Assessment of a Ternary Model for the Binding of Agonists to Neurohumoral Receptors†

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ABSTRACT: A frequently cited variant of the "mobile receptor" hypothesis has been examined for its ability to describe the binding of agonists at neurohumoral receptors that operate via a guanylyl nucleotide binding protein. The model involves a reversible association between the receptor (R) and the G protein (G). Agonists (A) bind with different affinity to R and to the RG complex; similarly, G differentiates between R and the AR complex. Theoretical binding curves calculated according to the model have been analyzed in terms of the Hill equation and as a mixture of independent and noninteracting sites. The model is shown to be compatible in some respects with reported data on the binding of agonists to the β-adrenergic receptor but not to the muscarinic cholinergic or D_2 dopaminergic receptors. It is difficult to reconcile with the reported effects of guanylyl nucleotides, magnesium, and *N*-ethylmaleimide on the binding of agonists at any neurohumoral receptor.

Neurohumoral receptors that regulate the activity of adenylate cyclase bind agonists and antagonists in a characteristic manner (Stadel et al., 1982; Smigel et al., 1984; Sokolovsky et al., 1984): the former reveal a dispersion of affinities characterized by Hill coefficients (n_H) markedly less than 1; in contrast, the latter typically reveal Hill coefficients indistinguishable from 1, although somewhat lower values can be obtained with receptors that attenuate the enzyme (Hulme et al., 1980; De Lean et al., 1982b; Yeung & Green, 1983). The changes in binding brought about by guanylyl nucleotides, magnesium, and pretreatment of the receptors with sulfhydryl reagents indicate that deviations from rectangular hyperbolic behavior reflect an interaction between the receptor and a stimulatory or inhibitory G protein. Sites of higher affinity for agonists appear to reflect a complex between the receptor and the G protein: purified or semipurified receptors generally are of lower affinity; also, reconstitution with purified G protein yields sites of higher affinity that reveal the sensitivity to guanylyl nucleotides characteristic of receptors in the native membrane [see Cerione et al. (1984), Florio & Sternweis (1985), and Haga & Haga (1985) and references cited therein]. While G proteins thus contribute to the properties of receptors revealed in the binding of neurohumoral agonists, the nature of that contribution remains unclear.

Low values of the Hill coefficient have prompted many investigators to interpret their data in terms of two or more classes of independent sites differing in their affinity for the agonist. Such a model is not universally consistent with experimental data, however, as pointed out by Kent et al. (1980) for the β-adrenergic receptor and by Wong et al. (1986) for the muscarinic cholinergic receptor. It is particularly difficult to reconcile with the observation that the relative size of each class of sites can differ for different agonists, since no process is implied whereby the ligand promotes the interconversion of sites from one class to another. The limitations of models based on classical saturation functions prompted De Lean et al. (1980) to propose that the low Hill coefficients arise from an interaction among agonist, receptor, and an additional component of the membrane to yield a ternary complex; the additional component was identified tentatively as the nucleotide-specific G protein believed to link the β-adrenergic receptor with adenylate cyclase (Swillins & Dumont, 1980; Ross & Gilman, 1980; Rodbell, 1980). The ternary complex model is analogous to the mobile receptor hypothesis of Jacobs and Cuatrecasas (1976) and to similar proposals of other investigators (Boeynaems & Dumont, 1975; De Haen, 1976). A spontaneous equilibrium is thought to exist within the membrane between the receptor (R) and free G protein (G) on the one hand and a heterodimeric complex (RG) on the other; agonists bind with higher affinity to RG than to R, while antagonists either are indifferent or exhibit the reverse preference. Guanylyl nucleotides, magnesium, and other agents that perturb the binding of agonists are thought to act via an effect on the affinity of G for R.

The ternary model of De Lean et al. (1980) has been used implicity by many investigators to rationalize the binding patterns of neurohumoral agonists. In most reports, however, the data have been analyzed in terms of a multisite model that assumes a heterogeneous mixture of noninterconverting and noninteracting sites. This is a questionable practice, since the mathematical expressions of the two models cannot be reduced to a common function; accordingly, there is no direct relationship in most situations between the parameters obtained from one model and those obtained from the other. Recent attempts to apply the ternary model in an explicit and quantitative manner have met with mixed success. Wreggett and De Lean (1984) have concluded that the model can describe the binding of agonists to D_2 dopaminergic receptors in bovine anterior pituitary gland if the number of receptors exceeds the number of available G proteins and if the latter are predominantly in the coupled (RG) form even in the absence of agonists; in that study, however, the model suggested that guanylyl imidodiphosphate (GMP-PNP) reduces the total number of available G proteins. The binding properties of α_2-adrenergic receptors and the large excess of inhibitory G proteins in human platelet membranes imply that some G

