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Perspectives in Pharmacology

Stoichiometry and Compartmentation in G Protein-Coupled Receptor Signaling: Implications for Therapeutic Interventions Involving G_s[1]

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ABSTRACT

There is great therapeutic interest in manipulating (either enhancing or suppressing) G protein-coupled receptor (GPCR) signal transduction. However, most current strategies are limited to pharmacological activation or blockade of receptors. Human gene therapy, including both overexpression and antisense approaches, may allow manipulation of GPCR signaling at steps distal to receptors. To fully understand the impact of such therapy, the transduction of signals between the multiple components of GPCR signaling and their interaction with other cellular molecules must be understood in the context of both normal physiology and disease. Defining the stoichiometric relationship among multiple components of GPCR signaling is a first step. We summarize data showing the substantial excess of $G_{\alpha s}$ relative to both β-adrenergic receptors and adenylyl cyclase. A predominant idea regarding signaling via GPCRs has for over 20 years emphasized the concept of random movement and collision ("collision coupling") of proteins within the lipid bilayer of the plasma membrane. This notion does not readily account for the rapidity and fidelity of signal transduction by the multiple components involved in GPCR-G protein-effector systems, especially considering the low abundance of these proteins in cells. Recently, many components involved in signal transduction by GPCRs have been shown to exist primarily in microdomains of the plasma membrane, in particular, caveolae. These and other structures may serve to compartmentalize signals, thereby optimizing signal transduction between an agonist and specific effectors. The formation, organization, and maintenance of such structures may prove to be altered in disease states associated with disregulated signaling. In addition, we speculate that identification of genetic polymorphisms of and therapy targeted to components that are critical for determining efficacy (e.g., effectors such as adenylyl cyclase) will provide important future therapeutic strategies.

The transduction of signals from the extracellular environment across the plasma membrane barrier and into the intracellular milieu is a fundamental aspect of cellular regulation. Nature has evolved a variety of means to accomplish this feat, in particular, via the use of many different types of ligands and receptors. One can generalize that such signal transduction pathways fall into four basic paradigms: 1) membrane receptors that function as ion channels, 2) membrane receptors that are enzymes, 3) intracellular receptors that recognize lipophilic ligands that diffuse across the plasma membrane, and 4) G protein-coupled receptors (GPCRs). In contrast with the other three systems, GPCR systems involve membrane interaction of components in addition to the receptor to initiate transduction of extracellular signals into the cell. Additional molecules are required to mediate feedback regulation and to integrate such signals with other cellular inputs and events. Therapeutic manipulations of GPCR systems have thus far been limited primarily to pharmacological blockade or activation of the receptors. Although GPCRs are useful as drug targets because of their patterns of distribution on different cell types and the pref-

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ABBREVIATIONS: GPCR, G protein-coupled receptor; AC, adenylyl cyclase; RGS, regulator of G protein signaling; AC6, adenylyl cyclase type 6; PGE_2, prostaglandin E_2; PKA, cAMP-dependent protein kinase; AKAP, A-kinase anchoring protein; PKC, protein kinase C; AR, adrenergic receptor; GRK, G protein receptor kinase; RACK, receptor for activated C kinase; RAMP, receptor activity modifying protein; PLC, phospholipase C.

erential role of particular GPCR subtypes in mediating specific responses, postreceptor components are also potential therapeutic targets. If one wishes to alter GPCR signaling pathways in novel ways, it is necessary to understand the dynamics of activation for each component in the pathway and the subsequent interactions among these components.

