

Receptors, G Proteins, and Their Interactions

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Membrane receptors coupling to intracellular G proteins (G protein-coupled receptors) form one of the major classes of membrane signaling proteins. They are of great importance to the practice of anesthesiology because they are involved in many systems of relevance to the specialty (cardiovascular and respiratory control, pain transmission, and others) and many drugs target these systems. In recent years, understanding of these signaling systems has grown. The structure of receptors and G proteins has been elucidated in more detail, their regulation is better understood, and the complexity of interactions between the various parts of the system (receptors, G proteins, effectors, and regulatory molecules) has become clear. These findings may help explain both actions and side effects of drugs. In addition, these newly discovered targets are likely to play important roles in disease states of relevance to anesthesiologists.

G PROTEIN-coupled receptors (GPCRs) form one of the major families of cellular signaling proteins and have great importance to the anesthesiologist. Many drugs target these systems. In fact, the broad range of physiologic functions associated with GPCRs explains why at least 50% of currently available medications target this receptor family. Among the GPCRs that are particularly important to anesthesiology are receptors for opiates, adenosine and related compounds, serotonin and related compounds, and α_2 -adrenergic agonists. In addition, many other drugs used in anesthetic practice—including volatile and local anesthetics—affect GPCR signaling and may cause some of their beneficial and adverse effects through this mechanism. During the past years, our knowledge of GPCR signaling has grown substantially, and the signaling cascades involved have been found to be much more complex than envisioned a decade ago. Some of these findings may help explain both effects and side effects of drugs. For example, a class of GPCR regulatory molecules has been discovered, termed *regulators of G-protein signaling* (RGSs), which seem to be

involved in development of drug tolerance and have been found to be upregulated after administration of opiates. Some of these newly discovered targets are likely to play important roles in disease states. RGS proteins provide another example here. G-protein overstimulation induces cardiac hypertrophy. RGS proteins have been shown to mitigate this process and have become targets for possible drug therapy. An understanding of the complexity of GPCR signaling is of importance for interpretation of study results. For example, experimental data that seem to indicate interference of a drug with G proteins may in fact be a result of an interaction of that drug with RGS proteins.

In this article, the current understanding of GPCR signaling is reviewed. The structure of GPCRs and G proteins, as well as the structural basis of their coupling, is described. GPCR signaling is summarized, emphasizing current models of the process. Allosteric interactions and modulation of GPCR signaling are described. Finally, several examples of anesthetic interactions with GPCR signaling are provided—a section that largely serves to show that our understanding of these molecular interactions still woefully lacks detail.

G Protein-coupled Receptors

G protein-coupled receptors constitute one of the largest known protein families responsible for signal transduction: approximately 2% (about 800) of the genes present in a mammalian genome code for these types of receptors. They are membrane proteins that, through changes in their conformation, report the presence of an extracellular ligand to the intracellular environment. These receptors respond to stimuli such as light, gustatory compounds, odorants, neurotransmitters, neuropeptides, hormones, and glycoproteins. When an extracellular agonist binds to a GPCR, it induces a change in conformation of the receptor. This, in turn, leads to coupling to and activation of one or more G proteins inside the cell. It is the activated G proteins that subsequently regulate intracellular enzymes, *e.g.*, leading to changes in cyclic AMP concentrations or degree of phosphorylation of proteins.

Structure of GPCRs

Recently, the high resolution structure of bovine rhodopsin was elucidated (fig. 1).¹ Although this is a rather

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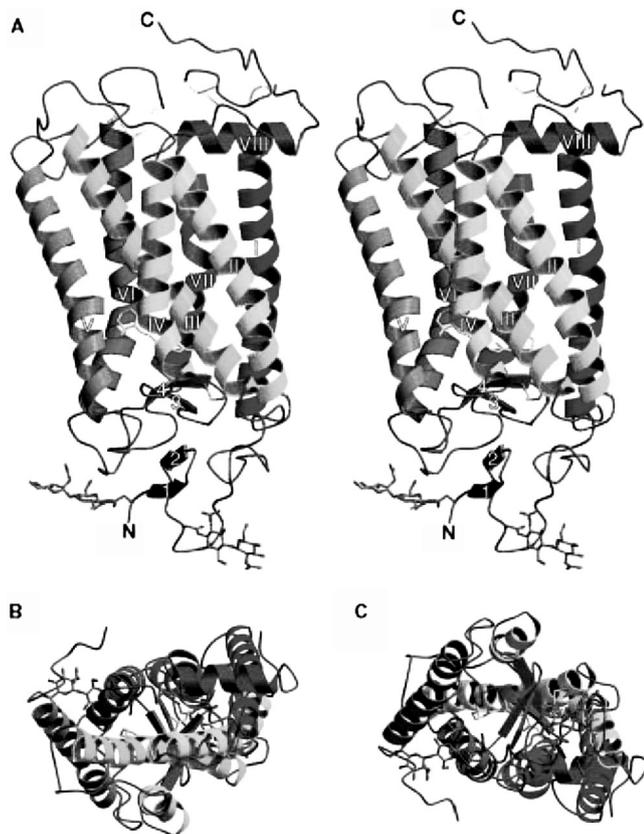


Fig. 1. Three-dimensional structure of a G protein-coupled receptor. The structure of bovine rhodopsin as determined by x-ray crystallography. (A) A stereoview of the receptor as seen parallel to the plane of the membrane. A view into the membrane as seen from the intracellular (B) and extracellular (C) sides. Reprinted with permission from Palczewski *et al.*:¹ Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* 2000; 289:739–45. Copyright 2000 American Association for the Advancement of Science.

unusual GPCR (as it transduces light), this structure provides details about how GPCRs bind to their ligands and how they may activate G proteins. GPCRs have a very recognizable structure (fig. 2), consisting of an extracellular amino acid tail (*N-terminal segment*), an intracellular amino acid tail (*C-terminal segment*), and a common three-dimensional structure consisting of seven *transmembrane domains* (TM I–VII) of sufficient length to span the lipid bilayer. The TM domains are linked by alternating intracellular (i1–i3) and extracellular (e1–e3) loops, resulting in a serpentine arrangement through the membrane. A fourth cytoplasmic loop can be formed when the C-terminal segment is connected to the membrane by attaching a lipid moiety to the amino acid chain (*palmitoylation*). The ligand binding pocket is often envisioned as residing within a funnel-like structure created by the TM domains, but this is not necessarily the case.

The extracellular domain is highly variable among GPCRs. The size of the N-terminal segment is highly variable and can be from as few as 4 to more than 50 amino acid residues in length. It frequently contains

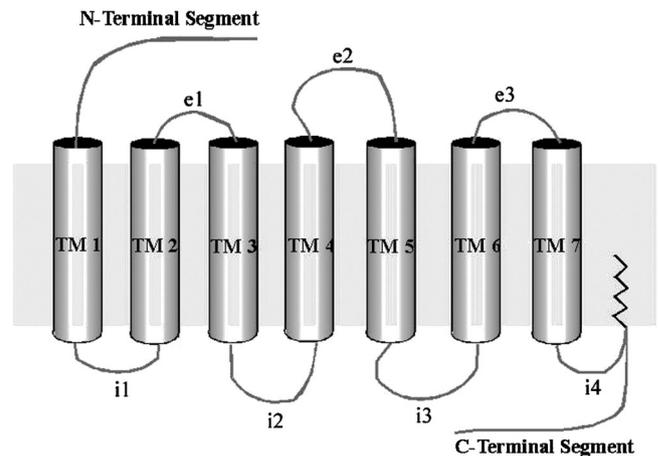


Fig. 2. Schematic of G protein-coupled receptors. Cartoon of a typical G protein-coupled receptor with its extracellular N-terminal segment and an intracellular C-terminal segment. The seven transmembrane (TM) spanning domains (TM 1–7) are linked by alternating intracellular (i1–i3) and extracellular (e1–e3) loops. A fourth cytoplasmic loop (i4) is formed when the C-terminal segment is palmitoylated at cysteine.

sequences where sugar moieties can be attached (*N-linked glycosylation*). Glycosylation modulates various activities of GPCRs. Dependent on receptor type, it may affect surface expression of the receptor, ligand binding potency, long-term down-regulation, and cross-talk to other receptors.² Among the three extracellular loops, e1 has the most consistent loop size, ranging from 3 amino acids up to 18 amino acid residues. The other two extracellular loops (e2 and e3) have more variable loop sizes.³

In contrast, the cytoplasmic side (cytoplasmic loops and C-terminal segment) is similar among GPCRs. Intracellular loop 1 consists of 5–7 amino acids, i2 has a loop size of 10–12 amino acids, whereas i3 and the C-terminus (which determine G-protein specificity^{4,5}; see next section) show the greatest variation in amino acid length among the GPCRs. The most frequent length of this region is approximately 50 amino acid residues.³ The C-terminus contains consensus sequences for phosphorylation and palmitoylation, which are important for functional regulation, such as desensitization and internalization of receptors.⁶ The relative conservation of the intracellular domains suggests a common mechanism by which GPCRs activate G proteins and is probably related to the fact that hundreds of GPCRs activate only dozens of G proteins. The C-terminus is the site of functional desensitization upon agonist stimulation.⁷

The arrangements of the seven TM domains are different at the extracellular and cytoplasmic faces of the receptor, as a consequence of tilting of the domains within the membrane (primarily TM III). This orientation of TM domains seems to be conserved among GPCRs.³

Each of the seven TM domains is generally composed of 20–27 amino acids. TM III contains an amino acid (immediately after a conserved cysteine), which indicates

the ligand type for the receptor: If this amino acid is basic, the ligand is most likely a peptide, whereas if it is acidic, a biogenic amine ligand can be expected. A number of conserved amino acids in the various transmembrane domains probably play an essential role in maintaining the structure of the protein, whereas other amino acids conserved only among major classes of receptors may determine their unique functional properties.⁶

In summary, GPCRs are one of the major protein families in mammals and humans responsible for signal transduction and are targets for a host of drugs used in anesthetic practice. They consist of seven transmembrane domains, linked by extracellular and intracellular loops, as well as an extracellular N-terminus and intracellular C-terminal segment, mainly involved in functional receptor regulation. Extracellular domains, which vary among the different classes of receptors, contribute to ligand recognition and binding, but coupling to G proteins is determined mainly by interactions with intracellular domains.