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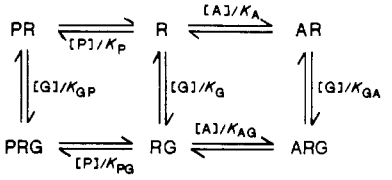
Scheme I



proteins have no access to receptors if the ternary model is to fit the data of Neubig et al. (1985). Muscarinic receptors are reported to behave in a manner consistent with the model in homogenates of rabbit heart (Ehlert, 1985) but not in homogenates of hamster heart (Wong et al., 1986). The present report describes the limitations of the ternary model and considers several points of disagreement between the model and the binding properties of guanylyl nucleotide sensitive receptors. Preliminary reports of this work have appeared elsewhere (Wells et al., 1982; Wells, 1983).

Theory and Methods

Description of the Ternary Complex Model. If the system is at thermodynamic equilibrium, binding to a population of identical and noninteracting sites is expected to be rectangular hyperbolic with respect to the concentration of free ligand irrespective of the number of conformational equilibria that may occur. If another component is present, however, various constraints on the interactions between that component and the receptor could result in more complex patterns characterized by Hill coefficients less than 1. The present analysis is based on schemes described previously and the model shown below (Scheme I) (Boeynaems & Dumont, 1975; De Haen, 1976; Jacobs & Cuatrecasas, 1976; De Lean et al., 1980). In this representation, R is the receptor and G the nucleotide-specific G protein that binds reversibly to form RG. Agonists or antagonists, shown as A, may occupy a site on the receptor forming either AR or the ternary complex ARG. Many drugs cannot be measured directly, however, and therefore are investigated via their inhibitory effect on the binding of a radiolabeled probe, shown as P. K_A, K_{AG}, K_G, and K_{GA} represent the equilibrium dissociation constants for the binding of A to R and to RG, and for the binding of G to R and to AR; K_P, K_{PG}, and K_{GP} represent the corresponding constants for the binding of P. It is relevant to note that K_A/K_{AG} equals K_G/K_{GA} and that K_P/K_{PG} equals K_G/K_{GP}. In Scheme I and throughout the following discussion, brackets refer to the free molar concentration of the particular variable unless indicated otherwise by a subscript.

Binding data obtained at thermodynamic equilibrium typically are plotted on two-dimensional coordinates with the total bound probe shown on the ordinate and the concentration of unlabeled drug on the abscissa. For the ternary model, such a relationship is described by eq 1, in which the total specific binding is the quantity [PR] + [PRG]:

$$\frac{[PR] + [PRG]}{[R]_t} = \frac{[P]}{[P] + K_P'} \frac{K_A'}{[A] + K_A'} \quad (1)$$

where

$$K_P' = \frac{1 + [G]/K_G}{1/K_P + (1/K_{PG})([G]/K_G)}$$

$$K_A' = \frac{[P]/K_P + ([P]/K_{PG})([G]/K_G) + [G]/K_G + 1}{1/K_A + (1/K_{AG})([G]/K_G)}$$

$$[R]_t = [R] + [RG] + [AR] + [ARG] + [PR] + [PRG]$$

Also

$$[G]_t = [G] + [RG] + [ARG] + [PRG]$$

$$[A]_t = [A] + [AR] + [ARG]$$

$$[P]_t = [P] + [PR] + [PRG]$$

Binding is a rectangular hyperbolic function of [A] provided that K_A' and K_P' are constant at all concentrations of the unlabeled ligand. K_P' is a function of [G], however, and K_A' is a function of both [G] and [P]; accordingly, the binding sites appear homogeneous only when the values of $[G]_t$, $[P]_t$, and the various constants are such that the fraction of $[G]_t$ or $[P]_t$ bound to the receptor is negligible or remains virtually unchanged under the conditions of the experiment. The quantities [G] and [P] otherwise constitute two variables that effectively are ignored when the four-dimensional system is mapped onto two-dimensional coordinates. The total concentration of radiolabeled probe can be both controlled and measured. Conditions thus can be adjusted to ensure that depletion of the free radioligand is negligible; alternatively, the effects of depletion can be accounted for, within limits, if binding is analyzed as a function of $[P]_t$ rather than [P].[1] In contrast, the total concentration of G protein in reversible equilibrium or otherwise associated with a particular receptor cannot be controlled in preparations of native membrane. The magnitude of [G] thus varies with [A] whenever [RG], [ARG], and [PRG] together constitute a significant fraction of $[G]_t$. If K_G is such that K_P' and K_A' also vary, the system will yield a Hill coefficient less than 1 when the data are plotted on two-dimensional coordinates.

