0026-895X/93/010245-12$03.00/0

MOLECULAR PHARMACOLOGY, 43:245-256

$\beta\gamma$ Subunits of Guanine Nucleotide-Binding Proteins and Regulation of Spontaneous Receptor Activity: Thermodynamic Model for the Interaction between Receptors and Guanine Nucleotide-Binding Protein Subunits

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Received April 23, 1992; Accepted October 5, 1992

SUMMARY

We used a thermodynamic model to examine the interactions between receptor, guanine nucleotide-binding protein (G protein), and their ligands. The model describes the interactions as multiple equilibria occurring between three distinct protein species (receptor, G_α subunit, and $G_{\beta\gamma}$ complex) and two small ligands, i.e., agonist (which interacts with receptor) and guanine nucleotide (which binds to G_α). The equilibrium distribution of free and complexed species is determined by the total concentration of the components, the affinities that govern the bimolecular reactions, and the allosteric interactions that ligands exert on each other when they are simultaneously bound to the same species. These allosteric factors are given in terms of free energy coupling. The model explains a number of experimental observations, as follows. (i) Both GTP and GDP can reduce agonist affinity, whereas the agonist enhances the net binding of GTP and diminishes that of GDP. (ii) $G_{\beta\gamma}$ is more effective in reducing agonist-independent than agonist-dependent receptor activity. (iii) Removal of guanine nucleotides increases the ratio between agonist-independent and -dependent activation of G protein. The model leads to a number of interesting predictions. (i) Not only G_α but also $G_{\beta\gamma}$ has effects on hormone binding. (ii) As long as the distribution of protein species is $[G_{\beta\gamma}] > [G_\alpha] > [\text{receptor}]$ (as often observed in the cell membrane), small changes in the concentration of $G_{\beta\gamma}$ do not alter the overall response induced by agonist. (iii) Agonist activity examined at low concentrations of guanine nucleotide is inevitably different from that observed at high concentrations, typical of intact systems. (iv) Differences in potencies and maximal effects for various guanine nucleotide analogues may reflect differences in their coupling constants that are experimentally measurable. The present model suggests several experimentally testable hypotheses that could be important in elucidating the activation mechanism and regulatory flexibility of G protein-dependent transduction systems.

G protein heterotrimers play a critical role in transferring signals from membrane receptors (R) to intracellular effectors (1). The process by which a hormone (H) induces activation of the G protein is often described in a stepwise cyclic fashion. (i) Agonist binds to the receptor to form a low affinity complex (HR). (ii) Occupied receptors bind to the G protein heterotrimer ($G_{\alpha\beta\gamma}$). (iii) GDP dissociates from and GTP associates with a common site on the G_α subunit of the G protein (nucleotide exchange). (iv) $G_{\beta\gamma}$ complexes dissociate from the G_α subunit. The free form of the GTP-bound α subunit, considered by many to be the active species, interacts with second messenger-generating effectors such as enzymes or ion channels (2–4). An inactivation phase follows and involves GTP hydrolysis, which restores the GDP-bound inactive species, reassociation of the heterotrimer, and dissociation of the receptor. Kinetic studies on purified G proteins in reconstituted system have clarified that the limiting step in the process of activation is nucleotide exchange rather than GTP hydrolysis (3).

Such a view of the G protein activation cycle is a powerful tool for intuitive descriptions of events, but it does not permit the quantitative interpretation of receptor-mediated biological responses, particularly when these are measured in nearly steady state experiments, as most often is the case. Further, the following three questions arising from the phenomenology of receptor-mediated activation of G protein cannot be easily explained on an intuitive basis. (i) One question regards the sequence of formation of the complexed species and their life spans. Many investigators believe that receptor-mediated acti-

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ABBREVIATIONS: G protein, guanine nucleotide-binding protein; Gpp(NH)p, guanosine 5′-(β,γ-imido)triphosphate; GTPγS, guanosine-5′-*O*-(γ-thio)triphosphate.

vation of G protein is a catalytic process; each hormone-receptor complex can activate (and thus induce to dissociate) several molecules of G proteins within the time-frame of its existence (2, 3, 5). This implies that HR must dissociate from G_α-GTP at a faster rate than it does from $G_{\alpha\beta\gamma}$-GDP. Because GTP tends to destabilize whereas GDP tends to stabilize the heterotrimeric $G_{\alpha\beta\gamma}$, one would expect to see triphosphate and diphosphate guanine nucleotides exerting opposite effects on the apparent affinity of the agonist. Instead, experimental evidence shows otherwise; every nucleotide studied to date invariably reduces agonist binding (6, 7). (ii) A second question regards the role of the agonist in the process of signal transmission. HR must have a higher probability of interacting with the G protein than does R in order to explain hormone-induced activation. As indicated by detergent solubilization experiments (8, 9), agonist-bound receptor-G protein complexes are more stable than unoccupied receptor-G protein complexes, suggesting that hormone binding reduces the rate of dissociation of the receptor from the G protein. However, a catalytic mechanism of activation implies that receptors are most efficient when they dissociate rapidly from the transducer and thus can activate a larger population of G proteins. How can hormone binding confer both increased stability and catalytic efficiency to the interaction of receptor with the G protein? (iii) A third question regards the effects of $\beta\gamma$ subunits on nucleotide exchange and hormone-mediated activation. The sequential nature of the activation reaction would predict that $G_{\beta\gamma}$, by stabilizing the GDP-bound heterotrimeric complex, should act as an inhibitor of both processes. Yet, available experimental evidence shows the opposite; increasing the concentration of $G_{\beta\gamma}$ subunits maximizes agonist-induced responses (10–12), and the presence of $G_{\beta\gamma}$ is essential for the observation of nucleotide exchange (13).

A quantitative model should be able to address explicitly the existence of allosteric effects linking the binding processes of the protein species involved. Such allosteric effects can be formulated in the framework of thermodynamic equilibrium as illustrated by Weber (14). A quantitative model of this kind (the ternary complex model) was proposed by DeLean *et al.* (15) to explain the binding properties of β-adrenoceptors and was then extended to other G protein-coupled receptor systems (16–18). The ternary complex model (a minimal representation of the G protein-receptor system) assumes that receptor interacts with hormone and G protein, as predicted by their respective equilibrium constants, and that hormone and G protein exert reciprocal effects on the binding of each other, as imposed by the law of conservation of standard free energy. Using this model, we have recently shown that drug efficacy can be expressed in thermodynamic terms (19).

The ternary complex model, however, is clearly incomplete, because it treats the heterotrimeric G protein as a single component and cannot explicitly address the contributions of guanine nucleotides and the association and dissociation of G protein subunits. Recent extension of the ternary complex model has been proposed by Ehlert and Rathbun (20) and by Mackay (21), who used a steady state treatment rather than an approach based on the assumption of equilibrium. However, these extended versions of the model, although including guanine nucleotide binding to G protein, still fail to address the dissociation of the two functional subunits of the G protein and the allosteric effects that they exert on each other.

In the present study, we used an equilibrium thermodynamic model in order to examine in detail the interactions between all the species involved. The steady state interactions were considered as multiple equilibria occurring between three distinct protein species (receptor and G_α and $G_{\beta\gamma}$ subunits of G protein) and two small ligands, i.e., agonist (which interacts with receptor) and nucleotide (GTP and/or GDP, which bind to and compete for the same binding site on G_α). As shown here, this model can explain a number of experimental observations and constitutes a potential tool to help one to understand the complex interactions that take place in receptor-G protein-mediated signal transduction.

Methods

Model. We shall consider the following components: (i) one trivalent species, G_α (A), that interacts with receptor, nucleotide, and $G_{\beta\gamma}$; (ii) one divalent species, receptor (R), that binds hormone and A; and (iii) two monovalent species, hormone (H) and nucleotide (N), that react with R and A, respectively. No direct interactions occur between B and R, R and N, N and B, or H and A or B. A multiple equilibria scheme is shown in Fig. 1. As explained in the next section, we excluded the interactions leading to a quinternary complex (HRANB). The concentrations of free and complexed species depend on four unconditional equilibrium constants (J, K, L, and M) and seven "coupling factors" (α, β, γ, δ, ϕ, ι, and π). These factors and the unconditional equilibrium constants are used to construct 14 conditional equilibrium constants. The coupling factors indicate the allosteric effects that ligands exert on each other when they are simultaneously bound to the same species. The unconditional constants describe the following equilibria:

$$\left(J(\mathrm{N}) = \frac{[\mathrm{NA}]}{[\mathrm{N}][\mathrm{A}]}\right) \mathrm{N} + \mathrm{A} \leftrightarrow \mathrm{NA} \tag{1a}$$

$$\left(K(\mathrm{H}) = \frac{[\mathrm{HR}]}{[\mathrm{H}][\mathrm{R}]}\right) \mathrm{H} + \mathrm{R} \leftrightarrow \mathrm{HR} \tag{1b}$$

$$\left(L(\mathrm{B}) = \frac{[\mathrm{BA}]}{[\mathrm{B}][\mathrm{A}]}\right) \mathrm{B} + \mathrm{A} \leftrightarrow \mathrm{BA} \tag{1c}$$

$$\left(M(\mathrm{R}) = \frac{[\mathrm{RA}]}{[\mathrm{R}][\mathrm{A}]}\right) \mathrm{R} + \mathrm{A} \leftrightarrow \mathrm{RA} \tag{1d}$$