Structural Basis of Receptor-G Protein Coupling Selectivity

When activated by the appropriate ligands, most GPCRs can usually recognize and activate more than one G protein (this promiscuity is discussed in more detail in the next paragraph) but interact with only a distinct subset of the many structurally similar G proteins that are expressed in a cell.^{5,8} Structural information encoded by the receptor and G-protein amino acid sequences is the primary basis for receptor-G protein recognition. Coupling to a G protein responsible for a particular effect on an intracellular signaling pathway requires a specific structure in the cytoplasmic loops and C-terminal region of the GPCR. For example, inhibition of adenylate cyclase by activation of the G_i protein requires a long third cytoplasmic loop and a short C-terminal region, whereas stimulation of phosphoinositide hydrolysis *via* the G_q protein requires a short third cytoplasmic loop and a long C-terminal region. Stimulation of adenylate cyclase by activation of the G_s protein requires a long carboxy-terminus with many serine and threonine residues. However, other factors (including receptor and G-protein density, and perhaps restricted localization of specific G proteins and receptors in the plasma membrane) may also contribute to coupling selectivity.^{8,9}

On the basis of their G protein-coupling preference, GPCRs can be broadly subclassified into G_s , $G_{i/o}$, and $G_{q/11}$ -coupled receptors (see α Subunits section regarding G-protein classes). Receptors that preferentially couple to G proteins of the $G_{12/13}$ family have not been identified. However, $G_{12/13}$ proteins can be activated by several different GPCRs, such as thrombin, thromboxane

A_2 , or thyrotropin-stimulating hormone receptors, but these are also capable of efficiently activating other classes of G proteins.^{10,11} Most GPCRs, although preferentially linked to a certain subfamily of G proteins, can also couple to other classes of G proteins—usually, however, with reduced efficiency.^{8,12} G-protein coupling preference displayed by an individual GPCR is therefore relative rather than absolute. For example, many primarily G_s -coupled receptors can also stimulate $G_{q/11}$ proteins,⁸ and many receptors that preferentially couple to $G_{i/o}$ proteins are able to interact with G_s , particularly when the receptors are expressed at relatively high levels or high concentrations of activating ligands are used. The most promiscuous GPCR identified to date seems to be the thyrotropin-stimulating hormone receptor, which has been shown to couple to members of all four major classes of G proteins.¹⁰ Conversely, G_{15} and G_{16} show very little receptor selectivity and can be activated by most GPCRs studied to date.¹³ The physiologic relevance of such receptor-coupling promiscuity remains to be determined. However, it does allow the receptor access to a wider array of intracellular signaling systems and therefore provides it with greater ability to regulate cellular processes. In most cases in which sufficient data are available, it seems that receptors are able to interact with most or all members of their preferred G-protein family, but there is evidence that some GPCRs are able to distinguish between different members of the $G_{q/11}$ family.^{14,15}

Work with the β_2 -adrenergic receptor has shown that the selectivity of receptor-G protein coupling may also be regulated by receptor phosphorylation, as protein kinase A phosphorylation reversed the G-protein coupling profile for those receptors: Unphosphorylated receptors activated primarily G_s , whereas the phosphorylated receptor showed a drastic reduction in its ability to couple to G_s and gained the ability to interact with G_i with increased efficacy.^{16,17} Therefore, protein kinase A phosphorylation might represent a switch mechanism for regulating receptor-G protein coupling selectivity.

In addition, receptor palmitoylation may play a role in regulating coupling selectivity. For endothelin receptors, palmitoylation is essential for coupling to G_q and G_i but not for coupling to G_s proteins.^{18,19}

G protein-coupled receptors can be divided into four major classes of receptors on the basis of their G protein-coupling preference. The process of coupling is relative rather than absolute, because the receptors are also able to couple to other classes of G proteins. Protein kinase A phosphorylation and palmitoylation seem to play a role in regulating the selectivity of G-protein coupling. A detailed understanding of physiologic relevance of coupling promiscuity remains to be established.

G Proteins

G proteins comprise a large family of regulatory proteins that are involved in both intracellular and intercellular signal transduction processes. They are molecular switches whose activities are determined by their interaction with guanine nucleotides, hence their name. G proteins cycle between an inactive, guanosine diphosphate (GDP)-bound form and an active, guanosine triphosphate (GTP)-bound form. This cycle is regulated on the one hand by a *nucleotide exchange factor* and on the other by a *guanosine triphosphatase (GTPase) activating protein (GAP)*. G proteins can be divided into two groups: Heterotrimeric (*i.e.*, consisting of three different subunits: α , β , and γ), membrane-bound proteins bind to GPCR. Smaller (20- to 30-kd), monomeric cytoplasmic G proteins are involved in regulation of various intracellular processes.

The structures of two different G-protein heterotrimers, $G\alpha_{i1}\beta_1\gamma_2$ ²⁰ and $G\alpha_i\beta_1\gamma_1$,²¹ have been resolved by x-ray crystallography. The two structures show that the α subunit consists of two structural components between which the bound guanine nucleotide is deeply buried. Activation of G proteins involves the release of GDP and rapid binding of GTP to the α subunit. The binding of GTP induces the dissociation of receptor and G protein and the dissociation of the G protein into α and $\beta\gamma$ subunits. The α_{GTP} or $\beta\gamma$ complex or both then interact with various effectors (*e.g.*, enzymes, ion channels), stimulating or inhibiting their activity. The activity of the α_{GTP} complex is terminated by hydrolysis of GTP by an enzymatic activity inherent in the α subunit itself. In the GDP-bound state, the α subunit reassociates with $\beta\gamma$ subunits and is subject to reactivation.

α Subunits

Historically, heterotrimeric G proteins are named after their α subunits. To date, more than 20 different α subunits (encoded by 16 distinct genes) have been identified. On the basis of amino acid similarity, the G protein α subunits can be grouped into four major families: G_s , $G_{i/o}$, $G_{q/11}$, and $G_{12/13}$.^{22,23} The G_s family includes several splice variants of α_s , as well as α_{olf} , which is primarily expressed in olfactory epithelia but also has been found in high concentrations in several regions of the brain, especially the striatum. The $G_{i/o}$ family consists of three different α_i species (α_{i1} , α_{i2} , and α_{i3}) and α_o (which exists in two splice variants, α_{o1} and α_{o2}), the two retinal transducins (α_{t1} and α_{t2}), an α subunit found in gustatory epithelium (α_{gust}), and α_z . The individual members of the $G_{q/11}$ family are α_q , α_{11} , α_{14} , α_{15} , and α_{16} (α_{15} and α_{16} seem to be the murine and human versions of the

same gene). The $G_{12/13}$ family consists of only two members, α_{12} and α_{13} .

The α subunit consists of a polypeptide with a molecular weight of 40–46 kd. Most G-protein α subunits are tightly associated with the cytosolic face of the plasma membrane. One source of this association is an anchoring effect of the $\beta\gamma$ subunits; α subunits do not associate with phospholipid vesicles unless $\beta\gamma$ subunits are present.²⁴ A second source of this association may be covalent modification of the protein α subunits with fatty acids. The N-terminal region is particularly important both for association between α subunit and $\beta\gamma$ and for membrane association. In contrast, the C-terminal domain of the α subunit seems to play an important role in interactions with receptor and effector structures.²⁵

G proteins are regulatory proteins, consisting of an α subunit carrying a guanine nucleotide, and an associated $\beta\gamma$ complex. The G protein exists in either an inactive, GDP-bound or an active, GTP-bound form. In the presence of the $\beta\gamma$ dimer and because of interactions with fatty acids, α subunits remain tightly bound to the plasma membrane.

β and γ Subunits

The β subunit consists of an N-terminal helical domain and seven similar β sheets, each composed of four anti-parallel β strands that form the blades of a “propeller” structure. The γ subunit binds to one side of the β propeller. There seem to be no direct interactions between the α and γ subunits, whereas extensive contacts occur between α and β .

The β (35 or 36 kd) and γ (8 or 9 kd) subunits remain closely associated with each other and are therefore usually regarded as one functional unit. The different β subunits share a relatively high degree of sequence identity, but the various γ subunits are structurally more diverse. Six different G-protein β subunits and 12 distinct γ subunits have been described, allowing, at least theoretically, formation of 72 different $\beta\gamma$ complexes.²⁶ The potential functional importance of such diversity is unclear, particularly because most $\beta\gamma$ complexes (except $\beta_1\gamma_1$) are thought to exhibit similar functional properties. Most studies of G-protein effector coupling do not support selectivity of $G\beta\gamma$ action. However, recent evidence does indicate that the β isoform in the $\beta\gamma$ dimer can determine the specificity of signaling at both the receptor and at the effector enzyme regulated by the G protein. Selective activation of the phospholipase C β pathway by $G\beta\gamma$ complexes containing β_1 subunits has been demonstrated, whereas β_2 -containing complexes produced no activation.²⁷ A specialized role for the β_5 subunit in cell signaling has also been suggested, because β_1 and β_2 isoforms interact with α subunits from the α_i , α_s , and α_q families, whereas the structurally divergent β_5 subunit only interacts with $G\alpha_q$.²⁸

Several functions have been ascribed to the $\beta\gamma$ dimer.

|| See www.rcsb.org/pdb. Accessed May 18, 2005.