Quantitative applications of the ternary model require that mutual depletion of the receptor and the G protein be considered. Concentrations of bound radioligand ([PR] and [PRG]) then appear as the roots of two, quadratic polynomials [f([PR]), eq 2; g([PRG]), eq 3] if one retains the simplification that the free concentrations of both ligands ([A] and [P]) are known:

$$f([PR]) = Q_f[PR]^2 + R_f[PR] + S_f \quad (2)$$

$$g([PRG]) = Q_g[PRG]^2 + R_g[PRG] + S_g \quad (3)$$

where

$$Q_f = K_A K_{AG} K_P^2 K_{GP} + K_A K_{AG}[P](K_P K_G + K_P K_{GP} + K_G[P]) + K_P^2 K_{GP}[A] \times (K_A + K_{AG} + [A]) + K_P[A][P](K_{AG} K_G + K_A K_{GP})$$

$$R_f = K_A K_{AG} K_P K_{GP}[P](K_G + [G]_t - [R]_t) + K_A K_{AG} K_G[P]^2(K_{GP} + [G]_t - [R]_t) + K_P K_{GP}[A][P](K_{AG} K_G + K_A[G]_t - K_A[R]_t)$$

$$S_f = -K_A K_{AG} K_G K_{GP}[P]^2[R]_t$$

$$Q_g = -K_{AG}^2[P](2K_{PG} + [P]) - K_{PG}^2[A](2K_{AG} + [A]) - 2K_{AG} K_{PG}[A][P] - K_{AG}^2 K_{PG}^2$$

$$R_g = K_{AG}^2[P]^2(K_{GP} + [G]_t + [R]_t) + K_{AG}^2 K_{PG}[P] \times (K_G + [G]_t + [R]_t) + K_{AG} K_{PG}[A][P](K_{GA} + [G]_t + [R]_t)$$

$$S_g = -K_{AG}^2[P]^2[G]_t[R]_t$$

Total binding (B_{obsd}) is obtained according to eq 4, in which C represents the fraction of unbound probe that appears as

[1] The consequences of depletion have been well documented for other models [see, for example, Jacobs et al. (1975), Chang et al. (1975), and Wells et al. (1980)].

nonspecific binding following physical separation of the homogenate into the soluble and insoluble fractions.

$$B_{obsd} = [PR] + [PRG] + C[P] \quad (4)$$

Simulation and Analysis of Data. The ternary model predicts that a drug may bind with higher affinity either to the G-coupled form ($K_A/K_{AG} > 1$) or to the uncoupled form ($K_A/K_{AG} < 1$) of the receptor. The fraction of receptors coupled to G protein thus will be either increased ($K_G/K_{GA} > 1$) or decreased ($K_G/K_{GA} < 1$) upon addition of the drug. In practice, neurohumoral agonists appear to bind with higher affinity to receptors in their G-coupled form irrespective of whether the system activates or attenuates adenylate cyclase; antagonists often appear to be indifferent to the presence of the G protein or, in some systems, to show the opposite preference. For the purpose of the present discussion, the model described by eq 2–4 has been simplified by assuming that K_P equals K_{PG}; this in turn implies that K_G equals K_{GP}.

The model thus simplified has been investigated by numerical solution of eq 2 and 3 to yield values of [PR] and [PRG] that subsequently were combined according to eq 4. The ratio $[P]/K_P$ was set equal to 10^{-5}, thereby ensuring that effects of the radioligand on the position of the resulting curve are negligible. Higher values of $[P]/K_P$ would cause the simulated, semilogarithmic curves to be shifted in a parallel fashion to higher values of log [A]. The constant C in eq 4 was set to zero. Unless indicated otherwise, 100 data points were calculated at equal increments of log [A] over the range of specific binding from at least 99.5% to less than 0.5% of the maximal value. The curves presented in part A of each figure have been calculated with $K_A/K_{AG} > 1$ and thus correspond to events as currently understood for agonists at all cyclase-linked receptors. Those in part B have been calculated with $K_A/K_{AG} < 1$ and are included primarily for purposes of comparison; they may correspond to the binding of antagonists in some systems that attenuate adenylate cyclase (De Lean et al., 1982b; Burgisser et al., 1982; Yeung & Green, 1983), although presumably not that studied by Wong et al. (1986). Further to the latter point, however, it is noteworthy that the data in part B are simulated by assuming competition with a radioligand for which $K_P = K_{PG}$; such a situation may not be experimentally possible when agonists and antagonists both distinguish between G-coupled and uncoupled receptors.