One approach to identify novel therapeutic strategies is to examine the stoichiometry, i.e., absolute concentrations or relative proportions of each component, of a GPCR signal transduction pathway expressed in a given cell. Identifying the components that determine potency (sensitivity, EC_{50}, etc.) and efficacy (maximal response) can lead to insights as how to best enhance or suppress a disregulated system. Such studies have been completed for the G_s-linked adenylyl cyclase (AC) pathway in cardiac myocytes (described below). Moreover, the recent evidence that many signaling molecules are enriched in specialized microdomains of the plasma membrane increases the likelihood that GPCR signaling is highly compartmentalized in cells (for reviews, see Neubig, 1994; Chidiac, 1998; Okamoto et al., 1998; Shaul and Anderson, 1998). Considering the rapidity and fidelity of signal transduction by GPCR systems, it has been suggested that the essential molecules of such pathways are held in close association with one another and not freely floating or dependent on random collision to interact. The evidence supporting this idea and the therapeutic implications of stoichiometric expression and compartmentation are the focus of this Perspective.

Components of GPCR Signaling: GPCR-G_s/G_i-AC as a Paradigm

In addition to GPCRs as the initial components that interact with extracellular hormone or neurotransmitter, GPCRs transduce signals by coupling to heterotrimeric (α-, β-, γ-subunit-containing) GTP-binding (G) proteins that regulate effector molecules. There are four principal G protein families (G_s, G_i, $G_{q/11}$, $G_{12/13}$), each identified by structurally similar α-subunits that preferentially regulate specific classes of effector molecules. $G_{q/11}$ stimulates phospholipase C (PLC), G_s stimulates AC, and G_i inhibits AC and activates K^+ channels, although family members can regulate multiple types of effector enzymes, ion channels, and transporters. The intrinsic GTPase activity of G_i and G_q α-subunits can be enhanced by regulators of G protein signaling (RGS) proteins (Dohlman and Thorner, 1997), and G_s α-subunit GTPase activity can be enhanced by AC (Scholich et al., 1999). The G_β and G_γ subunits function as a heterodimer to regulate effector molecules and other proteins involved in GPCR signaling and also to restrain G_α action by forming inactive $G_{\alpha\beta\gamma}$ complexes.

Among the various G protein-regulated effectors, AC is arguably the most well studied and has provided a particularly useful system to examine GPCR stoichiometry. AC, regulated by G_s and G_i, synthesizes cAMP from ATP, which in turn regulates cell function via activation of cAMP-dependent protein kinase (PKA). PKA phosphorylates serine residues on substrates to initiate cellular actions of cAMP, and phosphatases reverse such phosphorylation and actions. Cells "target" the relatively nonspecific kinase activity of PKA via A-kinase anchoring proteins (AKAPs) so that the kinase preferentially phosphorylates specific substrates (Colledge and Scott, 1999). A substantial number of different AKAP proteins have been identified. These include molecules that show preference for individual isoforms of PKA and for interaction with specific types of substrates. An example is AKAP250 (also known as gravin), which interacts with the β-adrenergic receptor (β-AR) and targets activated PKA to phosphorylate the receptor, thus permitting specific feedback regulation of receptor activity (Shih et al., 1999). Targeting mechanisms also exist for G protein receptor kinases (GRKs) that phosphorylate β-ARs and other GPCRs. Targeting of GRK to activated receptors is mediated by $G_{\beta\gamma}$ subunits, which appear to enhance the specificity of GRK for agonist-occupied receptors (Krupnick and Benovic, 1998; Lefkowitz, 1998). By phosphorylating receptors, GRKs impair interaction of GPCRs with G proteins and induce the recruitment of β-arrestin, a protein that acts as an adapter between the receptor and clathrin and thereby initiates internalization via clathrin-coated pits (Ferguson et al., 1996; Goodman et al., 1996).

Stoichiometry of AC Pathway

As discussed above, there is great interest in therapeutic efforts to modulate GPCR signaling. This has been particularly true for β-AR signal transduction. Given the multicomponent interactions required for GPCR signaling, it is intriguing to imagine that each of the components has the potential to be a therapeutic target. Developing new approaches to accomplish this depends on understanding which component(s) of these signal transduction pathways are most critical in determining efficacy and potency of the system. Classical receptor theory predicts that expression of receptor, as the site of interaction with the agonist, determines potency, and indeed some studies confirm this theoretical prediction (Milligan, 1996).