It promotes the association of the α subunit with the receptor. It decreases nucleotide exchange on free G proteins, thereby decreasing inadvertent signal transduction through the G-protein cycle. It anchors the G protein to the cytosolic surface of the membrane. Finally, it plays direct roles in activating or inhibiting signal transduction pathways. Details on the specificity of $\beta\gamma$ subunits for the different signal transduction pathways are rapidly emerging, and it is becoming abundantly evident that $\beta\gamma$ subunits are as important as α subunits in regulating the activity of cellular effector mechanisms (see next section).

Beta and γ subunits function as a single unit. They not only promote association of α subunits with the receptor or specific recognition of a coupling G protein but also regulate activity on downstream cellular effectors after dissociation from the α subunit.

G-protein Function

Activation of heterotrimeric G proteins by GPCRs leads to the formation of free α -GTP and $\beta\gamma$ subunits that are able to interact with a diverse array of effector enzymes, ion channels, or both to stimulate or inhibit the activity of these effector proteins. Within the α subunit resides the binding site for guanine nucleotides, the GTPase catalytic activity that converts GTP to GDP, amino acid residues that are substrates for adenosine diphosphate ribosylation by bacterial toxins (cholera and pertussis toxins), and sites involved in interactions with both receptors and effector mechanisms. Therefore, most attention has focused on the α subunit.

Activated α subunits are known to interact selectively with a specific set of effector molecules. For example, activated α_s and α_i subunits regulate intracellular cyclic AMP concentrations by mediating the activation and inhibition, respectively, of distinct isoforms of adenylyl cyclase. All α subunits of the $G_{q/11}$ family can activate different isoforms of phospholipase $C\beta$ (resulting in the breakdown of phosphatidylinositol 4,5-bisphosphate and the generation of the second messengers, inositol 1,4,5-trisphosphate and diacylglycerol), and α_t mediates the stimulation of cyclic GMP phosphodiesterase.^{22,23} It has become clear during the past years that the $\beta\gamma$ subunits, in a fashion similar to the activated α subunits, can also bind to a great variety of effector molecules to regulate their activity. In many cases, α and $\beta\gamma$ subunits bind to the same effectors to mediate opposing or synergistic effects. However, there are also examples in which distinct effector molecules are exclusively regulated by either α or $\beta\gamma$ subunits.²⁶ Most effector molecules are members of protein families, and the individual members of these families frequently show different patterns of regulation by α and $\beta\gamma$ subunits. For example, all eight isoforms of adenylyl cyclase can be stimulated by α_s , whereas two isoforms, AC-II and AC-IV, can be activated synergistically by both α_s and $\beta\gamma$ subunits.²⁹

On the other hand, the activity of one isoform, AC-I, is inhibited by $\beta\gamma$ complexes. In contrast to the effects of $\beta\gamma$ complexes on the activity of different adenylyl cyclase isoforms, $\beta\gamma$ can activate different phospholipase $C\beta$ species independent of α subunits.^{30,31} However, the different phospholipase $C\beta$ subtypes vary considerably in their sensitivity to stimulation by different subtypes of the $\alpha_{q/11}$ family and by $\beta\gamma$ subunits. The pattern of biochemical responses of a particular cell to stimulation of a given GPCR is quite complex because of the diversity of G-protein subunits and downstream effector molecules. The nature of the observed responses critically depends on which G-protein heterotrimers are recognized by the receptor and which effector molecules are present in specific tissue. Moreover, the magnitude of these responses is also modulated by the relative concentrations of the various components of the different receptor-G protein signaling pathways.

Activation of G proteins results in dissociation of the α and $\beta\gamma$ subunits, both now able to interact with a variety of downstream effector mechanisms. Because of possible binding to the same effector, leading to either synergistic, additive or even opposing effects, cellular responses to GPCR stimulation can be quite complex.

Signal Transduction

Signal transduction begins with binding of a ligand to the receptor. Once an agonist is bound, the receptor can be activated. Receptor activation may be divided into at least three steps: signal generation, TM signal transduction, and signal transfer to cytoplasmic signal molecules.

Ligand Binding

Ligand binding can take place at a variety of locations on the receptor: to the TM core exclusively (photons, biogenic amines, eicosanoids, and lipids such as lysophosphatidic acid and sphingosine-1 phosphate), to both the core and extracellular loops (peptides ≤ 40 amino acids), to extracellular loops and N-terminal segment (polypeptides ≤ 90 amino acids), or exclusively to the N-terminal segment (glycoproteins ≥ 30 kd). Interactions of ligands and receptors seem to involve hydrogen bonds, ion pairs, and hydrophobic contacts.

In biogenic amine receptors, TM III is believed to be the primary site for ligand binding. Agonists for neuropeptide receptors bind to both TM and extracellular loop regions, whereas those for glycoprotein or metabotropic receptors bind almost exclusively to the extracellular N-terminal domain of the receptor. Lipophilic drugs can approach the receptor *via* the lipid membrane and interact with amino acid regions at the lipid-protein interface; some can even interact with cytoplasmic regions. Small ligands bind in a pocket located in the extracellular half of the TM formed by the helical bundle.

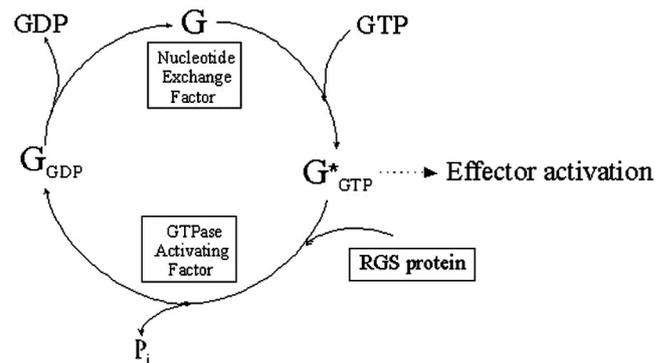
Ligands can bind GPCRs in a variety of locations. The binding site may be located extracellularly, intracellularly, or both, depending on the type of ligand activating the receptor.

Receptor Domains Involved in G-protein Coupling

Data mainly derived from studies with muscarinic and adrenergic receptors strongly suggest that the selectivity of G-protein recognition is determined by multiple intracellular receptor regions. The most critical regions are the i2 loop and the N- and C-terminal areas of the i3 loop (referred to as the *Ni3* and *Ci3* segments, respectively).⁵ These regions are thought to act in a cooperative fashion.³² It has been shown that short synthetic peptides corresponding to the i2, Ni3, and Ci3 regions can mimic or inhibit receptor interactions with G proteins.^{33,34} Different regions of the cytosolic loops of GPCRs activate different G proteins.^{35,36} The relative contributions of different intracellular receptor domains to the selectivity of G-protein recognition varies among different classes of GPCRs and even among structurally closely related members of the same receptor subfamily. A large body of evidence indicates that the C-terminal portions of $G\alpha$ can directly contact the receptor protein³⁷ and that the C-terminal five amino acids of $G\alpha$ subunits play a key role in dictating the specificity of receptor-G protein coupling.³⁸ Receptor coupling selectivity of G-protein α subunits can be altered by single amino acid substitution in this region.³⁸ Although precise positions of the C-terminal $G\alpha$ residues that are intimately involved in determining the selectivity of receptor-G protein interactions vary between different functional classes of $G\alpha$ subunits, in both $\alpha_{i/o}$ and $\alpha_{q/11}$ subunits, the -3 residue is of fundamental importance for proper receptor recognition. The residues at this position are perfectly conserved in individual $G\alpha$ subfamilies and can correctly predict the coupling profile of a given $G\alpha$ subunit. However the C-terminal segment of $G\alpha$ is not the only structural determinant of receptor-G protein coupling selectivity. The N-terminal portion of $G\alpha$, which lies adjacent to its C-terminus, has been shown to contribute also to receptor binding and to the selectivity of receptor-G protein interactions. For example, when the N-terminal six-amino acid extension characteristic for α_q and α_{11} subunits was removed, the resulting mutant α_q subunit gained the ability to be activated by various different G_s - and $G_{i/o}$ -coupled receptors that normally do not couple efficiently to wild-type α_q .

G-protein $\beta\gamma$ complexes can also modulate the selectivity of receptor-G protein interactions.^{8,26} G-protein $\beta\gamma$ subunits increase the affinity of GPCRs for the G-protein α subunits, and residues on the β subunit as well as on the γ subunit may contribute to receptor binding. The different β subunits share a high degree of amino acid homology, whereas the various γ subunits are structurally quite diverse, as mentioned before. The structural

G protein activation



G protein inactivation

Fig. 3. Cassel-Selinger G-protein activation cycle. Agonist-bound receptors activate guanosine diphosphate (GDP)-bound (inactive) G proteins (G) by facilitating GDP-guanosine triphosphate (GTP) exchange. Switching between the two states is controlled by nucleotide exchange and guanosine triphosphatase (GTPase) activating factors. In the resting state, G proteins are GDP bound and inactive (G_{GDP}). GDP release (slow, reversible) is followed by GTP binding and G-protein activation (G^*_{GTP}). The active complex is unstable: The G protein is a GTPase and deactivates spontaneously after irreversible GTP hydrolysis.

variability observed among γ subunits may be relevant for regulating receptor-G protein coupling selectivity.

A detailed overview of the relative contribution of different structural parts of GPCRs and G proteins for coupling selectivity is provided by Wess.³⁹

Different sites at the receptor and the G protein are involved in maintaining the specificity of G-protein coupling. In particular, intracellular loops of the receptor, the C- and N-termini of the α subunit, and the γ subunit of the $\beta\gamma$ dimer seem to play a key role in coupling selectivity.

