subunits and on the activity of their respective signal transduction pathways.

Other G Proteins

Of other known G proteins, Gαi is found to be expressed in the heart at a rather low level, mainly in the atria and even less in the ventricle,\textsuperscript{75} compared with its high level of expression in brain. Changes of Gαi in diseases of the heart have not been described.

A number of small GTP-binding proteins are found in the heart. Their functions, however, are not fully understood. Their involvement has been postulated in intracellular signaling processes such as secretion (e.g., of atrial natriuretic peptide) as has been described for intracellular transport mechanisms in yeast and mammalian cells.\textsuperscript{76} Further studies, including molecular cloning of their cDNAs or genes and structure-function analysis, are needed to evaluate their role in the regulation of cardiac function. Recently, there have been data to suggest that the ras protein and its GTPase-activating protein may
play a role in receptor-mediated modulation of ion channel activity.

\[\beta/\gamma\] Subunits of G Proteins

Upon activation of the G protein, the \(\beta\)- and \(\gamma\)-subunits remain tightly associated with each other after dissociation from the \(\alpha\)-subunit. They are probably shared among different \(\alpha\)-subunits. The functional association of an \(\alpha\)-subunit with a \(\beta/\gamma\) complex is required for the interaction of the \(\alpha\)-subunit with a catalytic receptor; thus, \(\beta/\gamma\) subunits play a major role in the activation step of a G protein. Their important role in modulating the activity of the \(\alpha\)-subunit has been discussed earlier. There can be no doubt that the stoichiometry of \(\beta/\gamma\) relative to \(\alpha\)-subunits is a crucial determinant of the steady state of G protein activation and deactivation. More quantitative measurements, however, are needed because the role of subunit stoichiometry in determining the overall efficiency of receptor-triggered G protein activation remains poorly defined.

A controversial issue relates to the role of \(\beta/\gamma\) in the activation of atrial muscarinic K⁺ channels. Logothetis and coworkers originally suggested that these subunits could directly gate atrial K⁺ channels. However, results from other investigators were in disagreement with these findings and indicated that the \(\alpha\)-subunit, not the \(\beta/\gamma\)-subunit, was responsible for K⁺ channel activation by G succès. There has, however, been evidence that the \(\beta/\gamma\)-subunits can activate cardiac K⁺ channels via activation of phospholipase A₂.

Two distinct forms of \(\beta\)-subunits have been known for some time to exist in G₁, G₂, and G₄—which migrate on SDS-PAGE as a close doublet of 35- and 36-kd bands. The cDNAs of the bovine transducin-\(\beta\) (\(\beta₁\)) and the bovine brain-\(\beta\) (\(\beta₂\)) forms have been cloned, and the predicted amino acid sequences are approximately 90% homologous. Similar results have been obtained from human \(\beta₁\) and \(\beta₂\) cDNAs. An additional third isoform has been shown to exist by cloning of an additional cDNA species from a bovine and a human retinal library. This isoform shares 81–83% homology with the other isoforms. It is still not known whether the \(\beta\)-isoforms are functionally distinct. There is evidence that retinal transducin is the only G protein using only one subunit, the 36-kd species \(\beta₁\), whereas in purified preparations of other G proteins, other isoforms are detectable. This supports the hypothesis that an as-yet-unknown functional specificity of the subunits may exist.

The functional properties of the \(\gamma\)-subunits are the least well characterized. Transducin-\(\gamma\), a polypeptide of 74 amino acids, appears to be distinct from the \(\gamma\)-subunits of other G proteins. The existence of a family of G protein \(\gamma\)-subunits, suggested initially by immunological studies, has now been proven by cDNA cloning. The \(\gamma\)-subunits are thought to play a role as a membrane anchor of the heterotrimer, possibly as a result of a posttranslational modification. Recently, they have been shown to undergo carboxy terminal isoprenylation, which probably plays a role in this process.