However, it is not so simple to prognosticate which component determines efficacy of the system. In part, this difficulty relates to what one may wish to define as "response". Most molecular pharmacologists focus on the initial event (i.e., generation of second messenger) and assess receptor occupancy relative to maximal formation of second messenger. We emphasize this approach in this Perspective. Others assess response as activation of "downstream" enzymes, channels, or physiological events. In these latter cases, downstream components that limit the rate or extent of response may prove to be as, or more, critical than upstream components that regulate second-messenger formation. For example, if one wishes to examine β-AR activation in the heart, one might relate receptor occupancy to G protein or AC activation, cAMP formation, PKA activation, ion channel activity, contractile protein or metabolic enzyme phosphorylation, or measures of inotropy, chronotropy, lusitropy, or dromotropy.

Studies to assess efficacy based on second-messenger formation necessitate quantification of each of the components involved. In the case of β-AR-G_s-AC, one can use radioligand binding to quantitate receptors and AC (using [^{3}H]forskolin for the latter) and radioimmunoassay or quantitative immunoblotting to quantitate G_s. Using this approach, we conducted initial studies to quantify β-AR signaling components of AC in S49 murine lymphoma cells. In these cells, the ratio of receptor:G protein:AC is approximately 1:100:3, and receptor activation of G_s appears to be the critical factor for am-

plification of signaling (Alousi et al., 1991). Subsequent studies in adult rat cardiac myocytes and with NG108-15 cells yielded comparable results (Post et al., 1995; Milligan, 1996). These results, together with assumptions regarding uniform accessibility and equivalent function of individual components, lead one to predict that either receptor or AC, but not G_s, would set the limit on the maximum efficacy of the system. Related data are consistent with the idea that other effectors (e.g., voltage-sensitive calcium channels) are expressed at a level akin to that of AC (Szabadkai et al., 1998). How the low absolute concentration of GPCR, G protein, and effector (in the femtomole to low picomole per milligram of protein range) favors rapid, high-fidelity interaction of components required for signal transduction in native cells and membranes is not clear. In addition, the stoichiometric expression of $\beta\gamma$-subunits, in particular different combinations of β- and γ-subunits, has not been evaluated carefully relative to other components, a major shortcoming considering their importance in GPCR signaling. Release of $\beta\gamma$-subunits should occur in molar equivalent to that of α-subunits on activation, but their subsequent cellular effects likely depend on the regulation of effectors by specific isoforms of $\beta\gamma$ (Hildebrandt, 1997).

Subsequent studies, primarily those conducted with cardiac preparations, have strongly suggested that AC is the critical component that limits maximal β-AR response. This is true whether one measures cAMP accumulation in isolated cells, AC activity in membranes, or functional parameters in whole hearts. Overexpression of β-AR subtypes or G_s in isolated cells or transgenic animals leads to small increases in the maximum cAMP and only modest enhancement in cardiac performance in response to β-AR activation (Milano et al., 1994; Gaudin et al., 1995). In contrast, overexpression of AC type 6 (AC6) increases maximal cAMP response in a manner proportional to the degree of overexpression of the enzyme (Gao et al., 1998). Furthermore, transgenic mice with cardiac-directed overexpression of AC6 display improved cardiac performance and β-AR-mediated cAMP formation (Gao et al., 1999; Roth et al., 1999).