Proposed Models for G-protein Activation

Two-state Model. The classic two-state model for the basic G-protein activation cycle (fig. 3) suggested that the resting G protein exists in its trimeric form, with GDP occupying the nucleotide binding site on the α subunit. Because it is unlikely that the nucleotide can be contacted by the intracellular loops of the receptor protein (which are rather short in many cases), GPCRs are thought to trigger GDP release by an allosteric mechanism (*i.e.*, the intracellular loops induce a conformational change in the α subunit, which in turn results in GDP release). Increasing understanding of those allosteric interactions within or between GPCRs have made the basic models of G-protein activation and signaling transduction far more complex, as described later in this section.

The association of G proteins and receptors has consequences for the receptor's binding properties. In general, the interaction between agonist binding and G-protein coupling is positively cooperative, *i.e.*, agonist binding results in a change of receptor conformation

that displays a higher affinity toward the G protein, thus favoring coupling. However, the binding of GTP to its site on the G protein results in a change of G-protein structure that is transmitted to the receptor's conformation as a negatively cooperative effect on agonist binding. This promotes the uncoupling of the activated G protein from the receptor, allowing signaling to proceed. These negatively cooperative effects of GTP on agonist binding underlie the so-called GTP shift observed in GPCR binding assays: When GTP is added to a binding assay, the resulting uncoupling of receptors and G proteins is noted as a decrease in agonist affinity.

There are two critical points in the G-protein cycle: the release of GDP from the α subunit and the hydrolysis of GTP. The lifetime of the α_{GTP} complex is several seconds, but the basal rate of GDP dissociation from the α subunit is even slower. Therefore, during resting conditions, most of the G protein is in the inactive, GDP-bound state.

Hydrolysis of GTP terminates $G\alpha$ regulation of the effector. This turnover is normally quite low. Hence, in the small signaling G proteins such as RAS, GTP hydrolysis requires the action of a separate GAP. It has been demonstrated that an effector such as phospholipase C can stimulate G-protein GTPase activity. Therefore, effectors can mediate the fast physiologic deactivation of G protein-mediated signaling and thereby terminate their own action. Another possibility is that the α subunit incorporates a GAP-like domain.

The two-state model is a simplistic description of signaling transduction via GPCRs: agonist binding \rightarrow G-protein activation \rightarrow GDP-GTP exchange \rightarrow dissociation of α and $\beta\gamma$ subunits \rightarrow interactions with various effectors \rightarrow termination by GTPase-hydrolysis \rightarrow reassociation of α and $\beta\gamma$ subunits. G-protein activation seems to be far more complex than suggested by this model, because of various allosteric interactions within the GPCRs.

Ternary Complex Model. G protein-coupled receptors can be constitutively active, *i.e.*, spontaneous coupling of receptors in active conformations to G proteins in the absence of ligands can occur.³⁵ This observation made clear that the classic model of receptor-G protein interaction was not sufficiently comprehensive. This resulted in development of the ternary complex model shown in figure 4. In this model, agonist-bound receptors activate GDP-bound (inactive) G proteins by facilitating GDP-GTP exchange. This reaction is catalytic (*i.e.*, each stimulated receptor activates several G proteins in turn). In the absence of GTP, agonists discriminate two GPCR binding states: the high- and low-affinity binding sites. The ability of the ligand to activate G proteins is related to its ability to discriminate between the two receptor states.^{40,41} Agonist potency, which is measured

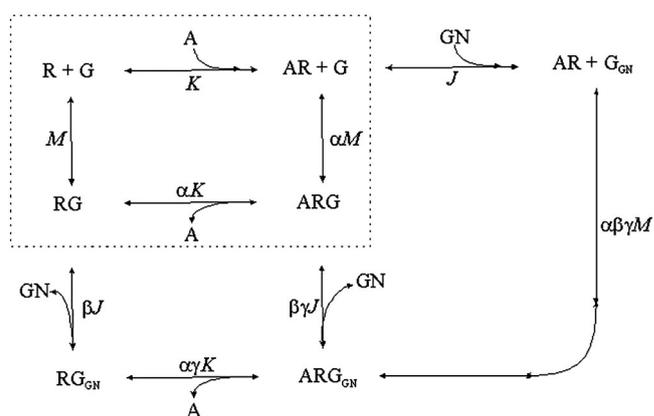


Fig. 4. The ternary complex model. The ternary complex model is shown in the *box*. R represents the receptor, G represents the G protein, A represents the corresponding agonist, and GN represents the guanyl nucleotide (GDP or GTP). The constants K , J , and M are affinity constants, and α , β , and γ represent allosteric factors. Free, uncoupled receptors have low affinities for the agonist and G protein (represented as K and M). Agonists increase the average receptor's affinity for the G protein (from M to αM) and *vice versa*. The allosteric factor α is therefore greater than 1. The agonist equilibrium dissociation constants from agonist-receptor-G protein complex (ARG) are $K_H = 1/\alpha K$ and $K_L = 1/K$. Guanyl nucleotides inhibit the binding of agonists; $\alpha\gamma K$ is lower than αK , so γ must be lower than 1. The affinity of GN for ARG ($\beta\gamma J$) is therefore lower than the affinity of GN for RG (βJ): Agonists inhibit the recognition of nucleotides by receptor-coupled G proteins.

by the method of partial receptor activation (EC_{50}), can correlate with the dissociation constants for either the high- or the low-affinity binding site, depending on the system studied.⁴² The ternary complex model remains the most parsimonious and most commonly used model for both prediction and quantification of allosteric interactions at GPCRs. It is adequate for analysis of binding studies; in the absence of GTP, it predicts the existence of two agonist-binding states: high affinity, corresponding to agonist-receptor-G protein, and low affinity, corresponding to agonist-bound receptors. The same model is used increasingly to define functional properties, including agonist efficacy and potency, which is not appropriate.

Further refinements of these models have led to exponentially more complex systems, including the extended ternary complex model and the cubic ternary model. Even these are not fully able to describe the interactions that occur when G-protein signaling is affected by allosteric interactions or modulators.

Development of models to describe the pharmacologic behavior of GPCRs has led to three essential characteristics for GPCRs: (1) GPCRs can exist in various conformations (active and inactive states); (2) GPCRs can spontaneously interact with G proteins in the absence of agonist ligands (constitutive activity); and (3) multiple active receptor states exist, each capable of inducing physiologic responses (ligand- and G protein-specific receptor conformations).

Allosteric Interactions and Modulators

An *orthosteric site* is the agonist binding site on a receptor, which comprises amino acids that form contacts with the endogenous agonist for that receptor; an *allosteric site* is an additional binding site that is distinct from the agonist binding site (and therefore not containing amino acids that make direct contact with the agonist) but that can modulate receptor activity. An interaction between two topographically distinct binding sites on the same receptor complex is termed an *allosteric interaction*. Such interactions can be positive (enhancing signaling) or negative (inhibiting signaling). Both negative allosteric modulators and competitive interactions at the orthosteric site shift the concentration-response curve for agonist to the right. Classic teaching⁴³ that right shift indicates competitive antagonism (*i.e.*, agonist and antagonist compete for the same binding site on the receptor) and that decrease in maximal effect indicates noncompetitive antagonism is therefore not necessarily correct. However, whereas the right shift is limited in the case of an allosteric modulator, it is theoretically unlimited in the case of a competitive antagonist.⁴⁴ That is, an allosteric modulator can only have a limited effect on potency, whereas a competitive inhibitor can completely reverse the action of the agonist.

Until recently, agonists, partial agonists, and antagonists were the primary types of receptor drugs considered, but chemicals interacting with allosteric sites might open a novel field of drug development. Allosteric enhancers can potentiate the effects of agonists either by enhancing agonist affinity, stabilization of agonist-receptor and receptor-G protein interaction, or by some other unspecified increase of efficacy. On the other hand, allosteric modulators could inhibit agonist stimulation of the receptor without interference with agonist binding to the receptor. Allosteric agonists might activate receptors without being a target for classic antagonists. Many anesthetics act as allosteric enhancers at the γ -aminobutyric acid type A (GABA_A) receptor.

Allosteric modulators of GPCRs may be categorized as *allosteric enhancers*, which exert their effects by increasing the affinity of the orthosteric ligand for its site on the receptor (G proteins themselves act as allosteric enhancers; see Proposed Models for G-protein Activation); *allosteric agonists*, which act by promoting G-protein coupling independent of any effects on orthosteric agonist binding; and *allosteric antagonists*, which produce their action by decreasing the affinity of the receptor for its orthosteric agonist, reducing the affinity of the receptor for its G protein(s), or both.

In addition to the allosteric effects of G proteins on orthosteric ligand binding, numerous compounds can affect GPCR binding and coupling properties by interacting with intracellular receptor regions. For example, suramin acts as an orthosteric antagonist on purinergic

receptors but has also been shown to uncouple opioid receptors, adrenoceptors, and adenosine and dopamine receptors from their G proteins. These effects are associated with an inhibition of agonist binding, whereas antagonist binding is unaffected or even increased.^{45,46} Interestingly, in the absence of G proteins or in preparations using stable GTP analogs (which uncouple G proteins), suramin and its analogs lack the effect on orthosteric ligand binding.⁴⁷ It is not clear whether those effects result primarily from binding to the receptor, the G protein, or equally to both, but it is known that suramin can bind to a site on the G-protein α subunit in the absence of any receptor coupling and modifies nucleotide binding properties,⁴⁶ supporting the hypothesis that allosteric effects by this compound are mediated primarily through its action on the G protein.