Changes in G Protein Function and Stoichiometry in Cardiovascular Disease

The adrenergic signaling mechanism can become dysfunctional in a variety of disease processes that affect the heart, including heart failure and ischemia. Changes in adrenergic drive have been described in heart failure in humans and in animal models of heart failure and include a downregulation of \(\beta\)-adrenergic receptor content and decreased adenylyl cyclase activity. These changes, resulting in decreased cAMP production, are thought to be responsible for a decreased responsiveness of the failing myocardium to endogenous as well as therapeutically administered catecholamines and for deteriorating myocardial function.

However, not all experimental models in which a decreased adenylyl cyclase activity is found also have a concomitant decrease in \(\beta\)-adrenergic receptor density. It is interesting that studies from the era before the discovery of G proteins supported the existence of a postreceptor defect in the adenylyl cyclase system in heart failure. In papillary muscles from patients with heart failure, the positive inotropic effect of glucagon, which activates adenylyl cyclase via a glucagon receptor and G₁ (i.e., independent of the \(\beta\)-adrenergic system), was markedly attenuated, as was the \(\beta\)-adrenergic effect. In more recent studies in a heart failure model of chronic pressure overload in dogs resulting from aortic stenosis, a significant decrease in high-affinity \(\beta\)-adrenergic agonist–binding sites has been found, pointing to a possible role of the coupling G protein, G₄, or to an alteration of the receptor itself. In the same model, G₄ activity was decreased by approximately 40–50% of control levels as determined by functional reconstitution using membranes from S49 cdc cells. Similar findings were obtained in conscious dogs in which ischemic ventricular myocardium was compared with the nonischemic region after 1 hour of coronary artery occlusion. Ischemia caused an attenuated adenylyl cyclase activity and a decrease in G₄ activity, whereas the number of \(\beta\)-adrenoceptors was increased. The majority of these receptors, however, were also uncoupled from G₄. Data describing a functional blockade of G₄ in human dilated cardiomyopathy have been presented by Rasnás et al, who used a specific antibody that recognized only the dissociated or activated \(\alpha\)-subunit of G₄. With this technique, the amount of G₄ activated by GTP was quantitated and found to be decreased in the failing hearts. Functional abnormalities of G₄ without concomitant decreases in G₄, \(\alpha\) protein levels were also reported in cardiomyopathic animals. Studies by Feldman and associates examined strains of hamsters that develop hypertrophy or dilated cardiomyopathy. In both experiments, a decreased functional activity of G₄ compared with control animals was
reported. The molecular reason for this loss in function has yet to be determined in the models studied. Mechanisms involving posttranslational modification such as ribosylation or phosphorylation may be important in this regulation of protein activity during the development of heart disease.

In an attempt to understand the processes that lead to the development of postreceptor defects of the adrenergic nervous system in the heart, the effect of chronic norepinephrine exposure on this same pathway was studied in a conscious animal model. Although the molecular details of the acute desensitization process have been extremely well defined, little information is available on the effects of chronic catecholamine exposure on the adrenergic signaling pathway in vivo. This is particularly important because the cardiac sympathetic nerves play a major role in regulating the exposure of postsynaptic receptors to circulating catecholamines. In fact, some of the changes that occur in human heart failure at the level of the \( \beta \)-adrenergic receptor may require abnormal or malfunctioning cardiac sympathetic nerves for development. In this model of prolonged norepinephrine exposure in conscious dogs, an actual increase in \( \beta \)-adrenergic receptor density was observed with a reduction in functional \( G_i \) and adenyl cyclase activity. The \( \beta \)-adrenergic receptors were also found to be in a predominantly low-affinity or uncoupled state. Thus, these different pathophysiological states (heart failure, ischemia, and prolonged agonist exposure) showed the same phenotype — increased \( \beta \)-adrenergic receptor density and decreased \( G_i \) and adenyl cyclase activities — suggesting that a common but unidentified mechanism may mediate these post-receptor abnormalities. Evidence from our laboratory indicates that a decrease in \( G_i \) functional activity occurs with the development of cardiac hypertrophy before heart failure ensues. Furthermore, this reduction is paralleled by a decrease of similar magnitude in \( G_i \alpha \) mRNA levels. However, as systolic heart failure and left ventricular dilatation ensue, an additional and even more marked decrease in adenyl cyclase activity occurs without a further reduction in \( G_i \) or its \( \alpha \)-subunit mRNA level. Clearly, these more recent data also implicate the cyclase catalytic unit itself as an entity whose malfunction may contribute to the development of a worsening contractile state.