Simulated data were described empirically by using eq 5 and 6, in which total binding of the radioligand (B_{obsd}) is the sum of n components. K_S represents the concentration of drug

$$B_{obsd} = B_{min} + (B_{max} - B_{min}) \sum_{S=1}^{n} \frac{F_S K_S}{[A] + K_S} \quad (5)$$

$$B_{obsd} = B_{min} + (B_{max} - B_{min}) \sum_{S=1}^{n} \frac{F_S K_S^{n_{HS}}}{[A]^{n_{HS}} + K_S^{n_{HS}}} \quad (6)$$

A that reduces component S by 50%, and F_S is the corresponding fraction of total binding reflected in K_S. B_{max} and B_{min} represent maximal ([A] = 0) and minimal ([A] → ∞) binding of the probe, respectively. These expressions were fitted to the simulated data by using an iterative procedure based on the nonlinear, least-squares algorithm of Marquardt (1963). No weighting factors were applied. During successive iterations, the value of B_{max} was fixed at the asymptotic value of eq 4 when [A] equals zero; B_{min} was fixed at zero. Equation 5 is analogous to eq 3 in Wong et al. (1986), but the latter expression includes the total concentration of the radioligand ($[P]_t$) and thus constitutes an explicit description of the multisite model. In subsequent references to eq 5 above, it is understood either that the radioligand is without effect on the parametric values (i.e., $[P] << K_P$) or that appropriate corrections have been made.

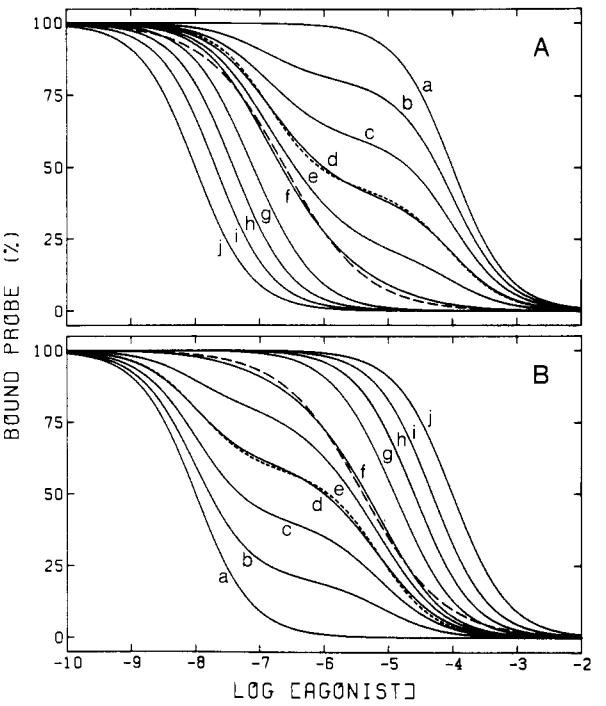


FIGURE 1: Effect of $[G]_t/[R]_t$ on the behavior of the ternary model. The solid lines were simulated according to eq 4 with the following values of $[G]_t/[R]_t$: a, 0.0; b, 0.2; c, 0.4; d, 0.6; e, 0.8; f, 1.0; g, 2.0; h, 4.0; i, 10.0; and j, 1000. Other parametric values were as follows: (A) log K_A = −4, log K_{AG} = −8, and log $([R]_t/K_G)$ = −1; (B) log K_A = −8, log K_{AG} = −4, and log $([R]_t/K_G)$ = +3. The dashed line represents the best fit of eq 6 (n = 1) to the simulated data of curve f; parametric values obtained by regression are as follows: (A) log IC_{50} = −6.60 and n_H = 0.70; (B) log IC_{50} = −5.40 and n_H = 0.69. Values of the Hill coefficient obtained for the curves in which $[G]_t$ exceeds $[R]_t$ are as follows: g, 0.91; h, 0.97; i, 0.99; and j, 1.0 (fitted curves not shown). The dotted line represents the best fit of eq 5 (n = 2) to the simulated data of curve d. Parametric values obtained by regression are as follows: (A) log K_1 = −6.85, log K_2 = −4.06, and F_2 = 0.42; (B) log K_1 = −7.95, log K_2 = −5.15, and F_2 = 0.58.