Weber (14) has shown that when two different ligands, X and Y, are bound to a protein P, their free energies of binding are mutually dependent upon the energy coupling ΔF_{xy}:

$$\Delta F_{xy} = \Delta F(X) - \Delta F(X \mid Y) = \Delta F(Y) - \Delta F(Y \mid X)$$

where $\Delta F(\mathrm{X})$ and $\Delta F(\mathrm{Y})$ are free energies of binding of X and Y in the absence of the other ligand and $\Delta F(\mathrm{X}|\mathrm{Y})$ and $\Delta F(\mathrm{Y}|\mathrm{X})$ are the conditional free energies of binding of X to YP and of Y to XP, respectively. Thus, a formal definition and a physical interpretation for a coupling factor, a, can be given as follows:

$$a = \frac{K(X \mid Y)}{K(X)} = \frac{K(Y \mid X)}{K(Y)} = e^{(-\Delta F_{xy}/RT)}$$

where $K(\mathrm{X})$ and $K(\mathrm{Y})$ are unconditional and $K(\mathrm{X}|\mathrm{Y})$ and $K(\mathrm{Y}|\mathrm{X})$ are conditional equilibrium constants, respectively. R and T have the usual meaning. Note that the coupling factor depends exponentially on the free energy of coupling, where 1 implies no coupling ($\Delta F_{xy} = 0$) and values less or greater than unity imply positive (ΔF_{xy}) < 0) or negative ($\Delta F_{xy} > 0$) couplings. Using the notation given above, the following expressions define the coupling factors used in the present model:

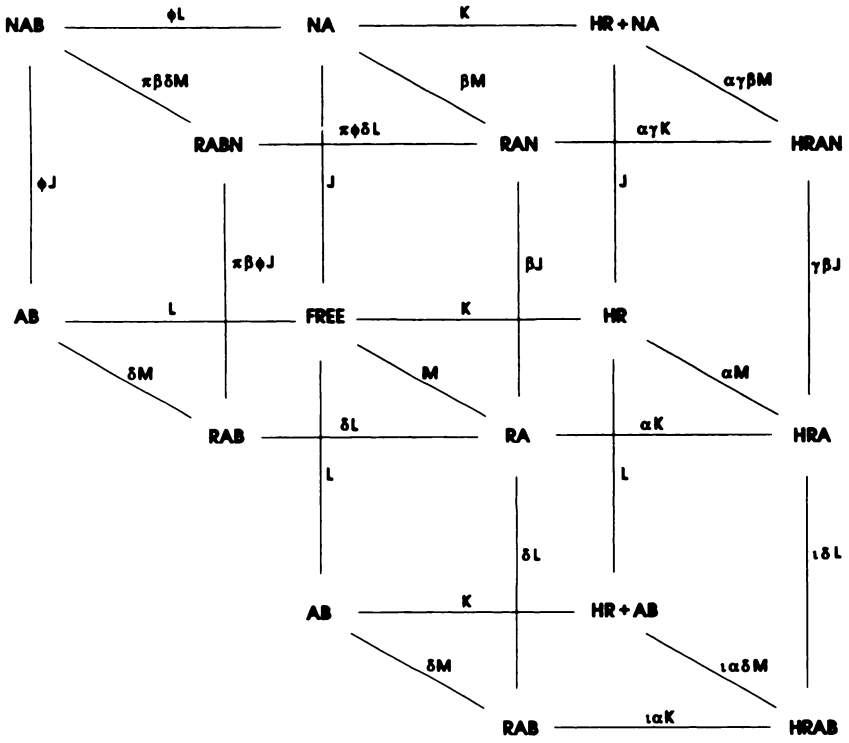

Fig. 1. Equilibrium scheme for the interaction between G_α (A), $G_{\beta\gamma}$ (B), receptor (R), receptor ligands (H), and nucleotides (N). *Lines*, reversible transitions between the states. *FREE*, state of (A + B + H + N + R). Nonreacting species at different branches are omitted for clarity. *J*, *K*, *L*, and *M* are unconditional affinities for the formation of NA, HR, AB, and RA complexes, respectively. α, β, γ, δ, ϕ, ι, and π are dimensionless coupling factors describing free energy coupling. Composite constants indicate the conditional affinities for the corresponding transitions. See "Methods" for detailed description of the coupling factors and conditional affinities.

$$\alpha = \frac{K(\mathrm{H}\,|\,\mathrm{A})}{K(\mathrm{H})} = \frac{M(\mathrm{A}\,|\,\mathrm{H})}{M(\mathrm{A})}$$ (H and A when bound to R)

$$\beta = \frac{M(\mathrm{R}\,|\,\mathrm{N})}{M(\mathrm{R})} = \frac{J(\mathrm{N}\,|\,\mathrm{R})}{J(\mathrm{N})}$$ (R and N when bound to A)

$$\delta = \frac{M(\mathrm{R}\,|\,\mathrm{B})}{M(\mathrm{R})} = \frac{L(\mathrm{B}\,|\,\mathrm{R})}{L(\mathrm{B})}$$ (R and B when bound to A)

$$\phi = \frac{J(\mathrm{N}\,|\,\mathrm{B})}{J(\mathrm{N})} = \frac{L(\mathrm{B}\,|\,\mathrm{N})}{L(\mathrm{B})}$$ (N and B when bound to A)

$$\gamma = \frac{K(\mathrm{H}\,|\,\mathrm{A},\,\mathrm{N})}{K(\mathrm{H}\,|\,\mathrm{A})} = \frac{J(\mathrm{N}\,|\,\mathrm{R},\,\mathrm{H})}{J(\mathrm{N}\,|\,\mathrm{R})}$$ (H and A when bound to RA)

$$\iota = \frac{K(\mathrm{H}\,|\,\mathrm{A},\,\mathrm{B})}{K(\mathrm{H}\,|\,\mathrm{A})} = \frac{L(\mathrm{B}\,|\,\mathrm{R},\,\mathrm{H})}{J(\mathrm{B}\,|\,\mathrm{R})}$$ (H and B when bound to RA)

$$\pi\beta = \frac{M(\mathrm{R}\,|\,\mathrm{N},\,\mathrm{B})}{M(\mathrm{R}\,|\,\mathrm{B})} = \frac{J(\mathrm{N}\,|\,\mathrm{R},\,\mathrm{B})}{J(\mathrm{N}\,|\,\mathrm{B})}$$ (R and N when bound to AB)

$$\pi\delta = \frac{M(\mathrm{R}\,|\,\mathrm{B},\,\mathrm{N})}{M(\mathrm{R}\,|\,\mathrm{N})} = \frac{L(\mathrm{B}\,|\,\mathrm{R},\,\mathrm{N})}{L(\mathrm{B}\,|\,\mathrm{N})}$$ (R and B when bound to AN)

$$\pi\phi = \frac{J(\mathrm{N}\,|\,\mathrm{B},\,\mathrm{R})}{J(\mathrm{N}\,|\,\mathrm{R})} = \frac{L(\mathrm{B}\,|\,\mathrm{N},\,\mathrm{R})}{L(\mathrm{B}\,|\,\mathrm{R})}$$ (N and B when bound to AR)

The allosteric effects that two ligands exert on each other when they are bound to the same species are given in parentheses. These interactions are schematically represented in Fig. 2. From the above definitions and Fig. 2, it is clear that the coupling factors given above can be divided into three categories, i.e., (i) first-order constants (α, β, δ, and ϕ), which describe an interaction between two binding sites and depend on only one condition; (ii) pseudo-first order constants (γ and ι), which describe an interaction between two binding sites and depend on two conditions; and (iii) second-order constants, which describe an interaction between three binding sites and depend on two conditions.

The pseudo-first order factors (γ and ι) describe the interactions between H and N and between H and B, respectively. Because the binding site of H is located on R, whereas those of N and B are located

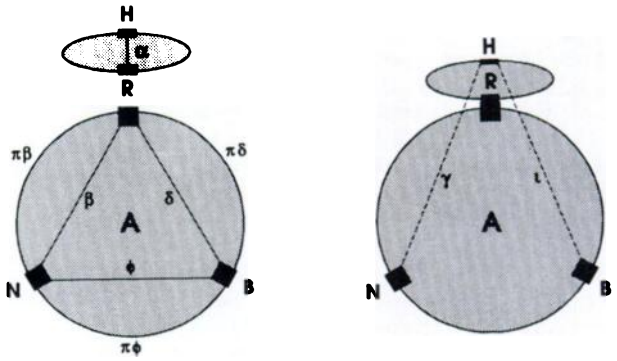

Fig. 2. Schematic representation of the interactions between the binding sites. H is the binding site located on the receptor protein (R) for receptor ligands. N and B are binding sites for nucleotides and $G_{\beta\gamma}$, respectively, located on G_α (A). Six interactions denoted by the coupling factors β, ϕ, δ, $\pi\beta$, $\pi\phi$, and $\pi\delta$ are possible between three binding site on A, whereas only one interaction (α) takes place on the receptor protein. Two additional interactions (γ and ι) become possible upon the binding of R to A (*right*).

on A, the binding of R to A is necessary for H to interact with N or B. This explains the existence of a second condition in the definition of these two constants. The last three constants describe triple interactions. Because all the binding sites involved in these interactions are on the same species, pairwise interactions are still possible in the absence of the third bound ligand. Binding of this third ligand constitutes the second condition. However, in the absence of the third bound ligand, pairwise interactions are given by β, δ, and ϕ instead of $\pi\beta$, $\pi\delta$, and $\pi\phi$. In other words, π (upon which $\pi\beta$, $\pi\delta$, and $\pi\phi$ are dependent) describes the effect imposed by any one of the three binding sites on the interaction between the other two. According to the conservation of free energy, if binding of N to A has an effect π on the coupling between B and R, then the binding of R or B to their binding sites should have the same effects on the coupling between B and N or between R and N sites, respectively. Using the above notation, π is given as

$$\pi = \frac{M(R \mid N, B) \cdot M(R)}{M(R \mid B) \cdot M(R \mid N)} = \frac{L(B \mid R, N) \cdot L(B)}{L(B \mid N) \cdot L(B \mid R)} = \frac{J(N \mid B, R) \cdot J(N)}{J(N \mid R) \cdot J(N \mid B)}$$

and indicates a triple allosteric effect when the three ligands are simultaneously bound to A. This effect is independent of the pairwise interactions of the three sites.