Allosteric modulators are able to alter GPCR signaling by interactions with specific binding sites other than the actual ligand binding site. Effects might be enhancing/agonistic or antagonistic. In addition, various compounds seem to affect GPCR binding and coupling properties by direct interactions with the receptor molecule. The possibility that anesthetics might exert some of their effects on GPCR signaling by this manner deserves further investigation.

Modulation of GPCR Function by Other Molecules

Regulators of G-protein Signaling (RGS)

In 1996, a large family of highly diverse, multifunctional signaling proteins that modulate signaling by G proteins was identified. These proteins, which bind directly to activated G α subunits and act like a GAP to rapidly deactivate G α , share a conserved domain and are termed *regulators of G-protein signaling* (RGSs). At least 30 members of the RGS protein family have been identified by now,^{48,49} targeting G proteins of the G_i, G_q, G_{12/13}, and G_s families. RGS proteins are quite promiscuous in that they can interact with several different G-protein α subunits.

In contrast to initial understanding, when RGSs were seen exclusively as negative regulators of G-protein signaling, more recent evidence suggests that these proteins may subtly act as regulated modulators and integrators of G-protein signaling. Next to accelerating (up to 1,000-fold) G α _{GTP} hydrolysis and thereby limiting the duration of action of G α _{GTP}, RGS proteins can also act independent of GAP activity as effector antagonists by binding to active G α and thereby preventing it from binding to its effector. Competitive inhibition of G α binding to effectors such as phospholipase C, for example, has been demonstrated.⁵⁰

The entire group of RGS proteins can be subdivided into simple RGSs, serving predominantly as negative regulators or modulators of G-protein signaling, and

larger, complex RGSs, which act either as integrators of G-protein signaling, novel G-protein effectors, or scaffolding proteins (*i.e.*, proteins that allow other proteins to attain the correct spatial orientation for interaction). Complex RGS proteins have been suggested to link active $G\alpha$ subunits to other signaling pathways and serve as multifunctional integrators of G protein signaling. They can link $G\alpha$ directly to nontraditional signaling cascades, *e.g.*, monomeric GTPases.

The RGS family contains a special group of proteins termed *G-protein receptor kinases*, which primarily phosphorylate GPCRs. These proteins limit $\beta\gamma$ signaling by binding $\beta\gamma$ subunits and are often used as tool to identify $G\alpha$ - versus $G\beta\gamma$ -mediated signaling in cells. In addition, G-protein receptor kinase 2 has been shown to decrease $G\alpha_q$ signaling, most likely by sequestering $G\alpha_q$ or interference with effector activation independent of an increased GTP hydrolysis.⁵¹ Interaction of local anesthetics with G-protein receptor kinase 2 might explain in part the observed inhibition of G_q protein function by those drugs.⁵²

RGS-PX1, a recently discovered and unique $G\alpha_s$ -specific GAP, has been demonstrated to regulate trafficking between intracellular compartments. In addition, it may also facilitate transport of internalized receptors.⁵³ RGS2, a selective GAP for $G\alpha_q$, has been shown to directly interact with certain subtypes of adenylyl cyclase, leading to inhibition of cyclic AMP production. Therefore, it must be assumed that RGS proteins have more signaling functions than suggested by their GAP activity. They modulate traditional GPCR signals, play important roles as scaffolds for receptors and components of the G-protein signaling cascade, integrate divergent signals, and facilitate ion channel regulation, intracellular transport, and cell morphology through G protein- and non-G protein-mediated signals.

Proteins as important for signal transduction as RGS have to be regulated by sophisticated cellular mechanisms. Mechanisms that have been demonstrated include (1) phosphorylation by protein kinase C or transmembrane tyrosine kinase, which can either increase or decrease GAP activity or affect RGS interactions with other proteins that compete with $G\alpha$ subunits for binding; (2) palmitoylation with varying effects on RGS protein function; (3) modulation of protein stability by, for example, direct phosphorylation or regulation of interaction with binding partners; and (4) regulation of RGS messenger RNA expression and protein translation.

RGS proteins might be involved in drug tolerance or dependence. Chronic administration of amphetamines was shown to induce up-regulation of RGS2 and RGS3.⁵⁴ Increased protein concentrations of RGS4 have been shown after stimulation of μ - and δ -opioid receptors.⁵⁵ Elimination of RGS activity dramatically increased opiate responses. These findings open potential routes for drug development. Because receptor desensitization, relevant

for development of opioid tolerance, occurs at high receptor occupancy, inhibition of RGS protein function might allow reduced agonist doses, which occupy only a small fraction of receptors, to produce analgesia, thus preventing tolerance and possibly dependence.

Increasing evidence suggest that RGS3 might also play a role in cell cycle and cell migration. Certain RGS proteins are thought to be involved in cell proliferation and apoptosis.^{56,57} Of particular interest is the modulation of cardiac development and output by RGS proteins.⁵⁸ Certain RGS proteins have been shown to be up-regulated in failed human hearts. Increased concentrations of RGS2, RGS3, and RGS4, effective GAPs for $G\alpha_q$ signaling (which is involved in heart failure⁵⁹), might indicate a negative feedback mechanism to prevent cardiac hypertrophy by preventing overactive signaling of those G-protein α subunits. In mice, overexpression of RGS4 led to reduced G_q -mediated cardiac hypertrophy in response to pressure overload, supporting involvement of this protein in cardiac growth.⁶⁰

RGS proteins are also thought to be sophisticated regulators of immune responses. Specific immune responses (*e.g.*, inhibition of chemokine-induced B-cell migration, B-cell maturation; RGS2 acting as immediate early gene involved in T cell activation) might require modulation by RGS proteins. Conversely, both simple and complex RGS proteins are regulated by immune activation.⁶¹⁻⁶³

RGS2 has been shown to contribute to development of synaptic connections in the hippocampus. Knockout animals deficient for RGS2 demonstrated an increased level of anxiety. Most antipsychotics exert their effect by reducing excessive stimulation of $G\alpha_{i/o}$ via dopamine D2 receptors. RGS4 would be able to limit $G\alpha_{i/o}$ signaling, and low concentrations of RGS4 were found to be a risk factor for development of schizophrenia.⁶⁴ RGS9-2, which specifically inhibits dopamine D2 receptor signaling, is thought to be involved in Parkinson disease.⁶⁵ RGS9-1, a potent GAP for transducin ($G\alpha_t$), is considered essential for proper vision.^{66,67}

RGSs are a large family of diverse, multifunctional signaling proteins. RGSs are widely known for terminating active $G\alpha$ subunits by accelerating $G\alpha_{GTP}$ hydrolysis or direct binding, but they are also involved in a variety of signaling processes, such as regulation of the cell cycle and immune responses.

GPCR Complexing

G protein-coupled receptors are able to form complexes among themselves, which leads to formation of dimers or even oligomers. This is similar to the situation which has been known to exist for receptors from other superfamilies (*e.g.*, growth factor receptors) or ion-channel linked receptors. In radioligand binding assays using β_2 adrenoceptors or m2 muscarinic receptors, orthosteric binding properties have been demonstrated that

suggest multiple affinity states (biphasic competition curves in the presence of G-protein coupling). Uncoupling of the receptor-G protein complex using a stable GTP analog in this experiment led to a bell-shaped binding curve for the agonist-antagonist interaction.⁶⁸ This observation cannot be explained with the ternary complex model but would fit with the assumption of receptor dimerization.

Subsequently, β_2 -adrenoceptor dimerization has been demonstrated *in vivo* in intact cells.^{69,70} In addition, it has been suggested that δ -opioid receptor dimers modulate the internalization process, whereas bradykinin B2 receptor dimers are required for agonist-mediated receptor activation and desensitization.⁷¹ GPCR dimerization is not restricted to *homodimers*. GABA_BR1 and GABA_BR2 monomeric receptor subtypes have been shown to function appropriately only when coexpressed in the same cell.⁷²

Recently, κ - and δ -opioid receptor heterodimers have been identified. In contrast to δ -opioid receptor homodimers, the κ - and δ -opioid receptor heterodimers showed only weak tendency to monomerize in the presence of agonist, suggesting a role of *heterodimerization* in the modulation of receptor function.⁷³ Strikingly, heterodimerization can lead to enhanced ligand affinity when measured in the presence of another ligand, which suggests a form of positive cooperativity for agonist binding to the heterodimer. An altered pharmacologic responsiveness of an angiotensin AT1/bradykinin B2 receptor heterodimer has been associated with the hypertension occurring in preeclampsia.⁷⁴

Similar to other receptor families, GPCRs are able to form complexes with proteins other than G proteins. Homodimers or heterodimers might produce allosteric interactions, thereby changing responsiveness to a stimulus.