The stoichiometry for other GPCR families may not be the same as for G_s-linked systems. For $G_{\alpha i}$, it is believed that levels of expression are somewhat greater than those of $G_{\alpha s}$, whereas receptor expression resembles that of G_s-linked GPCRs (Milligan, 1996). However, the fact that RGS proteins can regulate G_i activity, whereas no known RGS protein regulates G_s, may result in different kinetics of activation of this pathway. Stoichiometry in the G_q/PLC pathway is likely to be very different than that observed for G_s. Although there are no documented efforts to define the stoichiometric relationships of components in G_q-linked pathways, studies using receptor alkylating agents such as benzylilcholine mustard and phenoxybenzamine (selective for muscarinic and α-ARs, respectively) indicate that such GPCR systems possess a high degree of receptor reserve (Siegel and Triggle, 1982; Ruffolo, 1986). Data for $G_{q/11}$ suggest levels of expression akin to those for G_s, albeit with substantial decreases, at least in some tissues, during postnatal development (Mochizuki et al., 1995). Conceivably, the expression and kinetics of PLC activity may be key for signal amplification of those systems. The components that are most critical for potency and efficacy in $G_{q/11}$ and $G_{12/13}$ systems remain to be defined and may yield results different from those observed for G_s-linked systems.

Compartmentation of GPCR Signaling

Stoichiometric analysis of the overall cellular expression of components of signal transduction pathways may be an overly simplistic approach because such analysis fails to account for the potential compartmentation of molecules in cells. Such compartmentation is well known, wherein certain cells establish protein domains on one portion of the cell that are strikingly different from another. For example, epithelial cells have luminal and basolateral surfaces with a distinct segregation of proteins, including receptors and other signaling molecules (Wozniak et al., 1997). In addition, target cells innervated by neurons typically have subsynaptic regions enriched with high concentrations of certain receptors, transporters, and enzymes (Colledge and Froehner, 1998). Specific protein domains, such as PDZ (PSD-95, Discs large, ZO-1) domains, may be responsible for the differential targeting of molecules in neurons and epithelia (Kim, 1997) and for the clustering of multiple proteins into functional complexes (Fanning and Anderson, 1999). This type of compartmentation likely aids in amplification of signals and can contribute to the specialized responses of differentiated cells. Other observations at a single-cell level imply existence of subcellular compartments. For example, both β_1- and β_2-ARs stimulate production of cAMP and activate PKA in cardiac myocytes, but only β_1-AR-promoted PKA activity appears to lead to the phosphorylation of downstream effector molecules, such as phospholamban and troponin, proteins involved in the regulation of the contractile machinery (Kuschel et al., 1999).

Several targeting proteins have been identified that may help organize GPCR signaling and may contribute to the compartmentation of the signaling components. The aforementioned AKAPs target the PKA catalytic subunit to particular effector molecules (Colledge and Scott, 1999). RACKs, also known as receptors for activated C kinase, target PKC to particular phosphorylation targets (Mochly-Rosen and Gordon, 1998). RAMPs (receptor activity modifying proteins) serve as accessory proteins for a particular GPCR (which can be activated by calcitonin or adrenomedullin) and facilitate its transport to the cell surface and regulate its glycosylation and pharmacology (Foord and Marshall, 1999). Other as-yet-unidentified RAMPs may play similar roles for other GPCRs. AKAPs, RACKs, RAMPs, and other such proteins (probably many yet to be identified) facilitate the rapid and specific signaling characteristic of GPCR activation and may be specifically compartmentalized themselves.

Signaling Molecules in Caveolae and Coated Pits

Recent studies have emphasized the localization of GPCR-signaling components in specific membrane microdomains, caveolae, and the potential role of the caveolar protein caveolin as a scaffolding and regulatory molecule (Shaul and Anderson, 1998). Thus, the concept of "prearranged signaling complexes" has been put forth (Okamoto et al., 1998). Although this idea is controversial, if true, it would limit the utility of analyzing total cellular expression (and stoichiometry) of components because a given pool of receptors may be

physically and functionally "precoupled" to a G protein (or perhaps a small pool of total cellular G protein) and a specific effector. This concept would also be a mechanism to account for rapid, high-fidelity signaling of multicomponent systems such as that of GPCRs.

Caveolae, or "little caves", so-called because of their morphological identity as flask-like invaginations, are membrane regions enriched in particular proteins (caveolins and probably others) and lipids (cholesterol, sphingolipids). Caveolae were originally identified (almost 50 years ago) in endothelial cells, but are found in a wide variety of cell types where they are involved in potocytosis, endocytosis, and transcellular movement of molecules (Anderson, 1998). Endocytosis by caveolae differs from that mediated by another specialized region of the plasma membrane, clathrin-coated pits. These two vesicular structures differ biochemically and transport different types of molecules and thus represent parallel but distinct pathways.