In order to simulate experimental results, we defined two variables, (i) hormone binding, which is the sum of the equilibrium concentrations of all hormone-bound species (HR + HRA + HRAN + HRAB); and (ii) G protein activation, which is taken as the total concentration of α subunit that is GTP bound but free of $G_{\beta\gamma}$ (AN_{GTP} + RAN_{GTP} + $HRAN_{GTP}$). The following simplifying assumptions were made. (i) There is no direct coupling between hormone binding and the binding of N or B, i.e., R and HR are potentially equivalent in activating the system, and the unique action of hormone is to stabilize the interaction between receptor and G. This implies $\gamma = \iota = 1$ and $\alpha > 1$. (ii) We assumed that there is an overall negative coupling between the binding sites on A and that the probability for the accumulation of the triply liganded A (RANB) is extremely low (i.e., $\pi \ll 1$). Therefore, we neglected the concentrations of RANB and HRANB (see assumption 1) in the calculations. Calculations for the equilibrium are given in the Appendix.

Determination of parameter ranges. To simulate experimental data, three types of input parameters must be defined, (i) the concentrations of the reacting species, H, R, N, A, and B; (ii) the magnitudes of unconditional association constants J, K, L, and M; and (iii) the magnitudes of coupling factors. Some of these values can be set based on experimentally determined values. For instance, the concentration of A was set in excess with respect to the concentration of R, H and N were the varied input parameters. Likewise, some of the association constants have been experimentally measured. We used an average value of 10^7 M^{-1} for K (i.e., the estimate of the low affinity form of agonist binding) and a similar value for J for both GTP and GDP (22, 23). The values of L^{-1} and M^{-1} were chosen to be close to that of [A]. The coupling factor α, which indicates the efficacy of H, was set either to unity (for an antagonist) or larger than 1 (for an agonist) (19).

To explore systematically the propriety of the values of the remaining parameters (β_{GTP}, β_{GDP}, ϕ_{GTP}, ϕ_{GDP}, γ_{GTP}, γ_{GDP}, δ, ι, and B) we used an automated, computer-assisted procedure; R, A, α, J, K, L, and M, were held constant while the remaining nine parameters were allowed to assume three different values. Each set of the 3^9 possible combinations was used to simulate (i) binding isotherms for the agonist in the presence and absence of saturating concentrations of nucleotide, and (ii) activation of G protein in the presence and absence of agonist, at saturating concentrations of nucleotides. The nucleotide-induced shift in binding isotherms of agonist, and the ratios between agonist-independent and -dependent activation, computed from these results, were stored in a database linked to the corresponding parameter combination. For some of the coupling factors the range of values was constrained according to experimental clues. (i) It is known that binding of R to A increases the dissociation of GDP and $G_{\beta\gamma}$ from G_α and that GTP exerts a negative heterotropic effect on the agonist binding (and vice versa) (6, 24–27). This implies that β_{GTP}, β_{GDP}, and δ should be <1. (ii) It is also known that $G_{\beta\gamma}$ subunit increases the binding of GDP for A (and vice versa), which implies that $\phi_{GDP} > 1$ (28–30). On the other hand, the association of GTP induces dissociation of G protein subunits (and vice versa), which means that ϕ_{GTP} is <1 (30–33). ι and γ were unconstrained and were given values equal to less than, or greater than 1. The concentration of B was either smaller than, greater than, or equal to that of A.

The database was "filtered" by discarding all the parameter values associated with solutions that did not satisfy the following criteria: (i) reduction of agonist apparent affinity by GDP or GTP and (ii) low spontaneous activity (<10%) of agonist-dependent stimulation at saturating concentrations of GTP and GDP. The selection of a particular combination from the resulting set was arbitrary. The parameter values used in the following simulations are given in Table 1.

TABLE 1
Values of the coupling factors used in the present simulations

Constant	Meaning	Possible range according to experimental data	Values used
K	Hormone (H) binding to receptor (R)	10^6–10^9 M^{-1}	10^7 M^{-1}
J	Nucleotide (N) binding to α subunit (A)	10^7–10^8 M^{-1}	10^7 M^{-1}
M	Receptor (R) binding to α subunit (A)	?	$1/M \sim$ [A]
L	$\beta\gamma$ (B) binding to α subunit (A)	?	$1/L \sim$ [A]
α	Coupling between H and A on R	$\alpha_{agonist}$, >1	100–1000
		$\alpha_{antagonist}$, 1	1
		$\alpha_{negative\ antagonist}$, <1	Not used
β	Coupling between R and N on A	β_{GTP}, <1	0.5
		β_{GDP}, <1	0.01
ϕ	Coupling between N and B on A	ϕ_{GTP}, <1	0.9
		ϕ_{GDP}, >1	100
γ	Coupling between H and N on RA	γ_{GTP}, ≤1 (?)	1
		γ_{GDP}, ≤1 (?)	1
δ	Coupling between B and R on A	<1 (?)	0.1
ι	Coupling between H and B on RA	?	1
π	Coupling between R, B, and N on A	?	0

Results

Effect of guanine nucleotides on the binding isotherms of the agonist and on agonist-induced activation. The present model predicts that both agonist binding to the receptor and agonist-induced activation of G protein (i.e., binding of GTP to G_α and dissociation of subunits) are dependent on the concentration of guanine nucleotides (Fig. 3). To simulate a typical receptor binding experiment, we considered a situation in which the binding of a radiolabeled ligand, an antagonist ($\alpha = 1$) present at "tracer" concentration, is studied in the presence of increasing concentrations of an agonist ($\alpha = 500$) and GTP. Antagonist binding is not sensitive to GTP; in contrast, the half-saturation point for the competition isotherms of the agonist is progressively shifted towards higher concentrations as the concentration of GTP is increased (Fig. 3A).

The concentration-response curves for agonist-induced activation of G protein are also dramatically affected by GTP. Below certain concentrations of GTP the agonist is unable to produce activation, and the maximal effect induced by the agonist increases as the concentration of GTP is increased (Fig. 3B). GTP produces very low activation in the absence of agonist. Thus, the model predicts both the GTP dependence of hormonal effect and the synergism between hormone and guanine nucleotides that have been documented in many experimental studies on a variety of G protein-linked receptors (34–36).

Negative heterotropic effect of guanine nucleotides on agonist binding and agonist-induced nucleotide exchange. Any model describing the interaction of a receptor with a G protein must envision that agonist binding to the receptor results in GTP/GDP exchange on the α subunit. At the same time either nucleotide, upon occupation of the α subunit, should cause dissociation of the receptor from G_α and convert it to a lower affinity agonist binding state. Fig. 4 shows that the present model fulfills both these experimental obser-

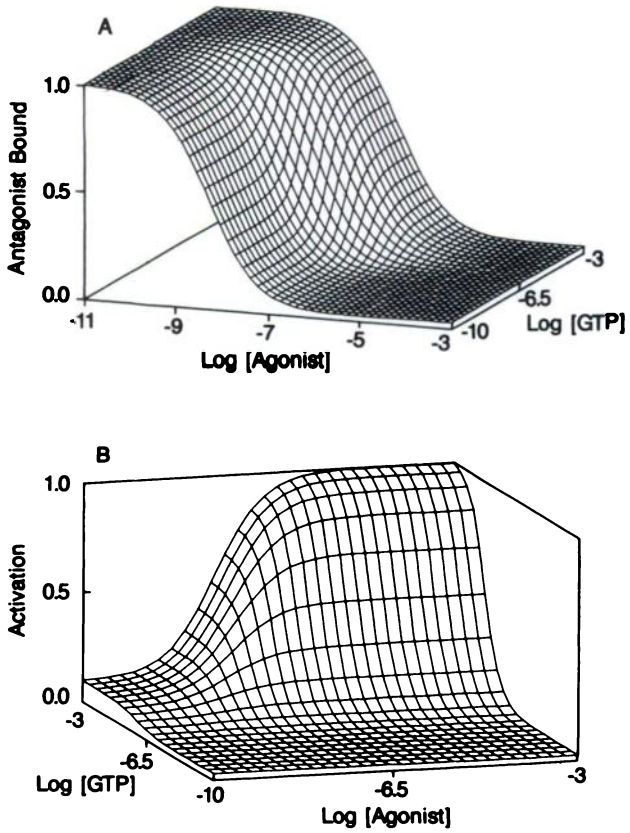

Fig. 3. Effect of GTP on the binding of an agonist (A) and on the dissociation of $G_{\beta\gamma}$ at different concentrations of agonist (B). A, Agonist binding (α = 500) is simulated as competition for a tracer antagonist (α = 1) at different concentrations of GTP. [GDP] is 10 nM in both panels.