Because it is evident now that the stimulation of adenyl cyclase is not the only role \( G_i \) plays in the myocardium and that ion channels can also be directly gated by \( G_\alpha \) changes in \( G_i \) activity in heart failure and ischemia could also influence ion movement in the myocyte. In the heart, in addition to the cAMP-dependent activation of voltage-dependent \( Ca^{2+} \) channels, which is mediated by phosphorylation of the channel, \( G_i \) can also more directly and rapidly interact with the channel to potentiate this effect. Voltage-dependent \( Na^+ \) channels in cardiac myocytes appear to be negatively regulated by \( G_\alpha \) again by direct membrane-delimited and indirect cAMP-dependent pathways. The alteration of such processes by changes in \( G \) protein activity or content could directly impact both electromechanical coupling in the cardiocyte and the maintenance of contractile function and rhythm in the intact organ.

Potential abnormalities in \( G_i \) have been studied by several groups in heart failure in patients with dilated cardiomyopathy who underwent cardiac transplantation. The authors found a significantly increased \( G_\alpha \) level in failing hearts compared with normal donor hearts using ribosylation techniques with pertussis toxin and/or immunoblotting for quantitation. An elevated level of \( G_\alpha \)-subunits but not \( \beta \)-subunits was also detected in a desensitization experiment using rat cardiocytes in culture that were exposed to noradrenaline for 3 days. However, whether the elevated \( G_\alpha \) levels are ultimately responsible for the defects in cAMP generation remains to be determined. Interestingly, in a study by Böhm et al, ischemic heart disease did not result in elevated \( G_i \) levels, whereas \( \beta \)-receptor density was decreased to an extent similar to that in dilated cardiomyopathy.

Taken together, these findings suggest that multiple defects can occur in the \( \beta \)-adrenergic signaling pathway in disease states of the heart that are characterized by etiologic and temporal differences in their development. However, we still have not identified the trigger that underlies these changes or even determined whether it is increased sympathetic activity and enhanced \( \beta \)-adrenergic receptor occupancy (common to states of cardiac stress) that mediate these processes. Finally, whether the dysfunctional activity of the components relates to a posttranslational event such as phosphorylation or to altered steady-state levels of their mRNA is only now being examined. The experimental methods necessary to answer these kinds of questions are made vastly more difficult by the very nature of the fact that whole animal models are required to recapitulate the disease processes.

Developmental changes in \( G_i \alpha \) and \( \beta \) isoform expression have been examined in rat heart during ontogeny, from the neonate to the adult animal. Clear differences were observed in the tissue-specific pattern of expression in atria and ventricle, indicating that the stoichiometry of these isoforms may have functional implications during the development of \( \alpha \)-adrenergic and parasympathetic signaling pathways in the heart. The principal isoform in ventricle, \( G_i \alpha _5 \), and its mRNA decreased significantly during development and aging, a process with regulatory consequences that are not understood. Developmental differences in \( \alpha \)-adrenoceptor control of cardiac pacemaker activity, however, may be related to the expression of a specific \( G_i \) isoform during development.