Accessory Proteins

Recent investigations discovered a family of single transmembrane accessory proteins able to change the phenotype of a receptor. These proteins, necessary for transport and ligand specificity, exist in three different subtypes and are called *receptor activity modifying proteins*. Receptor activity modifying proteins can associate with receptors and change their pharmacologic behavior to both agonists and antagonists. Receptor activity modifying protein 3, for example, has been shown to induce a change in calcitonin receptor coupling to G protein, because cotransfection of receptor activity modifying protein 3 with human calcitonin receptors led to inhibition of human calcitonin potency.⁷⁵

Clinical Implications

Increased understanding of GPCR signaling has made it clear that the role of these systems in clinical disease

states, and anesthetic interactions with these systems, are much more complex than previously envisioned. Our level of detail is still very inadequate.⁷⁶⁻⁷⁸

Anesthetic Actions on Airway Smooth Muscle

Airway smooth muscle cells express endothelin as well as m2 (and to a lesser extent m3) muscarinic receptors. Whereas m3 and endothelin receptors couple to G_q, resulting in increased activity of phospholipase C and increased concentrations of diacylglycerol and inositol trisphosphate, activation of m2 receptors inhibits adenylyl cyclase through members of the G_i family. Besides heterotrimeric G proteins, small G proteins of the Rho family play an important role in mediating calcium sensitivity in airway smooth muscle, as treatment with C3 exoenzyme (a Rho family inhibitor) reduced responses to stimulation with ET1, acetylcholine, and even GTP γ S.⁷⁹ Therefore, large and small G proteins are involved in airway smooth muscle contraction. In addition, cytokines are known to regulate airway smooth muscle tone. Tumor necrosis factor, for example, increases G_q and G_i protein concentrations (but is without any effect on expression of G_s protein).⁸⁰ Airway smooth muscle tone is in addition determined by reorganization of its cytoskeleton, a filamentous network consisting of strands of F-actin. Muscarinic receptors mediate carbachol-induced stress fiber formation solely *via* G_{i2},⁸¹ whereas lysophosphatidic acid and endothelin-1 receptors instead were shown to induce actin reorganization *via* G_i or G_q, as well as Rho proteins.⁸²

Volatile anesthetics were shown to directly relax airway smooth muscle by decreasing intracellular calcium concentrations and calcium sensitivity.^{83,84} Halothane reduces calcium sensitivity, most likely by interfering with G-protein function. This may represent an inhibition of G-protein dissociation,⁸³ but it has also been shown that volatile anesthetics inhibit GTP γ S binding to G α subunits, thereby preventing the exchange of GTP γ S for bound GDP.⁸⁵ This effect was observed for several subunits studied (including G_i and G_s) but not all (G_o and monomeric G proteins), suggesting potential anesthetic effects directly on the catalytic site that may be specific to the G α -subunit isotype. Further studies demonstrated that the anesthetic does not affect the binding of [³⁵S]GTP γ S to G α_i subunits. Therefore, the site of action seems to be the receptor itself or the interface between the receptor and the G protein.⁸⁶ A study in bovine retinal membranes demonstrated halothane binding to the photoreceptor but not to any G-protein subunit.⁸⁷

Interestingly, halothane was shown to exert dual effects on muscarinic receptors in airway smooth muscle: Calcium sensitivity was decreased in the presence of receptor stimulation by interference with G-protein function as described before but, surprisingly, was increased by the anesthetic in the absence of agonist. One possible explanation might be the fact that anesthetics

activate monomeric G proteins but inhibit receptor activation of heterotrimeric G proteins. In addition, several heterotrimeric G-protein families are known to mediate calcium sensitivity. Hence, volatile anesthetics might inhibit some and activate others.⁸⁸

Other Anesthetic-GPCR Interactions

Anthony *et al.* demonstrated that volatile anesthetics enhance [³H]methylscopolomine binding to brainstem muscarinic receptors⁸⁹ by presumed stabilization of receptor-G protein complexes and by disrupting G protein GTPase activity.^{90,91} Halothane, isoflurane, enflurane, and sevoflurane all inhibit GTP-GDP exchange and enhance dissociation of the nonhydrolyzable GTP analog GTP γ S.⁹² This effect was observed on G proteins of the G_i and G_s classes but not on G_o. Similar findings were obtained when the effects of halothane and isoflurane on the G protein-regulated muscarinic K channel were investigated.⁹² Myocardial preconditioning by volatile anesthetics is considered to be in part mediated by activation of G_i proteins, because pertussis toxin blocks the beneficial actions of isoflurane in this setting.⁹³ The modulation of cardiac Na channel function by volatile anesthetics seems to be particularly complex. Both halothane and isoflurane change Na channel behavior by interference with G proteins but apparently target G proteins of different classes.^{94,95} Sevoflurane (2 mM) inhibited cyclic AMP generation in rat myocardial membranes,⁹⁶ and it was concluded that the anesthetic reduces ligand-receptor binding and disrupts the "relationship between the receptor and G_s."

Local anesthetics also interact with signaling of GPCRs. Tetracaine was shown to activate GTP hydrolysis in HL60 cell membranes, whereas more hydrophilic local anesthetics were less stimulatory (lidocaine, bupivacaine) or even inhibitory (procaine).⁹⁷ These data were interpreted as an activation of the G_{i/o} protein GTPase. The effects of lidocaine on G protein-mediated modulation of K and Ca channels have been reported to take place at a site of action "between agonist binding and G protein activation." We have shown local anesthetics to interact with a variety of GPCR systems^{98,99} and have identified G α_q as a common target.^{52,100}

A variety of reports document interactions between anesthetics and GPCR signaling systems. Many of these reports are unfortunately somewhat conflicting. This apparent confusion may result in part from the fact that in essentially all the studies mentioned, attempts have been made to fit the observed data to the classic model of receptor-G protein interaction. In many ways, this model is inadequate, and the interactions occurring in the proximal signaling pathway of GPCRs are more complex than usually considered.

Summary

Recent experimental results have profoundly modified understanding of G protein-mediated transmembrane signaling. The concept of signal transduction pathways functioning in a linear fashion, *i.e.*, one receptor coupling to one G protein that activates one effector, is inadequate to explain recent findings. G protein-mediated signal transduction is a complex, highly organized signaling network with diverging and converging transduction steps at the ligand-receptor, receptor-G protein, and G protein-effector interfaces. Unraveling the complexities of these cascades will help in the design of experiments that eventually may reveal new mechanisms of disease states, targets for drugs, and sites and mechanisms of action of anesthetics on these important signaling pathways.

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References

1. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le TI, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M: Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* 2000; 289:739-45
2. Kelley LP, Kinsella BT: The role of N-linked glycosylation in determining the surface expression, G protein interaction and effector coupling of the alpha (alpha) isoform of the human thromboxane A(2) receptor. *Biochim Biophys Acta* 2003; 1621:192-203
3. Mirzadegan T, Benko G, Filipek S, Palczewski K: Sequence analyses of G-protein-coupled receptors: Similarities to rhodopsin. *Biochemistry* 2003; 42:2759-67
4. Hedin KE, Duerson K, Clapham DE: Specificity of receptor-G protein interactions: Searching for the structure behind the signal. *Cell Signal* 1993; 5:505-18
5. Wess J: G-protein-coupled receptors: Molecular mechanisms involved in receptor activation and selectivity of G-protein recognition. *FASEB J* 1997; 11:346-54
6. Coughlin SR: Expanding horizons for receptors coupled to G proteins: Diversity and disease. *Curr Opin Cell Biol* 1994; 6:191-7
7. Cideciyan AV, Zhao X, Nielsen L, Khani SC, Jacobson SG, Palczewski K: Null mutation in the rhodopsin kinase gene slows recovery kinetics of rod and cone phototransduction in man. *Proc Natl Acad Sci U S A* 1998; 95:328-33
8. Gudermann T, Kalkbrenner F, Schultz G: Diversity and selectivity of receptor-G protein interaction. *Annu Rev Pharmacol Toxicol* 1996; 36:429-59
9. Raymond JR: Multiple mechanisms of receptor-G protein signaling specificity. *Am J Physiol* 1995; 269:F141-58
10. Laugwitz KL, Allgeier A, Offermanns S, Spicher K, van Sande J, Dumont JE, Schultz G: The human thyrotropin receptor: A heptahelical receptor capable of stimulating members of all four G protein families. *Proc Natl Acad Sci U S A* 1996; 93:116-20
11. Offermanns S, Laugwitz KL, Spicher K, Schultz G: G proteins of the G12 family are activated via thromboxane A2 and thrombin receptors in human platelets. *Proc Natl Acad Sci U S A* 1994; 91:504-8
12. Gudermann T, Schoneberg T, Schultz G: Functional and structural complexity of signal transduction via G-protein-coupled receptors. *Annu Rev Neurosci* 1997; 20:399-427
13. Offermanns S, Simon MI: G alpha 15 and G alpha 16 couple a wide variety of receptors to phospholipase C. *J Biol Chem* 1995; 270:15175-80
14. Amatruda III, Gerard NP, Gerard C, Simon MI: Specific interactions of chemoattractant factor receptors with G-proteins. *J Biol Chem* 1993; 268:10139-44
15. Wu D, LaRosa GJ, Simon MI: G protein-coupled signal transduction pathways for interleukin-8. *Science* 1993; 261:101-3
16. Daaka Y, Luttrell LM, Lefkowitz RJ: Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature* 1997; 390:88-91
17. Okamoto T, Murayama Y, Hayashi Y, Inagaki M, Ogata E, Nishimoto I: Identification of a Gs activator region of the beta 2-adrenergic receptor that is autoregulated via protein kinase A-dependent phosphorylation. *Cell* 1991; 67:723-30