The recent renaissance in thinking of caveolae as centers for signal transduction has been aided by the discovery of a marker protein for these structures, caveolin, which has facilitated biochemical "purification" of caveolae and analysis of the proteins that reside therein. The separation of caveolar membranes depends on their enrichment for lipids that impart higher buoyancy than the rest of the plasma membrane and thus facilitate separation of caveolin-rich fractions on sucrose gradients. Such fractions may or may not contain morphologically distinct caveolae (Hooper, 1999). Use of certain detergents provides a rapid means to isolate caveolin-rich fractions, but detergents may alter retention of proteins in the resultant fractions. Thus, for studies of signaling components, detergent-free methods are preferred (Song et al., 1996; Oh and Schnitzer, 1999). Three different caveolins have been identified (called caveolin 1–3, caveolin-3 being a muscle-specific caveolin) that can be detected immunologically with commercially available antibodies. Researchers using this approach have defined a growing list of signaling molecules localized in caveolae or closely associated with caveolins (Table 1). GPCRs, as well as various receptor tyrosine kinases (including receptors for platelet-derived growth factor, epidermal growth factor, and nerve growth factor), have also been localized in caveolae, as have many of the molecules critical in transducing the signals initiated by these types of receptors. Most of the molecules involved in GPCR signaling (including GPCRs, G proteins, AC, PKA, and GRK) have been localized to caveolae, with the exception (thus far) of AKAPs and β-arrestins (Table 1). Therefore, caveolar microdomains concentrate signaling molecules and may also compartmentalize or segregate components.

Merely describing the expression of signaling proteins in caveolae is a long way from definitively demonstrating that the components exist in preassembled complexes. However, several different approaches have strongly suggested that this is the case. For example, disrupting caveolae using detergents, cholesterol-removing agents, or other methods can lead to altered signaling. Disruption of caveolae inhibits phophinositide hydrolysis by interfering with the interaction of the enzyme that mediates this hydrolysis (PLC) with its substrate (phosphatidylinositol 4,5-bisphosphate), which is also enriched in caveolae (Pike and Casey, 1996). By contrast, caveolar disruption can lead to increased activity of AC, perhaps because of removal of the enzyme from inhibition by caveolin (Toya et al., 1998).

TABLE 1
Signaling molecules expressed in caveolae[a]

	Method of Localization	
	Morphological	Biochemical
Receptors		
β-Adrenergic	+	+
Bradykinin	−	+
Endothelin	−	+
M_2 muscarinic acetylcholine	−	+
Adenosine A_1	+	+
Cholecystokinin	+	+
Platelet-derived growth factor	+	+
Epidermal growth factor	−	+
Insulin	+	−
Postreceptor components		
G proteins (α and $\beta\gamma$)	+	+
Endothelial nitric-oxide synthase	+	+
Mitogen-activated protein kinase	+	+
Adenylyl cyclase	+	+
PKA (catalytic subunit)	−	+
PKC (α)	+	+
Diacylglycerol	−	+
GRKs	−	+
Ras	−	+
Raf-1	+	+
Rac-1	−	+
RhoA	−	+
Src kinases	−	+
Shc	−	+
Calmodulin	−	+
IP_3 receptor	+	+

[a] Adapted from Shaul and Anderson (1998).