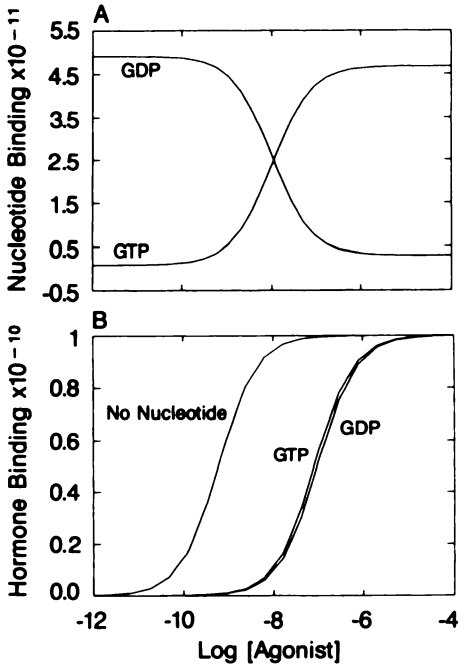

Fig. 4. Heterotropic interactions between agonist, GTP, and GDP. A, Effect of agonist on the binding of GDP and GTP. [GTP] = 1 mM, [GDP] = 10 μM. Binding is shown as deviation from the base line when [agonist] = 0. B, Binding of an agonist in the absence and presence of 10 mM GTP or GDP. Concentrations of receptor, G_α, and $G_{\beta\gamma}$ are 100 pM, 1 nM, and 10 nM, respectively, in both panels. Other parameters are as in Table 1.

vations. We assumed that the two nucleotides had identical affinities for G_α (i.e., $J_{GTP} = J_{GDP}$) and that both were negatively linked to the binding of R to G_α (β_{GTP} and β_{GDP} <1). They differed, however, in their effects on the binding of $G_{\beta\gamma}$ to G_α (i.e., $\phi_{GTP} < 1$ and $\phi_{GDP} > 1$). As the concentration of agonist is increased in the presence of a mixture of the two nucleotides, the binding of GTP is enhanced while that of GDP is decreased (Fig. 4A). On the other hand, the binding isotherm for the agonist is shifted rightward in the presence of either GTP or GDP (Fig. 4B). This asymmetry between nucleotide effects on agonist binding to R (which are qualitatively similar for any nucleotide) and agonist effect on nucleotide binding to A (which is negative for GDP and positive for GTP) depends entirely on the presence of $G_{\beta\gamma}$. When the concentration of $G_{\beta\gamma}$ was set very small with respect to G_α agonist-induced nucleotide exchange could no longer be observed (simulations not shown).

Different properties of guanine nucleotides and effects of guanine nucleotide-dependent coupling factors. Natural and synthetic guanine nucleotide analogues display wide differences in potencies and maximal effects in activating distinct types of G proteins or reducing the apparent affinity of the agonist (6, 37, 38). These differences are usually attributed to different affinities for distinct types of α subunits and to the ability to resist hydrolysis catalyzed by G_α. The present model, however, predicts that differences between nucleotides also depend on the magnitude of the nucleotide-dependent coupling factors β and ϕ, which indicate in which direction and to what extent the binding of any nucleotide to G_α is modified by the binding of receptor and/or $G_{\beta\gamma}$ (or vice versa), respectively. To explore the role of these allosteric factors we performed a variety of simulations in which nucleotide-dependent parameters (β, ϕ, and the affinity J) were systematically varied and the consequence of these variations was examined as ability of guanine nucleotide to reduce agonist binding and support agonist-induced dissociation of $\beta\gamma$ subunits.

Changes in the affinity constant J affected only the effective concentration range of the nucleotide, as intuition would predict (data not shown). In contrast, variations of the parameters β and ϕ affected not only the potency of nucleotides but also the qualitative character of the effect that agonist and nucleotide exert on each other. The results of these simulations are summarized in Fig. 5. As long as both β and ϕ are <1, nucleotide shifts the competition curves of the agonist rightward. The extent of the shift, however, depends on the combination of the values of the two coupling constants (Fig. 5A). The corresponding effects on the ability of the nucleotide to alter the ratio between agonist-dependent and independent activation are shown in Fig. 5B. It is interesting that the values of ϕ and β can dramatically influence the fraction of G protein activation due to the agonist and the extent of the shift in apparent agonist affinity produced by guanine nucleotide. In general, small values of ϕ and β are associated with the highest tendency of the nucleotide to activate the system in the absence of agonist (Fig. 5B), whereas large values of ϕ and small values of β cause the largest nucleotide effect on agonist binding (Fig. 5A). Thus, different combinations of the values of ϕ and β may account for the extensive differences in the effects of guanine nucleotides on different G protein systems reported in the literature.

Concentration of $\beta\gamma$ subunits and agonist-independent receptor activity. The concentration of $G_{\beta\gamma}$ influences both

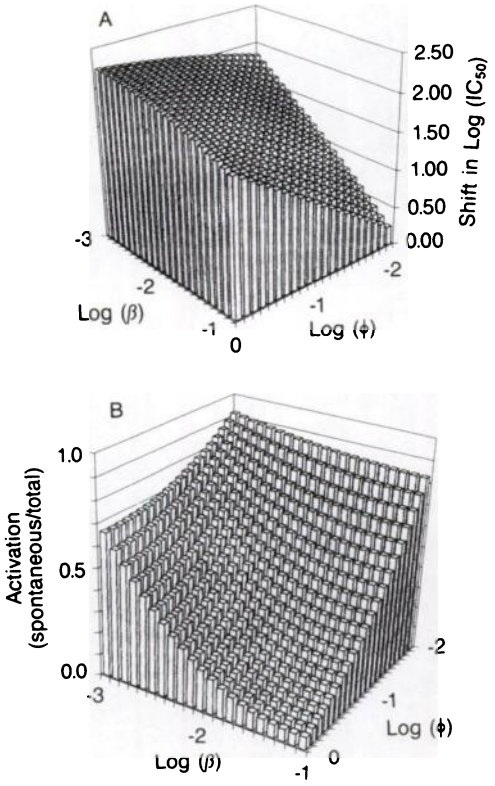

Fig. 5. Effect of different values of β and ϕ for GTP-like nucleotides on the ability of the nucleotide to induce a shift in the agonist binding isotherms (A) and to induce G_α dissociation in the absence of agonist (B). A, Nucleotide-induced shift in binding affinity is the difference between log(IC_{50}) obtained in the absence and presence of 10 mM nucleotide. [GDP] = 100 pM. B, Agonist-independent (spontaneous) activity is the fraction of maximal response induced at saturating concentrations of an agonist. Concentrations of GTP-like nucleotide and GDP are 1 mM and 10 μM, respectively.

agonist-dependent and agonist-independent dissociation of G protein subunits. However, $G_{\beta\gamma}$ suppresses spontaneous activity much more effectively than agonist-induced activity (Fig. 6A). The activation observed in the absence of ligand is inhibited by $G_{\beta\gamma}$ at much lower concentrations than those necessary to inhibit the activation induced at saturating concentrations of agonist. Such an effect of $G_{\beta\gamma}$ has been observed in several experimental studies (10–12). A consequence of this phenomenon is that the net effect due to the agonist (activation in the presence of agonist minus that in its absence) reaches a maximum at a certain optimal concentration of $G_{\beta\gamma}$ beyond which further increases in [$G_{\beta\gamma}$] result in reduction (Fig. 6B). Therefore, $G_{\beta\gamma}$ effectively maximizes signals generated upon binding of the agonist and minimizes those resulting from the spontaneous interaction between "empty" receptors and/or nucleotide with G_α. This "buffering" ability of $G_{\beta\gamma}$ depends on the concentration of guanine nucleotides. At high concentrations of a mixture of GTP and GDP (1000:1) the maximum is broader and occurs at higher [$G_{\beta\gamma}$] than in the presence of low concentrations of guanine nucleotides (compare Fig. 6, C and D).

Although these results predict and explain the ability of $G_{\beta\gamma}$ to suppress spontaneous activation and optimize agonist-mediated activity, they do not tell whether this role of $G_{\beta\gamma}$ is purely casual or necessary, i.e., whether it is the result of a fortuitous combination of values chosen for the parameters or is determined by the direction of the allosteric effects linking the interactions between the various species. To clarify this point we have addressed the following question: what is the distribution of spontaneous and hormone-stimulated activities at any concentration of $G_{\beta\gamma}$? The values of [GTP], [GDP], [$G_{\beta\gamma}$], β, ϕ, γ, and δ were allowed to vary randomly within wide ranges (see legend to Fig. 7), and each set of resulting parameter combinations (500,000 in total) was used to compute the corresponding level of both spontaneous and total minus spontaneous activities. A subset of the results is shown in Fig. 7, where both spontaneous activation (Fig. 7A) and agonist-induced net activation (Fig. 7B) are plotted as a function of the ratio $G_{\beta\gamma}/G_\alpha$. It is clear that as long as the concentration of G_α exceeds that of $G_{\beta\gamma}$ spontaneous activity will be relatively high, regardless of the value of coupling factors and the concentration of guanine nucleotides. Only when $G_{\beta\gamma}$ is greater than G_α is it possible for induced activation to be larger than spontaneous activation. Thus, the relative concentrations of $\beta\gamma$ and α subunits, but not the values of the allosteric constants or the concentration of guanine nucleotides, are the critical factors that ensure agonist dependence of activation.

Potencies of guanine nucleotides and synergism with $G_{\beta\gamma}$. If the concentration of $\beta\gamma$ subunit exceeds that of α subunits, the proportion of agonist-dependent activation depends on the concentration of guanine nucleotides. Parallel reductions in the concentration of GTP and GDP increases the ratio between spontaneous and agonist-dependent activity (Fig. 8). Interestingly, the concentrations of guanine nucleotide that maximize the ratio between agonist-dependent and agonist-independent activation is about 4 orders of magnitudes greater than the equilibrium dissociation constant of GTP for G_α ($1/J$ = 100 nM in this example). Thus, the ability of guanine nucleotides to support ligand-mediated activation requires concentrations much higher than those sufficient to saturate the binding site on α subunits, in the absence of the other components.

The same parameters of Fig. 8 were used to generate concentration-response curves for guanine nucleotide-mediated reductions of agonist binding. The apparent IC_{50} of these curves was much closer to the dissociation constant of the nucleotide (data not shown). Thus, even with the same receptor-G protein system, a given guanine nucleotide can display remarkable differences in potency depending on whether its effect is studied as regulation of agonist affinity or facilitation of agonist-mediated activation of the effector system.

The synergism between concentration of guanine nucleotides and $G_{\beta\gamma}$ is also evident when their effects on agonist binding to the receptor are examined (Fig. 9). The binding isotherms of the agonist are shifted to the right as the concentration of $G_{\beta\gamma}$ is increased, and this effect is much more pronounced at high (Fig. 9A) than at low (Fig. 9B) concentrations of guanine nucleotides.