18. Horstmeyer A, Cramer H, Sauer T, Muller-Esterl W, Schroeder C: Palmitoylation of endothelin receptor A: Differential modulation of signal transduction activity by post-translational modification. *J Biol Chem* 1996; 271:20811-9
19. Okamoto Y, Ninomiya H, Tanioka M, Sakamoto A, Miwa S, Masaki T: Palmitoylation of human endothelin B: Its critical role in G protein coupling and a differential requirement for the cytoplasmic tail by G protein subtypes. *J Biol Chem* 1997; 272:21589-96
20. Wall MA, Coleman DE, Lee E, Iniguez-Lluhi JA, Posner BA, Gilman AG, Sprang SR: The structure of the G protein heterotrimer Gi alpha 1 beta 1 gamma 2. *Cell* 1995; 83:1047-58
21. Lambright DG, Sondak J, Bohm A, Skiba NP, Hamm HE, Sigler PB: The 2.0 A crystal structure of a heterotrimeric G protein. *Nature* 1996; 379:311-9
22. Conklin BR, Bourne HR: Structural elements of G alpha subunits that interact with G beta gamma, receptors, and effectors. *Cell* 1993; 73:631-41
23. Neer EJ: Heterotrimeric G proteins: Organizers of transmembrane signals. *Cell* 1995; 80:249-57
24. Sternweis PC: The purified alpha subunits of Go and Gi from bovine brain require beta gamma for association with phospholipid vesicles. *J Biol Chem* 1986; 261:631-7
25. Masters SB, Sullivan KA, Miller RT, Beiderman B, Lopez NG, Ramachandran J, Bourne HR: Carboxyl terminal domain of Gs alpha specifies coupling of receptors to stimulation of adenylyl cyclase. *Science* 1988; 241:448-51
26. Clapham DE, Neer EJ: G protein beta gamma subunits. *Annu Rev Pharmacol Toxicol* 1997; 37:167-203
27. Diverse-Pierluissi M, McIntire WE, Myung CS, Lindorfer MA, Garrison JC, Goy MF, Dunlap K: Selective coupling of G protein beta gamma complexes to inhibition of Ca²⁺ channels. *J Biol Chem* 2000; 275:28380-5
28. Fletcher JE, Lindorfer MA, DeFilippo JM, Yasuda H, Guilford M, Garrison JC: The G protein beta5 subunit interacts selectively with the Gq alpha subunit. *J Biol Chem* 1998; 273:636-44
29. Sunahara RK, Dessauer CW, Gilman AG: Complexity and diversity of mammalian adenylyl cyclases. *Annu Rev Pharmacol Toxicol* 1996; 36:461-80
30. Exton JH: Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. *Annu Rev Pharmacol Toxicol* 1996; 36:481-509
31. Rhee SG, Bae YS: Regulation of phosphoinositide-specific phospholipase C isozymes. *J Biol Chem* 1997; 272:15045-8
32. Blin N, Yun J, Wess J: Mapping of single amino acid residues required for selective activation of Gq/11 by the m3 muscarinic acetylcholine receptor. *J Biol Chem* 1995; 270:17741-8
33. Munch G, Dees C, Hekman M, Palm D: Multisite contacts involved in coupling of the beta-adrenergic receptor with the stimulatory guanine-nucleotide-binding regulatory protein: Structural and functional studies by beta-receptor-site-specific synthetic peptides. *Eur J Biochem* 1991; 198:357-64
34. Okamoto T, Nishimoto I: Detection of G protein-activator regions in M4 subtype muscarinic, cholinergic, and alpha 2-adrenergic receptors based upon characteristics in primary structure. *J Biol Chem* 1992; 267:8342-6
35. Wade SM, Lim WK, Lan KL, Chung DA, Nanamori M, Neubig RR: G(i) activator region of alpha(2A)-adrenergic receptors: Distinct basic residues mediate G(i) versus G(s) activation. *Mol Pharmacol* 1999; 56:1005-13
36. Gomez J, Joly C, Kuhn R, Knopfel T, Bockaert J, Pin JP: The second intracellular loop of metabotropic glutamate receptor 1 cooperates with the other intracellular domains to control coupling to G-proteins. *J Biol Chem* 1996; 271:2199-205
37. Bourne HR: How receptors talk to trimeric G proteins. *Curr Opin Cell Biol* 1997; 9:134-42
38. Kostenis E, Conklin BR, Wess J: Molecular basis of receptor/G protein coupling selectivity studied by coexpression of wild type and mutant m2 muscarinic receptors with mutant G alpha(q) subunits. *Biochemistry* 1997; 36:1487-95
39. Wess J: Molecular basis of receptor/G-protein-coupling selectivity. *Pharmacol Ther* 1998; 80:231-64
40. De Lean A, Stadel JM, Lefkowitz RJ: A ternary complex model explains the agonist-specific binding properties of the adenylyl cyclase-coupled beta-adrenergic receptor. *J Biol Chem* 1980; 255:7108-17
41. Costa T, Ogino Y, Munson PJ, Onaran HO, Rodbard D: Drug efficacy at guanine nucleotide-binding regulatory protein-linked receptors: thermodynamic interpretation of negative antagonism and of receptor activity in the absence of ligand. *Mol Pharmacol* 1992; 41:549-60
42. McKinney M, Anderson D, Vella-Rountree L: Different agonist-receptor active conformations for rat brain M1 and M2 muscarinic receptors that are separately coupled to two biochemical effector systems. *Mol Pharmacol* 1989; 35:39-47
43. Ross EM: KTP: Pharmacodynamics: Mechanisms of drug action and the relationship between drug concentration and effect. Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition. Edited by Hardman JG, Limbird LE. New York, McGraw Hill Publishers, 2002, pp 31-44
44. Christopoulos A, Kenakin T: G protein-coupled receptor allosterism and complexing. *Pharmacol Rev* 2002; 54:323-74
45. Huang RR, Dehaven RN, Cheung AH, Diehl RE, Dixon RA, Strader CD: Identification of allosteric antagonists of receptor-guanine nucleotide-binding protein interactions. *Mol Pharmacol* 1990; 37:304-10
46. Beindl W, Mitterauer T, Hohenegger M, Ijzerman AP, Nanoff C, Freissmuth M: Inhibition of receptor/G protein coupling by suramin analogues. *Mol Pharmacol* 1996; 50:415-23
47. Freissmuth M, Boehm S, Beindl W, Nickel P, Ijzerman AP, Hohenegger M, Nanoff C: Suramin analogues as subtype-selective G protein inhibitors. *Mol Pharmacol* 1996; 49:602-11
48. Neubig RR, Siderovski DP: Regulators of G-protein signalling as new central nervous system drug targets. *Nat Rev Drug Discov* 2002; 1:187-97
49. Hollinger S, Hepler JR: Cellular regulation of RGS proteins: Modulators and integrators of G protein signaling. *Pharmacol Rev* 2002; 54:527-59
50. Huang C, Hepler JR, Gilman AG, Mumby SM: Attenuation of Gi- and Gq-mediated signaling by expression of RGS4 or GAIP in mammalian cells. *Proc Natl Acad Sci U S A* 1997; 94:6159-63
51. Usui H, Nishiyama M, Moroi K, Shibasaki T, Zhou J, Ishida J, Fukamizu A, Haga T, Sekiya S, Kimura S: RGS domain in the amino-terminus of G protein-coupled receptor kinase 2 inhibits Gq-mediated signaling. *Int J Mol Med* 2000; 5:335-40
52. Hollmann MW, Wiczorek KS, Berger A, Durieux ME: Local anesthetic inhibition of G protein-coupled receptor signaling by interference with Gq protein function. *Mol Pharmacol* 2001; 59:294-301
53. von Zastrow M, Mostov K: Signal transduction: A new thread in an intricate web. *Science* 2001; 294:1845-7
54. Burchett SA, Bannon MJ, Granneman JG: RGS mRNA expression in rat striatum: Modulation by dopamine receptors and effects of repeated amphetamine administration. *J Neurochem* 1999; 72:1529-33
55. Nakagawa T, Minami M, Satoh M: Up-regulation of RGS4 mRNA by opioid receptor agonists in PC12 cells expressing cloned mu- or kappa-opioid receptors. *Eur J Pharmacol* 2001; 433:29-36
56. Dulin NO, Pratt P, Tiruppathi C, Niu J, Voyno-Yasenetskaya T, Dunn MJ: Regulator of G protein signaling RGS3T is localized to the nucleus and induces apoptosis. *J Biol Chem* 2000; 275:21317-23
57. Lu Q, Sun EE, Klein RS, Flanagan JG: Ephrin-B reverse signaling is mediated by a novel PDZ-RGS protein and selectively inhibits G protein-coupled chemotraction. *Cell* 2001; 105:69-79
58. Sierra DA, Popov S, Wilkie TM: Regulators of G-protein signaling in receptor complexes. *Trends Cardiovasc Med* 2000; 10:263-8
59. Takeishi Y, Jalili T, Hoit BD, Kirkpatrick DL, Wagoner LE, Abraham WT, Walsh RA: Alterations in Ca²⁺ cycling proteins and G alpha q signaling after left ventricular assist device support in failing human hearts. *Cardiovasc Res* 2000; 45:883-8
60. Rogers JH, Tamirisa P, Kovacs A, Weinheimer C, Courtois M, Blumer KJ, Kelly DP, Muslin AJ: RGS4 causes increased mortality and reduced cardiac hypertrophy in response to pressure overload. *J Clin Invest* 1999; 104:567-76
61. Reif K, Cyster JG: RGS molecule expression in murine B lymphocytes and ability to down-regulate chemotaxis to lymphoid chemokines. *J Immunol* 2000; 164:4720-9
62. Beadling C, Druey KM, Richter G, Kehrl JH, Smith KA: Regulators of G protein signaling exhibit distinct patterns of gene expression and target G protein specificity in human lymphocytes. *J Immunol* 1999; 162:2677-82
63. Oliveira-Dos-Santos AJ, Matsumoto G, Snow BE, Bai D, Houston FP, Whishaw IQ, Mariathasan S, Sasaki T, Wakeham A, Ohashi PS, Roder JC, Barnes CA, Siderovski DP, Penninger JM: Regulation of T cell activation, anxiety, and male aggression by RGS2. *Proc Natl Acad Sci U S A* 2000; 97:12272-7
64. Mirnics K, Middleton FA, Stanwood GD, Lewis DA, Levitt P: Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia. *Mol Psychiatry* 2001; 6:293-301
65. Tekumalla PK, Calon F, Rahman Z, Birdi S, Rajput AH, Hornykiewicz O, Di Paolo T, Bedard PJ, Nestler EJ: Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease. *Biol Psychiatry* 2001; 50:813-6
66. Chen CK, Burns ME, He W, Wensel TG, Baylor DA, Simon MI: Slowed recovery of rod photoresponse in mice lacking the GTPase accelerating protein RGS9-1. *Nature* 2000; 403:557-60
67. Lyubarsky AL, Naarendorp F, Zhang X, Wensel T, Simon MI, Pugh Jr EN: RGS9-1 is required for normal inactivation of mouse cone phototransduction. *Mol Vis* 2001; 7:71-8
68. Wreggett KA, Wells JW: Cooperativity manifest in the binding properties of purified cardiac muscarinic receptors. *J Biol Chem* 1995; 270:22488-99
69. Angers S, Salahpour A, Bouvier M: Biochemical and biophysical demonstration of GPCR oligomerization in mammalian cells. *Life Sci* 2001; 68:2243-50
70. Angers S, Salahpour A, Joly E, Hilairet S, Chelsky D, Dennis M, Bouvier M: Detection of beta 2-adrenergic receptor dimerization in living cells using bioluminescence resonance energy transfer (BRET). *Proc Natl Acad Sci U S A* 2000; 97:3684-9
71. Abdalla S, Zaki E, Lother H, Quitterer U: Involvement of the amino terminus of the B(2) receptor in agonist-induced receptor dimerization. *J Biol Chem* 1999; 274:26079-84
72. Marshall FH, Jones KA, Kaupmann K, Bettler B: GABAB receptors: The first 7TM heterodimers. *Trends Pharmacol Sci* 1999; 20:396-9
73. Jordan BA, Devi LA: G-protein-coupled receptor heterodimerization modulates receptor function. *Nature* 1999; 399:697-700
74. Abdalla S, Lother H, el Massiery A, Quitterer U: Increased AT(1) receptor heterodimers in pre-eclampsia mediate enhanced angiotensin II responsiveness. *Nat Med* 2001; 7:1003-9
75. Armour SL, Foord S, Kenakin T, Chen WJ: Pharmacological characterization of receptor-activity-modifying proteins (RAMPs) and the human calcitonin receptor. *J Pharmacol Toxicol Methods* 1999; 42:217-24