### Where To Go From Here

Quantitation of \( G \) protein subunits, \( G \) protein activity, receptor numbers, and effector activities has revealed changes in transmembrane signaling in both physiological and pathophysiological processes during
development and disease. In addition, quantitation of steady-state mRNA has shed light on mechanisms regulating gene expression at the transcriptional and posttranscriptional levels. However, for more detailed analysis of these mechanisms, the quantitation of mRNA synthesis, processing, and degradation rates as well as factors contributing to translational efficiency of the individual proteins are necessary. Studies in intact organs and intact animals will be required in conjunction with in vitro models using cultured cells. These approaches provide unique and complementary information.

A new approach to addressing the role of changes in the stoichiometry of G proteins and their subunits and isoforms for different effector systems is the transfection of a genetically recombined cDNA encoding for a particular subunit into cultured cells. Through placement of heterologous promoter elements upstream of coding regions of cDNAs that drive high levels of transcription, the overexpression of the encoded polypeptide in these cells is made possible. In addition to the examination of the functional characteristics of a G protein α-subunit, this approach will emphasize the potentially important role of G protein stoichiometry in the regulation of a cellular signaling pathway.

However, with specific regard to cardiac physiology and, in particular, pathophysiology, such in vitro studies are not applicable. First, continuous cardiac cell lines are not available for this kind of approach. More important, cardiac function ultimately must be examined in the intact animal, where the effects of loading and innervation can be monitored. Hence, the establishment of a transgenic animal line overexpressing a particular G protein or receptor isoform in the heart may prove uniquely useful in assessing the tissue-specific functional consequences of changes in gene expression. The altered gene expression would be independent of pathophysiological mechanisms regulating the expression of the native gene. For example, in cardiac hypertrophy in which decreases in the levels of Gα and its mRNA content were observed, the role of increased expression of this protein on cardiac performance could be examined. By the very nature of this method, which uses a heterologous promoter and controlling element (enhancer), this expression would be independent of the transcriptional rate of the endogenous gene. In addition, inducible promoters (like the metallothionein promoter or hormone response elements) could be used to boost the level of gene transcription to higher levels after the onset of a pathophysiological state. Tissue specificity could be accomplished by inserting a regulatory element that is known to confer expression in a particular cell type. For example, myosin light and heavy chains are regulated in a tissue- and developmentally specific manner, and these promoters may be useful regulatory elements for this kind of approach.95

The transgenic animal model is not limited to mouse models, which, because of the small size of the animal, would have disadvantages in the assessment of physiological parameters. Data from several groups have indicated that transgenic miniswine can be developed in which the transgene is expressed at high levels.96,97 These animals are sufficiently large to allow careful physiological examinations in the intact animal and thus might be useful for the examination of cardiac performance under physiological and pathophysiological conditions. Furthermore, a larger species would be more amenable to the study of chronic processes (e.g., pressure overload–induced heart failure) that may require months to years to develop, depending on the model. These transgenic models are based on the idea of overexpression of a certain protein in the cardiocyte. However, transgenic animals could also be generated in which the expression of a G protein isoform is reduced or totally abolished. Deletion of the specific allele is accomplished by targeting a mutation to the gene locus using the method of homologous recombination.98 A mutation is generated within the sequence of the desired gene while simultaneously introducing selectable markers that permit the recombination event to be tracked. The mutation causes the disruption of the native gene sequence, which leads to premature termination of gene transcription or mRNA translation. Because the mutated sequence is in large part homologous to the endogenous gene, it will, in rare cases, be integrated into the genome of transfected embryonic stem cells in the place of the desired allele. The selectable markers (i.e., drug resistance genes) are now used to discriminate and select cells that have integrated the mutated sequence at the desired location. This mutated stem cell can now be microinjected into blastocysts; thus, germ-line chimeras can be generated. Finally, by interbreeding heterozygous individuals, one could obtain homozygotes in which both alleles are deleted. This assumes that growth and development can still occur in the absence of the particular gene product. Such deletion models may provide insight into the role and dosage effect of specific gene products in determining the ability of the heart to respond to various pathological states, including volume, pressure overload, and ischemia.

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