Caveolin exists in a hairpin-like structure with both the carboxy and amino tails intracellular separated by a turn in the membrane. On the N-terminal portion of caveolin-1 and -3 is a putative caveolin scaffolding domain, in part based on its interaction with binding motifs that exist in numerous signaling proteins as a conserved sequence of aromatic residues (Table 2). Interestingly, the binding of caveolin-1 or -3 (or peptide fragments thereof) to these signaling molecules generally results in their inactivation. Therefore, caveolin appears to be a negative regulator of signal transduction. Activities of G proteins, AC, GRK, PKC, multiple tyrosine kinases, and endothelial nitric-oxide synthase are each sup-

TABLE 2
Signaling molecules containing a caveolin binding motif[a]

	Caveolin Binding Motif	
	First Residue	Sequence ($\Phi X \Phi XXXX \Phi XX \Phi$)
Receptors		
β-Adrenergic	359	--FVFFNWLGY
Endothelin	146	WPFDHNDFGVF
Muscarinic acetylcholine	422	--WTIGYWLCY
Platelet-derived growth factor	887	WSFGILLWEIF
Epidermal growth factor	898	WSYGVTVW---
Insulin	1220	WSFGVVFW---
Effectors		
Endothelial nitric-oxide synthase	347	--FPAAPFSGW
Mitogen-activated protein kinase	124	--YIVGFYGAF
Transduction molecules		
G proteins (α)	190	FTFKDLHFKMF
PKC-α	656	FSYVNPQF---
Src kinases	425	WSFGILLY---

[a] Adapted from Couet et al. (1997).

pressed on interacting with caveolin or caveolin peptides (Couet et al., 1997; Toya et al., 1998; Carman et al., 1999). These observations are thus consistent with the notion that caveolins serve both as scaffolding molecules and as regulators of cell signaling.

Clathrin-coated pits are involved in internalization of GPCRs and desensitization of receptor responses as well as in endocytosis and vesicular transport. Coated pits form from the interaction of clathrin with other specific proteins such as adaptins and dynamin. These proteins, and their associated cargo, initiate the budding process and pinching off of clathrin-containing vesicles. Certain GPCRs are targeted to clathrin-coated pit domains, perhaps in part by $G_{\beta\gamma}$-activated GRK phosphorylation and the subsequent recruitment of β-arrestin, which escort the receptors into these domains (Krupnick and Benovic, 1998; Lefkowitz, 1998). In addition, muscarinic cholinergic, bradykinin, and β-AR receptors (at least in some cells) can translocate to caveolar membranes on agonist activation, implicating this structure as a means of internalizing certain GPCRs (Raposo et al., 1989; de Weerd and Leeb-Lundberg, 1997; Feron et al., 1997). In contrast, adenosine A_1 receptors translocate out of caveolae on exposure to agonist (Lasley et al., 2000). Clathrin-coated pits and caveolae differ in their intracellular destinations and thus may impart different fates for the internalized molecules. Therefore, although endocytosis of certain receptors (e.g., low-density lipoprotein receptors, certain GPCRs, and receptor tyrosine kinases) occur via clathrin-coated pit endocytosis, caveolae likely act as a separate, but nonredundant, route for internalization of other receptors. It is further possible that certain types of receptors may initially localize to caveolae on activation but subsequently exit these domains during the desensitization process. Additional work is needed to understand the precise roles of these two specialized membrane microdomains in signal transduction, including assessment as to whether other components involved in regulation of GPCR signaling (e.g., RACKs, RAMPs, RGS proteins, AKAPs, and other "partners") exist in caveolar microdomains.

Therapeutic Implications

The evolving ideas regarding stoichiometry and compartmentation of signaling molecules have numerous implications in terms of development and use of therapeutic agents. We briefly address two of these: choice of therapeutic targets and role of genetic polymorphisms.