Discussion

Usefulness of the Model

The model presented here provides for the first time a complete description of the multiple equilibria for the interactions of ligand, receptor, G protein subunits, and different nucleotides. The present model enables us to explain a number of poorly understood aspects of receptor-mediated signal transduction. These include (i) qualitative and quantitative differences in the allosteric effects of guanine nucleotide analogues, (ii) dissimilarity between agonist and guanine nucleotide in

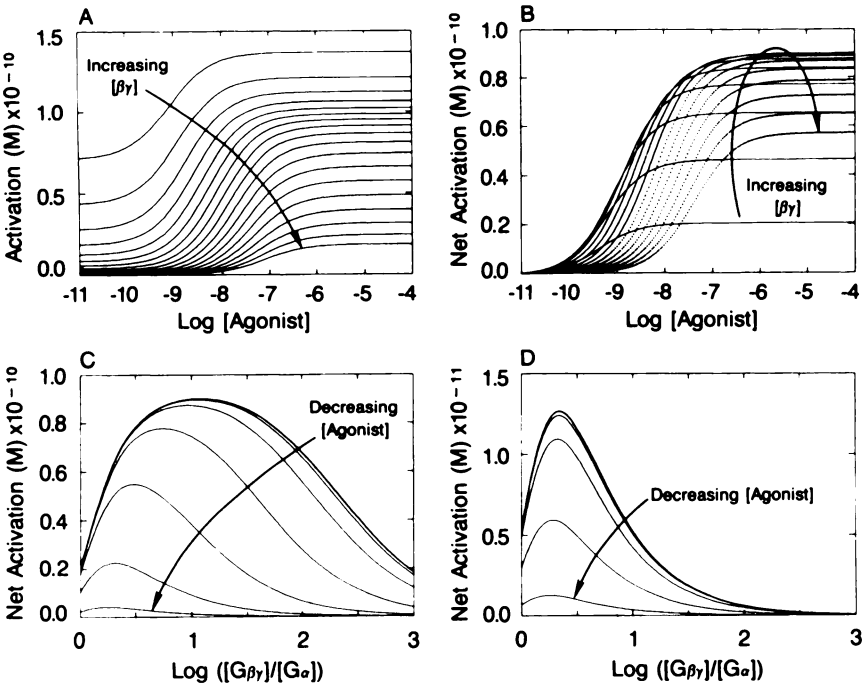

Fig. 6. Effect of [$G_{\beta\gamma}$] on the dissociation of G_{α}. A, Total activation is plotted against log[total concentration of agonist] at different concentrations of $G_{\beta\gamma}$. log[$G_{\beta\gamma}$] ranges from −8.7 (*uppermost curve*) to −6 (*lowermost curve*) in logarithmically equidistant steps. At the lowest concentration of $G_{\beta\gamma}$, agonist-independent activation is about 50% of the maximum, whereas at the intermediate concentrations agonist-independent activation is suppressed and agonist-induced activation is increased. B, Activation is plotted after subtraction of agonist-independent activity. log[$G_{\beta\gamma}$] ranges from −9 to −6.8 in logarithmically equidistant steps. *Solid curves*, range of concentrations of $G_{\beta\gamma}$ where maximum net activation increases as [$G_{\beta\gamma}$] increases (from −9 to −7.9); *dotted curves*, region where maximum net activation decreases (from −8 to −6.8). C and D, Net activation at various concentrations of agonist is plotted against log[total $G_{\beta\gamma}$] at high concentrations of guanine nucleotides ([GTP] = 1 mM, [GDP] = 10 μM) (C) and low concentrations of guanine nucleotides ([GTP] = 1 mM, [GDP] = 10 nM) (D).

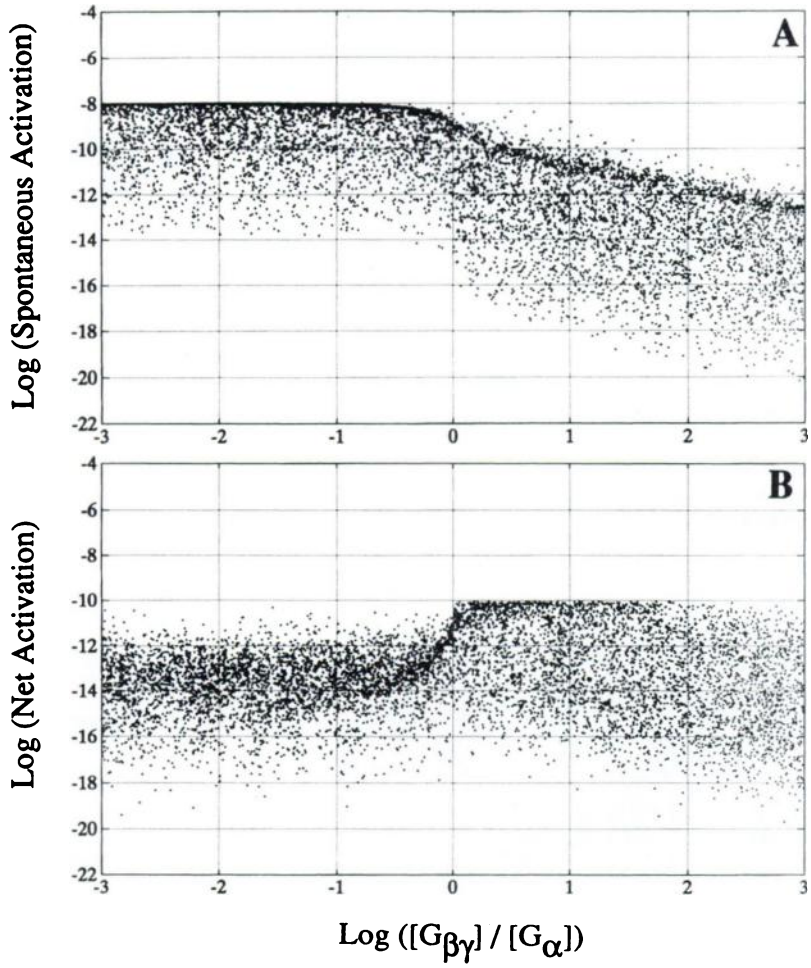

Fig. 7. Effect of [$G_{\beta\gamma}$] on agonist-independent and -dependent activities. Spontaneous activation (A) and total minus spontaneous activation (B) are plotted against the concentration of $G_{\beta\gamma}$ relative to that of G_{α}. Each *point* represents a response value calculated from a combination of randomly distributed values of [total $G_{\beta\gamma}$], β_{GTP}, β_{GDP}, δ, ϕ_{GTP}, ϕ_{GDP}, [GDP], and [GTP]. Thus, the two plots represent the dependence on [$G_{\beta\gamma}$]/[G_{α}] of the conditional distributions of both spontaneous and hormone-dependent activation. Each of the variables is log-uniformly and independently distributed in the following ranges: 10^{-11}-10^{-5} for [$G_{\beta\gamma}$], 0.001–1 for β_{GTP}, β_{GDP}, δ, and ϕ_{GTP}, 1–100 for ϕ_{GDP}, and 10^{-9}-10^{-3} for [GTP] and [GDP]. Each graph consisted originally of 0.5 × 10^{6} points, a 1:100 random sample of which is shown in the figure. The plots are, nonetheless, a faithful representation of the original displays. Note that the distribution of the responses at any given value of [$G_{\beta\gamma}$] shifts towards the region where agonist induction is favored as the ratio [$G_{\beta\gamma}$]/[G_{α}] increases. These data indicate that $G_{\beta\gamma}$ can be viewed as the "principal component" in the conditional distribution of agonist-dependent responses.

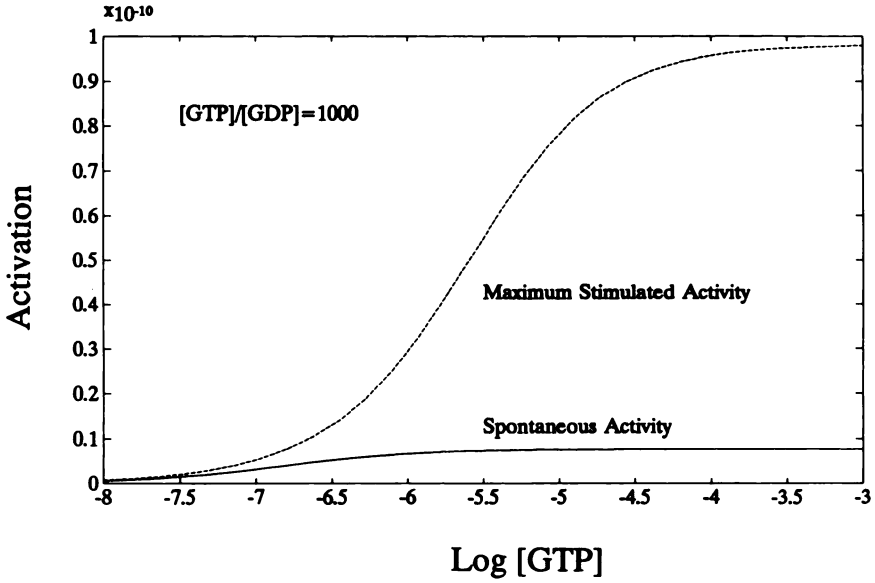

Fig. 8. Dependence of agonist-induced and spontaneous activities on the concentrations of guanine nucleotides. The total concentration of GTP is given on the *abscissa*. [GDP] = 1/1000th of that of GTP.