76. Hollmann MW, Durieux ME: Local anesthetics: Effects on inflammation, wound healing and coagulation. *Progr Anesthesiol* 2000; 14:291-304
77. Hollmann MW, Durieux ME: Local anesthetics: Effects on the central nervous system and bronchial reactivity. *Progr Anesthesiol* 2000; 14:323-36
78. Ishizawa Y, Pidikiti R, Liebman PA, Eckenhoff RG: G protein-coupled receptors as direct targets of inhaled anesthetics. *Mol Pharmacol* 2002; 61: 945-52
79. Croxton TL, Lande B, Hirshman CA: Role of G proteins in agonist-induced Ca²⁺ sensitization of tracheal smooth muscle. *Am J Physiol* 1998; 275:L748-55
80. Hotta K, Emala CW, Hirshman CA: TNF-alpha upregulates G α and G β protein expression and function in human airway smooth muscle cells. *Am J Physiol* 1999; 276:L405-11
81. Hirshman CA, Togashi H, Shao D, Emala CW: G α 2 is required for carbachol-induced stress fiber formation in human airway smooth muscle cells. *Am J Physiol* 1998; 275:L911-6
82. Hirshman CA, Emala CW: Actin reorganization in airway smooth muscle cells involves G α and Gi-2 activation of Rho. *Am J Physiol* 1999; 277:L653-61
83. Kai T, Jones KA, Warner DO: Halothane attenuates calcium sensitization in airway smooth muscle by inhibiting G-proteins. *ANESTHESIOLOGY* 1998; 89:1543-52
84. Emala CW, Liu F, Hirshman CA: G α but not G β is linked to activation of p21(ras) in human airway smooth muscle cells. *Am J Physiol* 1999; 276:L564-70
85. Pentylala SN, Sung K, Chowdhury A, Rebecchi MJ: Volatile anesthetics modulate the binding of guanine nucleotides to the alpha subunits of heterotrimeric GTP binding proteins. *Eur J Pharmacol* 1999; 384:213-22
86. Streiff J, Jones K, Perkins WJ, Warner DO, Jones KA: Effect of halothane on the guanosine 5' triphosphate binding activity of G-protein α subunits. *ANESTHESIOLOGY* 2003; 99:105-11
87. Ishizawa Y, Sharp R, Liebman PA, Eckenhoff RG: Halothane binding to a G protein coupled receptor in retinal membranes by photoaffinity labeling. *Biochemistry* 2000; 39:8497-502
88. Yoshimura H, Jones KA, Perkins WJ, Warner DO: Dual effects of hexanol and halothane on the regulation of calcium sensitivity in airway smooth muscle. *ANESTHESIOLOGY* 2003; 98:871-80
89. Anthony BL, Dennison RL, Aronstam RS: Disruption of muscarinic receptor-G protein coupling is a general property of liquid volatile anesthetics. *Neurosci Lett* 1989; 99:191-6
90. Aronstam RS, Anthony BL, Dennison RL: Halothane effects on muscarinic acetylcholine receptor complexes in rat brain. *Biochem Pharmacol* 1986; 35:667-72
91. Aronstam RS, Dennison RL: Anesthetic effects on muscarinic signal transduction. *Int Anesthesiol Clin* 1989; 27:265-72
92. Pentylala SN, Sung K, Chowdhury A, Rebecchi MJ: Volatile anesthetics modulate the binding of guanine nucleotides to the alpha subunits of heterotrimeric GTP binding proteins. *Eur J Pharmacol* 1999; 384:213-22
93. Toller WG, Kersten JR, Gross ER, Pagel PS, Wartier DC: Isoflurane preconditions myocardium against infarction *via* activation of inhibitory guanine nucleotide binding proteins. *ANESTHESIOLOGY* 2000; 92:1400-7
94. Weigt HU, Kwok WM, Rehmert GC, Bosnjak ZJ: Sensitization of the cardiac Na channel to α -adrenergic stimulation by inhalation anesthetics: Evidence for distinct modulatory pathways. *ANESTHESIOLOGY* 1998; 88:125-33
95. Weigt HU, Kwok WM, Rehmert GC, Bosnjak ZJ: Modulation of the cardiac sodium current by inhalational anesthetics in the absence and presence of β -stimulation. *ANESTHESIOLOGY* 1998; 88:114-24
96. Sanuki M, Yuge O, Kawamoto M, Fujii K, Azuma T: Sevoflurane inhibited β -adrenoceptor-G protein bindings in myocardial membranes in rats. *Anesth Analg* 1994; 79:466-71
97. Hagelucken A, Grunbaum L, Nurnberg B, Harhammer R, Schunack W, Seifert R: Lipophilic beta-adrenoceptor antagonists and local anesthetics are effective direct activators of G-proteins. *Biochem Pharmacol* 1994; 47:1789-95
98. Nietgen GW, Chan CK, Durieux ME: Inhibition of lysophosphatidate signaling by lidocaine and bupivacaine. *ANESTHESIOLOGY* 1997; 86:1112-9
99. Hollmann MW, Fischer LG, Byford AM, Durieux ME: Local anesthetic inhibition of m1 muscarinic acetylcholine signaling. *ANESTHESIOLOGY* 2000; 93: 497-509
100. Herroeder S, Durieux ME, Hollmann MW: Inflammatory responses after surgery. *Hosp Med* 2002; 63:99-103

Glossary

- Allosteric agonist:** a compound that promotes G-protein coupling independent of any effects on *orthosteric* agonist binding.
- Allosteric antagonist:** a compound that decreases the affinity of the *GPCR* for its *orthosteric* agonist and/or reduces the affinity of the receptor for its G protein(s).

Allosteric enhancer: a compound that increases the affinity of the ligand binding site on the receptor.

Allosteric interaction: an interaction between two topographically distinct binding sites on a single receptor.

Allosteric site: a binding site on a receptor distinct from the agonist binding site. It does not contain amino acids that make direct contact with the ligand when bound.

C-terminal (carboxy-terminal) segment: the part of the amino acid sequence of a *GPCR* that ends inside the cell.

G protein-coupled receptor (GPCR): member of a class of membrane proteins that transduce binding of an extracellular agonist to activation of an intracellular GTP-binding protein (G protein).

Guanosine triphosphatase (GTPase) activating protein (GAP): a protein that catalyzes conversion of a guanosine triphosphate to a guanosine biphosphate on a G protein. GAP activity is contained within the structure of some G proteins themselves.

Heterodimer: dimer consisting of two different component molecules.

Homodimer: dimer consisting of two identical component molecules.

N-linked glycosylation: attachment of sugar moieties to the *N-terminal* segment of a *GPCR*.

N-terminal (amino-terminal) segment: the part of the amino acid sequence of a *GPCR* that ends outside the cell.

Nucleotide exchange factor: a protein that facilitates exchange of a guanosine biphosphate by a guanosine triphosphate on a G protein. Nucleotide exchange factor activity is contained within the structure of some G proteins themselves.

Orthosteric site: the ligand-binding site on a receptor. This site contains amino acids that make contact with the agonist when bound.

Palmitoylation: attachment of a palmitic acid (lipid) moiety to the *C-terminal* segment of a *GPCR*. This allows attachment of that segment to the membrane and may regulate G-protein selectivity of the receptor.

Regulators of G-protein signaling (RGS): a class of proteins that modulates functioning of *GPCR*. These proteins may be of relevance in development of drug tolerance and mitigation of adverse effects of G-protein overstimulation.

Transmembrane domain: the part of the amino acid sequence of a *GPCR* that is located within the membrane. *GPCRs* contain seven of such domains.