If one accepts the notion of the critical role for expression of receptors and effectors as determinants of potency and efficacy, respectively, then therapy directed to each of those components may have very different effects on cell signaling. Receptor blockade by competitive antagonists will produce the expected patterns of rightward shifts in agonist concentration-response curves but will not alter maximal response. Furthermore, settings with higher concentrations of agonists would require higher concentrations of antagonists (i.e., higher IC_{50} values) to achieve equivalent decreases in signaling. For example, a higher concentration of a β-AR antagonist would be required to decrease β-AR stimulation of cardiac cells in a physiological setting, such as exercise, in which circulating catecholamines are increased. Enhancement of receptor number would be expected to produce a leftward shift in the agonist concentration-response curve, thereby sensitizing cells to endogenous or exogenous agonist, but having only a minor effect on maximal levels of signal transduction. This idea has been borne out in efforts to increase β-AR number in the heart via generation of transgenic animals or by gene transfer to cardiac myocytes. Increased expression of β-AR subtypes leads to increases in "basal" levels of cAMP, leftward shift of agonist concentration-response curves, and only minimal (if any) increase in maximal response to agonist activation (Milano et al., 1994; Zolk et al., 1998), the predicted result if expression of the effector AC determines maximal response.

Although the concepts related to receptors and receptor agonists and antagonists are well known in pharmacology, the impact of changing G protein or effector concentration has not been as carefully considered. Given the large stoichiometric excess of G proteins, we believe that attempts to manipulate their level may have only minimal impact on the usual types of cell signaling, but may considerably perturb cells via less "traditional" mechanisms. For example, transgenic animals that overexpress $G_{s\alpha}$ in the heart have a very small increase in ability to generate cAMP but develop decreased cardiac function, perhaps because of changes in Ca^{2+} dynamics resulting from G_s interaction with molecules other than AC (Lader et al., 1998). On the other hand, if only a portion of a cell's content of a G protein is appropriately compartmentalized with cognate receptors and effectors, adding to or subtracting from this specific population of G protein might be expected to have a substantial impact on signaling. In addition, if particular combinations of α-, β-, and γ-subunits link to different receptors and effectors (Hildebrandt, 1997), then manipulating the levels of specific G protein subunits might achieve selectivity in the regulation of responses to endogenous agonists.

Manipulation of expression of effector molecules would be expected to have a major impact on efficacy. There are a number of disease entities in which such an effect might be desirable. Three examples related to β-AR-mediated cAMP formation via ACs are: 1) asthma, wherein one might enhance cAMP formation in airway epithelial and smooth muscle cells; 2) cystic fibrosis, in which increased cAMP formation might enhance expression and function of the cystic fibrosis transmembrane regulator; and 3) congestive heart failure, for which enhanced responsiveness of AC might improve cardiac metabolism and contractility (Post et al., 1999). Limited data are available, but recent biochemical and functional findings related to congestive heart failure indicate that transgenic overexpression of AC6 has a beneficial or protective effect (Roth et al., 1999). Unlike effects studied in mice overexpressing β-AR or G_s, the effects of AC6 overexpression appear long-lived and without significant side effects or changes in basal cAMP formation, perhaps because of the tight regulation of AC activity even when overexpressed. Other settings may provide additional opportunities in which manipulating the expression of AC or other effectors could have therapeutic benefit. Both the stoichiometric relationship and potential compartmentation of components should be considered in particular cells and tissues in developing new gene therapy approaches targeted to GPCR signaling.

Genetic polymorphisms in signaling molecules are likely to prove of considerable importance in pharmacology. Recent data have emphasized coding polymorphisms in several dif-

ferent GPCRs, some of which alter the ability of the receptors to activate signaling pathways, whereas others have changes that perturb ability of the receptors to be desensitized (e.g., Büscher et al., 1999; Liggett, 1999). Other recent data have suggested that G protein subunits may have polymorphisms in coding or noncoding regions that may be linked to alterations in disease susceptibility or response to pharmacological agents (Siffert et al., 1998; Jia et al., 1999). To date, essentially nothing is known about polymorphisms in effector molecules. Polymorphisms in molecules involved in GPCR signaling that impart alterations in function or expression could have profound effects if that component is key for determining the potency or efficacy of the response. The ideas related to the critical role of effector molecules in determining maximal responses to agonists presented above suggest that studies designed to identify and characterize such polymorphisms should produce useful results. We hypothesize that polymorphic alterations changing expression or function of effectors will have an important impact on signaling and response in cells and will effect the use of therapeutic agents.

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