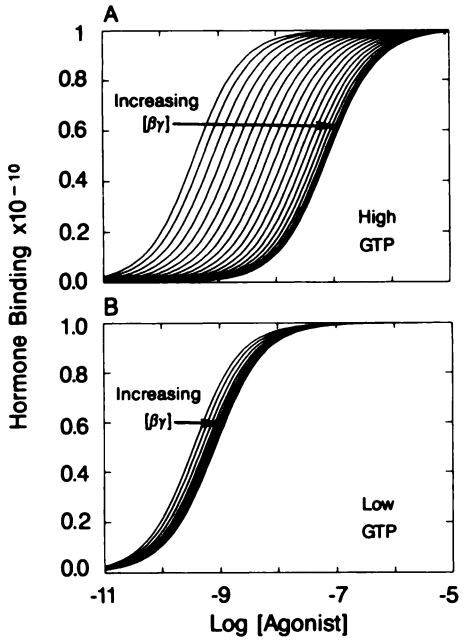

Fig. 9. Agonist-binding curves at various concentrations of $G_{\beta\gamma}$ and guanine nucleotides. A, [GTP] = 1 mM, [GDP] = 1 μM. B, [GTP] = 100 nM, [GDP] = 0.1 nM. Different curves represent different concentrations of $G_{\beta\gamma}$ in both panels. [$G_{\beta\gamma}$] ranges from 1 nM to 1 mM in logarithmically equidistant steps.

affecting the binding of each other and (iii) the ability of $G_{\beta\gamma}$ to optimize agonist-induced activation of G_α.

Allosteric effect of guanine nucleotides. The binding of guanine nucleotides to G_α influences the binding of receptor and that of $G_{\beta\gamma}$. Therefore, the overall effect of a guanine nucleotide depends not only on its affinity for G_α but also on the magnitudes and the signs of the free energy couplings that link the binding of nucleotides to that of the other ligands. In the absence of agonist, the ability of a nucleotide to activate the G protein increases as its ϕ value decreases, i.e., as its ability to dissociate $G_{\beta\gamma}$ increases. Thus, the smaller the value of ϕ the greater the tendency of the nucleotide to favor agonist-independent activation. This quality resembles the properties of some nonhydrolyzable analogues of GTP, such as GTPγS or Gpp(NH)p (37–41). An important implication of this result is that differences in the coupling factors of guanine nucleotides are sufficient to account for the qualitative differences between GTP and its analogues in activating distinct G protein systems, regardless of their resistance to hydrolysis. For instance, GTP might have larger ϕ and β values than GTPγS or Gpp(NH)p, which would explain why GTP displays a lesser ability to induce dissociation of $G_{\beta\gamma}$ from G_α than does GTPγS. A low intrinsic tendency of GTP to induce dissociation of the heterotrimer could be an important physiological control mechanism. Considering the high intracellular concentration of GTP and slow intrinsic catalytic rate of GTPase of G_α, it would be difficult to imagine how, under physiological conditions, constitutive activation of G protein could be effectively minimized otherwise.

Dissimilarity of the interactions between agonist and GTP/GDP. One central question addressed in this study is why the effects that agonist and guanine nucleotide exert on each other when interacting via receptor and G protein are not symmetrical; both GTP and GDP dissociate the agonist from the receptor, whereas the agonist increases the binding of GTP and decreases that of GDP. This apparent paradox finds a thermodynamically sound explanation if we consider the panel of energetic interactions that may exist when three ligands (receptor, guanine nucleotide, and $G_{\beta\gamma}$ subunit) simultaneously interact with three distinct sites of the same protein (G_α subunit). $G_{\beta\gamma}$ and both guanine nucleotides are negatively coupled with the binding of receptor to G_α. However, the coupling between binding of $G_{\beta\gamma}$ and guanine nucleotide is positive for GDP and negative for GTP. Hence, any type of guanine nucleotide will always induce dissociation of receptor from G_α thus favoring a low affinity state for agonist binding. In contrast, the effect of agonist-bound receptor on the binding of guanine nucleotides is critically determined by the membrane concentration of $G_{\beta\gamma}$ and by the type of mixture of guanine nucleotides. Agonist-induced binding of receptor to G_α facilitates the dissociation of $G_{\beta\gamma}$ from G_α because there is negative coupling between receptor and $G_{\beta\gamma}$. If both GTP and GDP are present simultaneously, as is typical under physiological conditions, the tendency of $G_{\beta\gamma}$ to dissociate from G_α facilitates

the dissociation of GDP and the association of GTP, because $G_{\beta\gamma}$ is coupled positively to the binding of GDP and negatively to that of GTP. If this effect of $G_{\beta\gamma}$ on guanine nucleotides is large enough to overcome the direct negative coupling between receptor and nucleotides, GTP and GDP will exchange on G_α upon agonist stimulation. Therefore, the model predicts that no nucleotide exchange can occur in the absence of $G_{\beta\gamma}$. Such a prediction is supported by experimental data obtained in a reconstituted system of muscarinic acetylcholine receptors and G_o (13).

Role of $G_{\beta\gamma}$. The individual roles that G_α and $G_{\beta\gamma}$ play in receptor-G protein systems remain one of the most controversial issues in signal transduction. Two conflicting views have been proposed; one is that the subunits, once dissociated from each other, are both capable of transmitting signals to effector molecules. Accordingly, receptor-induced dissociation of subunits would result in the simultaneous activation of two distinct transduction protein that spread the signal to distinct second messenger-generating processes (42–44). A second view is that G_α is the only subunit that transfers the stimulus to the effector molecule, whereas $G_{\beta\gamma}$ has only an accessory role. As proposed by Birnbaumer and collaborators (see Ref. 4 for review), $\beta\gamma$ subunits may serve as a buffer that prevents receptor-mediated activation of the system in the absence of ligand. Although the present theoretical study obviously cannot address the question of whether both G protein subunits are capable of interacting with effector systems, the model presented here provides a rational explanation for the ability of $G_{\beta\gamma}$ to suppress spontaneous activation. As shown here, the complex heterotropic interactions between three distinct binding sites on G_α imply that $G_{\beta\gamma}$ is able to suppress agonist-independent and nucleotide-dependent activation of G_α and thus maximize the net activation induced by the agonist. The optimum concentration of $G_{\beta\gamma}$ and the absolute value of the maximum depends on the concentration of nucleotide (see Fig. 6). At a constant concentration of $G_{\beta\gamma}$, a proportional increase of GTP and GDP will further reduce the ratio between spontaneous and stimulated activation (see Fig. 8). Thus, at optimal concentration of $G_{\beta\gamma}$ the "dynamic range" for a G protein to transduce the agonist-generated signal will be larger in the presence of high concentrations of nucleotides. Interestingly, the difference between "noise" and "signal" tends to approach its maximum as the concentration of nucleotide reaches the millimolar range, i.e., physiological intracellular concentrations. Thus, with the aid of $G_{\beta\gamma}$ both dynamic range and the signal to noise ratio of G protein-mediated signals are optimized for the intracellular environment. This provides a rational explanation for another question of signal transduction, i.e., why do guanine nucleotides have dissociation constants for G_α several orders of magnitudes lower than their intracellular concentrations? The present model suggests that such discrepancy is necessary to ensure the agonist dependency of the signals.

Experimental evidence in line with this role of $G_{\beta\gamma}$ has been presented. Itoh and Gilman (45) have shown that the GTPase activity of isolated $G_{s\alpha}$ is sharply reduced as the concentration of $\beta\gamma$ subunits exceeds that of G_α. For atrial muscarinic receptors, which can be coupled to potassium channel conductance via at least three distinct types of G_i (46), Birnbaumer and collaborators (11) have shown that $G_{\beta\gamma}$ inhibits agonist-independent channel activity much more efficiently than in the presence of saturating concentrations of carbachol. Similar observations have been made for purinergic receptor-mediated control of phospholipase C, which involves a G protein that is not a substrate of pertussis toxin (47), and for the β-adrenergic receptor and G_s-mediated activation of adenylate cyclase (10, 12). The finding that the total membrane concentration of $\beta\gamma$ subunits is higher than that of α subunits (39) also suggests that an excess of $G_{\beta\gamma}$ has functional relevance, as indicated by the present model.

Limitations of the Model

Although this model explains several aspects of receptor-mediated signaling via G proteins, it cannot explicitly address some nonequilibrium features of the process of activation, such as GTP hydrolysis at the guanine nucleotide binding site of G_α. However, such limitation should only minimally affect the conclusions drawn from simulations based on this model. Considerable experimental evidence suggests that nucleotide exchange but not the catalytic rate of GTP hydrolysis is the limiting step in receptor-mediated G protein activation (3). Thus, even if the model presented here were to apply rigorously only to guanine nucleotides that cannot be hydrolyzed by the α subunit [e.g., GTPγS and Gpp(NH)p], it can be generalized to any G protein-mediated response in the presence of any guanine nucleotide as long as nucleotide exchange and dissociation of α and $\beta\gamma$ subunits are examined as steady state measurements.

A second nonequilibrium feature that the model cannot address is the possibility that receptors activate G proteins catalytically, i.e., a single molecule of R can collide with several molecules of $G_{\alpha\beta\gamma}$ within the life span of its active conformation. Such a mechanism has been formulated in several "collision-coupling" models of hormone action (5, 48–51), and it is often implied in the interpretation of data of many experimental studies. Experimental evidence for a collision-coupling mechanism of activation has been provided for the rhodopsin-transducin system, where data suggest that a single photo-bleached rhodopsin can activate up to 100 molecules of transducin (2). By analogy with transducin, a similar mechanism has been proposed for several other G protein-linked receptors (3, 4), although in these cases experimental evidence is based on the determination of the apparent stoichiometry of the components obtained by equilibrium binding studies. No collision-coupling model, however, can satisfactorily explain the fact that in the membrane agonist-bound receptors and G protein can form stable intermediates that are dissociated by both GTP (which destabilizes $G_{\alpha\beta\gamma}$) and GDP (which stabilizes the heteotrimer), as observed in radioligand binding studies. To the best of our knowledge, the model presented here is the only one available to date that can describe the interaction of agonist and guanine nucleotides simultaneously and in both directions.

Experimental Verification

The model presented here offers several testable hypothesis, some of which correspond to experimental results already reported in the literature, as discussed above. Others suggest experiments that have not been yet performed, and what follows is a brief outline of those we find more interesting.

The first hypothesis is that the observed effects of guanine nucleotides on G protein-linked receptors depend on the concentration of $\beta\gamma$ subunits. This yields the following predictions. (i) Guanine nucleotide effects on agonist affinity should be altered when the concentration of $\beta\gamma$ subunits is changed. (ii)

Transfection of cells with a given type of G_α, if this results in a substantial change in the stoichiometric ratio with $G_{\beta\gamma}$, should increase spontaneous responses and reduce the net effect of the agonist. (iii) Permeabilization of cells and the consequent drop in the concentrations of guanine nucleotides should also result in an increased proportion of spontaneous activity. In general, the model predicts that receptor responses studied in intact cells (where the concentration of guanine nucleotide is high) should differ significantly from those studied in isolated preparations. This has implications not only for biochemical studies, where isolated membranes and intact cells have often been assumed to be interchangeable, but also for electrophysiological studies, where receptor responses are studied either in excised patches of membrane or in whole-cell and cell-attached configurations (52). Differences in expression levels of G protein subunits and, consequently, in $G_\alpha/G_{\beta\gamma}$ ratios among different tissues can change both binding characteristics and response properties of a given receptor. This factor is particularly relevant for studies in which receptors are expressed in foreign cells.

A second hypothesis is that the energetic terms β, δ, ϕ, and π, which describe the interactions between the binding sites on G_α control the responsiveness of the system even more than do the unconditional affinity constants for the pairwise interactions between each component. This would be particularly important for understanding the consequences of mutations on the functional properties of the protein species involved. Although envisioning the exact meaning of these parameters is complicated on both theoretical and intuitive bases, the actual measurement of their magnitude is experimentally accessible. In the case of a first-order constant, the ratio of apparent affinities measured in the absence and presence of saturating concentrations of the second ligand yields the corresponding coupling factor. For instance, the apparent dissociation constant (K_d) of a guanine nucleotide obtained from the binding isotherm of the nucleotide with pure G_α is a reasonable estimate of J^{-1}. The corresponding dissociation constant for the same nucleotide K_{d_1} determined in the presence of saturating concentrations of $G_{\beta\gamma}$ gives $(\phi J)^{-1}$, because the concentration of free G_α is negligible compared with $G_{\alpha\beta\gamma}$ when the concentration of $G_{\beta\gamma}$ is saturating. Thus, ϕ can be estimated simply from the ratio K_d/K_{d_1}. This principle should apply to any branch of the scheme given in Fig. 1, provided that the concentration of auxiliary ligand(s) is saturating. The coupling factors for the triple allosteric effect (π) can be estimated from the apparent nucleotide dissociation constants obtained using (i) pure G_α (K_d), (ii) G_α plus saturating $G_{\beta\gamma}$ ($K_{d_1} \approx (\phi J)^{-1}$), (iii) G_α plus saturating receptor ($K_{d_2} \approx (\beta J)^{-1}$), and (iv) G_α plus both saturating $G_{\beta\gamma}$ and receptor ($K_{d_3} \approx (\pi\beta\phi J)^{-1}$). Thus, the value of π can be estimated from the ratio $(K_{d_1} K_{d_2})/(K_{d_3} K_d)$.

In conclusion, the present theoretical study introduces a reasonably simple mathematical model that can be used to gain insights into hormone-receptor-G protein systems. By considering explicitly all the proteins and ligands participating in the first step of receptor-mediated signal transfer, the model is comprehensive but also involves a large number of parameters, many of which have never been experimentally measured. We have in part offset this pitfall by selecting among the massive number of possible parameter combinations only those compatible with current experimental evidence from many laboratories. In this sense, the study represents a "rough fitting" of the model to the available biochemical literature on receptor action. Further refinements will require experiments designed *ad hoc* and use of model systems in which the stoichiometry of the reacting species is under the experimenter's control.

Appendix

In order to calculate free and bound species starting from the total concentration of the components, we used the following equilibrium and conservation equations, along with the eqs. 1a-d given in the model for the unconditional constants:

$$A_T = [A] + [AB] + [RA] + [NA] + [RAB] + [BAN] + [RAN] + [HRA] + [HRAB] + [HRAN]$$

$$B_T = [B] + [AB] + [RAB] + [NAB] + [HRAB]$$

$$R_T = [R] + [HR] + [RA] + [HRA] + [RAN] + [RAB] + [HRAN] + [HRAB]$$

$$N_T = [N] + [NA] + [NAB] + [RAN] + [HRAN]$$

$$H_T = [H] + [HR] + [HRA] + [HRAB] + [HRAN]$$

$$\beta J = \frac{[RAN]}{[RA][N]}$$

$$\alpha K = \frac{[HRA]}{[H][RA]}$$

$$\delta L = \frac{[RAB]}{[RA][B]}$$

$$\alpha M = \frac{[HRA]}{[HR][A]}$$

$$\iota\alpha\delta M = \frac{[HRAB]}{[HR][AB]}$$

$$\phi J = \frac{[NAB]}{[N][AB]}$$

$$\alpha\gamma K = \frac{[HRAN]}{[H][RAN]}$$

$$\phi L = \frac{[NAB]}{[NA][B]}$$

$$\beta M = \frac{[RAN]}{[R][AN]}$$

$$\alpha\beta\gamma M = \frac{[HRAN]}{[HR][AN]}$$

$$\beta\gamma J = \frac{[HRAN]}{[HRA][N]}$$

$$\iota\alpha K = \frac{[HRAB]}{[H][RAB]}$$

$$\iota\delta L = \frac{[HRAB]}{[HRA][B]}$$

$$\delta M = \frac{[RAB]}{[R][RA]}$$

Using the conversion equations we can write:

$$[A] = \frac{A_T}{1 + SA} \quad (2)$$

$$[R] = \frac{R_T}{1 + SR} \quad (3)$$

$$[H_i] = \frac{H_{Ti}}{1 + SH_i} \quad (4)$$

$$[B] = \frac{B_T}{1 + SB} \quad (5)$$

$$[N_j] = \frac{N_{Tj}}{1 + SN_j} \quad (6)$$

Subscript T signifies total concentration of corresponding component; indices $i = 1 \ldots n$ and $j = 1 \ldots m$ denote n and m different ligands competing for hormone and nucleotide binding sites, respectively. SA, SB, SR, SN, and SH are total concentrations of the bound forms of corresponding components divided by their free concentrations and, after rearranging, are given as follows:

$$SA = L[B] + M[R]\left(1 + \sum_{i=1}^{n} \alpha_i K_i [H_i](1 + \delta\iota_i L[B]) + \delta L[B]\right)$$

$$+ \sum_{j=1}^{m}\left(J_j[N_j]\left(1 + \phi_j L[B] + \beta_j M[R]\left(\sum_{i=1}^{n} \alpha_i \gamma_{ij} K[H_i] + 1\right)_j\right)\right)$$

$$SB = L[A]\left(1 + \sum_{j=1}^{m} \phi_j J_j[N_j] + \delta M[R]\left(1 + \sum_{i=1}^{n} \alpha_i \iota_i K_i[H_i]\right)\right)$$

$$SR = M[A]\left(1 + \delta L[B] + \sum_{j=1}^{m} \beta_j J_j[N_j]\right)$$

$$+ \sum_{i=1}^{n}\left(K_i[H_i]\left(1 + \alpha_i M[A]\left(1 + \sum_{j=1}^{m} \beta_j \gamma_{ij} J_j[N_j] + \delta\iota_i L[B]\right)\right)\right)$$

$$SN_j = J_j[A]\left(1 + \phi_j L[B] + \beta_j M[R]\left(1 + \sum_{i=1}^{n} \alpha_i \gamma_{ij} K_i[H_i]\right)\right)$$

$$SH_i = K_i[R]\left(1 + \alpha_i M[A]\left(1 + \sum_{j=1}^{m} \beta_j J_j[N_j] + \delta\iota_i L[B]\right)\right)$$

We used the following iterative procedure to obtain simultaneous solutions for $3 + m + n$ free species (eqs. 2–6). (i) Starting with the free concentrations equal to the corresponding totals, eqs. 2–6 were solved sequentially. (ii) Calculations were iterated by replacing the free concentrations with the solution, until the relative changes in [H] and [A] were less than 10^{-7} in successive iterations. For the parameter values used in the present study, the method never failed to converge. In some cases we also used the Newton-Raphson procedure to compare the converged solutions. We found no difference between the results of the two procedures. Concentrations of complexed species were calculated using the solution and the definition of equilibrium constants given above.

References

1. Rodbell, M. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. *Nature (Lond.)* **284**:17–22 (1980).
2. Stryer, L. Cyclic AMP cascade of vision. *Annu. Rev. Neurosci.* **9**:87–119 (1986).
3. Gilman, A. G. G proteins: transducers of G protein generated signals. *Annu. Rev. Biochem.* **56**:615–649 (1987).
4. Birnbaumer, L. G proteins in signal transduction. *Annu. Rev. Pharmacol. Toxicol.* **30**:675–705 (1990).
5. Levitzki, A. Regulation of adenylate cyclase by hormones and G proteins. *FEBS Lett.* **211**:113–118 (1987).
6. Lefkowitz, R. J., D. Mullikin, and M. G. Caron. Regulation of β-adrenergic receptors by guanyl-5′-yl imidodiphosphate and other purine nucleotides. *J. Biol. Chem.* **254**:4686–4692 (1976).
7. Rojas, F. J., and L. Birnbaumer. Regulation of glucagon receptor binding *J. Biol. Chem.* **260**:7829–7835 (1987).
8. Limbird, L. E., and R. J. Lefkowitz. Agonist-induced increase in β-adrenergic receptor size. *Proc. Natl. Acad. Sci. USA* **75**:228–232 (1978).
9. Senogles, S. E., J. E. Benovic, N. Amlaiky, C. Unson, G. Milligan, R. Vinitsky, A. M. Spiegel, and M. G. Caron. The D2-dopamine receptor of anterior pituitary is functionally associated with a pertussis toxin-sensitive guanine-binding protein. *J. Biol. Chem.* **262**:4860–4867 (1987).
10. Cerione, R. A., C. Staniszewski, M. G. Caron, R. J. Lefkowitz, J. Codina, and L. Birmbaumer. I. Role of N_i in the hormonal stimulation of adenylate cyclase. *Nature (Lond.)* **328**:293–295 (1985).
11. Okabe, K., A. Yatani, T. Evans, Y. Ho, J. Codina, L. Birnbaumer, and A. M. Brown. βγ dimers of G proteins inhibit atrial muscarinic K^+ channels. *J. Biol. Chem.* **265**:12854–12858 (1990).
12. Hildebrandt, J. D., and R. E. Kohnken. Hormone inhibition of adenylyl cyclase. *J. Biol. Chem.* **265**:9825–9830 (1990).
13. Florio, V. A., and P. C. Sternweis. Mechanism of muscarinic receptor action on G_o in reconstituted phospholipid vesicles. *J. Biol. Chem.* **264**:3909–3915 (1989).
14. Weber, G. Ligand binding and internal equilibria in proteins. *Biochemistry* **11**:864–878 (1972).
15. DeLean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled β-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
16. Wregget, K. A., and A. DeLean. The ternary complex model: its properties and application to ligand interaction with the D2-dopamine receptor of the anterior pituitary gland. *Mol. Pharmacol.* **26**:214–227 (1984).
17. Ehlert, F. J. The relationship between muscarinic receptor occupancy and adenylate cyclase inhibition in the rabbit myocardium. *Mol. Pharmacol.* **28**:410–421 (1985).
18. Leung, E., K. A. Jacobson, and R. D. Green. Analysis of agonist-antagonist interactions at A1 adenosine receptors. *Mol. Pharmacol.* **38**:72–83 (1990).
19. Costa, T., Y. Ogino, P. J. Munson, H. O. Onaran, and D. Rodbard. 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.* **41**:549–560 (1992).
20. Ehlert, F. J., and B. E. Rathbun. Signaling through the muscarinic-receptor adenylate cyclase system of the heart is buffered against GTP over a range of concentrations. *Mol. Pharmacol.* **38**:148–158 (1990).
21. Mackay, D. Interpretation of relative potencies, relative efficacies and apparent affinity constants of agonist drugs estimated from concentration-response curves. *J. Theor. Biol.* **142**:415–427 (1990).
22. Higashijima, T., K. M. Ferguson, and P. C. Sternweis. Regulation of hormone-sensitive GTP-dependent regulatory proteins by chloride. *J. Biol. Chem.* **262**:3597–3602 (1987).
23. Tamir, A., A. B. Fawzi, and J. K. Northup. Unique guanine nucleotide binding properties of the human placental GTP-binding protein G_p. *Biochemistry* **29**:6947–6954 (1990).
24. Rodbell, M., M. C. Lin, and Y. Salomon. Evidence for interdependent action of glucagon and nucleotides of the hepatic adenylate cyclase system. *J. Biol. Chem.* **249**:59–65 (1974).
25. Maguire, M. E., P. M. Van Arsdale, and A. G. Gilman. An agonist specific effect of guanine nucleotides on binding to the *beta* adrenergic receptor. *Mol. Pharmacol.* **12**:335–339 (1976).
26. Cassel, D., and Z. Selinger. Mechanism of adenylate cyclase activation through the *beta*-adrenergic receptor: catecholamine-induced displacement of bound GDP by GTP. *Proc. Natl. Acad. Sci. USA* **75**:4155–4159 (1978).
27. Fung, B. K., and L. Stryer. Photolyzed rhodopsin catalyzes the exchange of GTP for bound GDP in retinal rod outer segments. *Proc. Natl. Acad. Sci. USA* **77**:2500–2504 (1980).
28. Brandt, D. R., and E. M. Ross. Catecholamine-stimulated GTPase cycle: multiple sites of regulation by β-adrenergic receptor and Mg^{2+} studied in reconstituted receptor-G_s vesicles. *J. Biol. Chem.* **261**:1656–1664 (1985).
29. Higashijima, T., K. M. Ferguson, P. C. Sternweis, M. D. Smigel, and A. G. Gilman. Effect of Mg^{2+} and the βγ-subunit complex on the interactions of guanine nucleotides with G-proteins. *J. Biol. Chem.* **262**:762–766 (1987).
30. Linder, M. E., D. A. Ewald, R. J. Miller, and A. G. Gilman. Purification and characterization of $G_{o\alpha}$ and three types of $G_{i\alpha}$ after expression in *Escherichia coli*. *J. Biol. Chem.* **265**:8243–8251 (1990).
31. Fung, B. K., J. B. Hurley, and L. Stryer. Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc. Natl. Acad. Sci. USA* **78**:152–156 (1981).
32. Northup, J. K., P. C. Sternweis, and A. G. Gilman. The subunits of the stimulatory regulatory component of adenylate cyclase: resolution, activity and properties of the 35,000-dalton β-subunit. *J. Biol. Chem.* **258**:11361–11368 (1983).
33. Linder, M. E., I. Pang, R. J. Duronio, J. I. Gordon, P. C. Sterweis, and A. G.

Gilman. Lipid modification of G protein subunits: myristoylation of $G_{o\alpha}$ increases its affinity for $\beta\gamma$. *J. Biol. Chem.* **266**:4654–4659 (1991).
34. Rodbell, M., L. Birnbaumer, S. L. Pohl, and H. M. J. Krans. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. *J. Biol. Chem.* **246**:1877–1882 (1971).
35. Pfeuffer, T. GTP-binding proteins in membranes and the control of adenylate cyclase activity. *J. Biol. Chem.* **252**:7224–7234 (1977).
36. Ross, E. M., M. E. Maguire, T. W. Sturgill, R. L. Biltonen, and A. G. Gilman. Relationship between the β-adrenergic receptor and adenylate cyclase. *J. Biol. Chem.* **252**:5761–5775 (1977).
37. Pfeuffer, T., and E. J. M. Helmreich. Activation of pigeon erythrocyte membrane adenylate cyclase by guanylnucleotide analogues and separation of a nucleotide binding protein. *J. Biol. Chem.* **250**:867–876 (1975).
38. Miller, R. T., S. B. Masters, K. A. Sullivan, B. Beiderman, and H. R. Bourne. A mutation that prevents GTP-dependent activation of α chain of G_s. *Nature (Lond.)* **334**:712–715 (1988).
39. Watkins, D. C., J. K. Northup, and C. C. Malbon. Regulation of G-proteins in differentiation: altered ratio of α- to β-subunits in 3T3-L1 cells. *J. Biol. Chem.* **262**:10651–10657 (1987).
40. Barrowman, M. M., S. Cockcroft, and B. D. Gompertz. Two roles of guanine nucleotides in the stimulus-secretion sequence of neutrophils. *Nature (Lond.)* **319**:504–507 (1986).
41. Stutchfield, J., and S. Cockcroft. Guanine nucleotides stimulate polyphosphoinositide phosphodiesterase and exocytic secretion from HL60 cells permeabilized with streptolysin O. *Biochem. J.* **250**:375–382 (1988).
42. Logothetis, D. E., Y. Kurachi, J. Galper, E. J. Neer, and D. E. Clapham. The $\beta\gamma$ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. *Nature (Lond.)* **325**:321–326 (1987).
43. Katada, T., K. Kusakabe, M. Oinuma, and M. Ui. A novel mechanism for the inhibition of adenylate cyclase via inhibitory GTP binding proteins. *J. Biol. Chem.* **262**:11897–11900 (1987).
44. Kurachi, Y., H. Ito, T. Sugimoto, T. Katada, and M. Ui. Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel. *Nature (Lond.)* **337**:555–557 (1989).
45. Itoh, H., and A. G. Gilman. Expression and analysis of $G_{s\alpha}$ mutants with decreased ability to activate adenylylcyclase. *J. Biol. Chem.* **266**:16226–16231 (1991).
46. Yatani, A., R. Mattera, J. Codina, R. Graf, K. Okabe, E. Padrell, R. Iyengar, A. M. Brown, and L. Birnbaumer. The G protein-gated atrial K^+ channel is stimulated by three distinct $G_{i\alpha}$ subunits. *Nature (Lond.)* **336**:680–682 (1988).
47. Boyer, J. L., G. L. Waldo, T. Evans, J. K. Northup, C. P. Downes, and T. K. Harden. Modification of $A1F_4^-$ and receptor stimulated phospholipase C activity by G-protein $\beta\gamma$ subunits. *J. Biol. Chem.* **264**:13917–13922 (1989).
48. Bergman, R. N., and O. Hechter. Neurohypophyseal hormone-responsive renal adenylate cyclase. IV. A random-hit matrix model for coupling in a hormone-sensitive adenylate cyclase system. *J. Biol. Chem.* **253**:3238–3250 (1978).
49. Tolkowski, A. M., and A. Levitzki. Mode of coupling between the β-adrenergic receptor and adenylate cyclase in turkey erythrocytes. *Biochemistry* **17**:3795–3810 (1978).
50. Macfarlane, D. E. Bidirectional collision coupling in the regulation of the adenylate cyclase: the alloenzyme hypothesis for receptor function. *Mol. Pharmacol.* **22**:580–588 (1982).
51. Gross, W., and M. J. Lohse. Mechanism of activation of A2 adenosine receptors. II. A restricted collision-coupling model of receptor-effector interaction. *Mol. Pharmacol.* **39**:524–530 (1991).
52. Sakman, B., and E. Neher. *Single Channel Recording.* Plenum Press, New York (1985).

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