RESULTS

Binding profiles predicted by the model depend in part upon the ratio of $[G]_t$ to $[R]_t$, a quantity that presumably remains constant within any one experiment. This is illustrated in Figure 1, where the solid lines have been calculated for values to $[G]_t/[R]_t$ ranging from 0 to 1000 and clearly are steeper at the extremes of this range. As expected from eq 1, the Hill coefficient is 1 when $[G]_t$ is 0 and tends to 1 as $[G]_t$ exceeds $[R]_t$ sufficiently that relatively little change can occur in the free concentration of G. When $[G]_t$ is less than $[R]_t$, the binding profiles can be distinctly biphasic with appropriate values of K_A/K_{AG} and $[R]_t/K_G$ (Figure 1, curves b–e). In such circumstances, the Hill equation (eq 6, n = 1) provides a poor description of the simulated data. Much better agreement is obtained if the data are assumed to arise from two classes of noninteracting sites (eq 5, n = 2) as illustrated in Figure 1 (curve d), and the implications of this are described below. When $[G]_t$ equals or exceeds $[R]_t$, both expressions provide at least a first approximation of the data, as illustrated in Figure 1 (curve f) for the Hill equation and in Figure 5 for eq 5 (n = 2). The following discussion considers the binding patterns expected from the ternary model for three situations that can exist in principle between the G protein and the receptor: $[G]_t$ equal to $[R]_t$, $[G]_t$ greater than $[R]_t$, and $[G]_t$ less than $[R]_t$.

Table I: Parametric Values Obtained by Fitting Equations 5 and 6 to the Simulated Data Illustrated in Figure 2[a]

	eq 4		eq 6 (n = 1)		eq 5 (n = 2)			
curve (Figure 2)	log (K_A/K_{AG})	log ($[R]_t/K_G$)	n_H	log (IC_{50}/K_A)	log (K_1/K_A)	log (K_2/K_A)	log (K_2/K_1)	F_2
e	5	−1	0.69	−3.60	−3.97	−2.29	1.68	0.25
d	4	−1	0.70	−2.60	−2.98	−1.37	1.61	0.26
c	3	−1	0.73	−1.64	−2.03	−0.68	1.35	0.31
b	2	−1	0.84	−0.78	−1.16	−0.28	0.88	0.43
a	1	−1	0.97	−0.21	−0.41	−0.07	0.33	0.58
	0		1.00	0.00				
a	−1	3	0.98	0.95	0.27	0.98	0.71	0.95
b	−2	3	0.90	1.79	0.65	1.90	1.25	0.90
c	−3	3	0.77	2.39	1.10	2.63	1.54	0.81
d	−4	3	0.69	2.61	1.35	2.97	1.62	0.75
e	−5	3	0.68	2.64	1.38	3.03	1.66	0.73

[a] Simulations were carried out according to the ternary model (eq 4) with $[G]_t = [R]_t$; other parametric values are listed in the table.

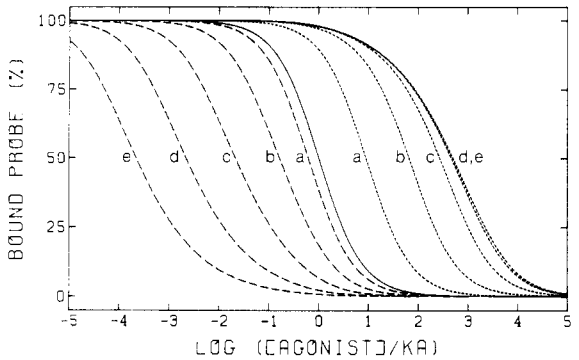


FIGURE 2: Effect of K_A/K_{AG} on the behavior of the ternary model. Lines were simulated according to eq 4 with $[G]_t$ set equal to $[R]_t$; other parameters were as follows: (—) log (K_A/K_{AG}) = 0; (---) log (K_A/K_{AG}) > 0 and log ($[R]_t/K_G$) = −1; (···) log (K_A/K_{AG}) < 0 and log ($[R]_t/K_G$) = +3. Individual values of log (K_A/K_{AG}) were as follows: a, ±1; b, ±2; c, ±3; d, ±4; and e, ±5. Parametric values for best fits of eq 5 (n = 2) and eq 6 (n = 1) to the simulated data are listed in Table I (fitted curves not shown).

$[G]_t$ Equal to $[R]_t$. For a system in which $[G]_t$ and $[R]_t$ are equal, the binding patterns obtained in competitive experiments between an agonist ($K_A \neq K_{AG}$) and a radiolabeled antagonist ($K_P = K_{PG}$) will be determined by the values of K_A, K_{AG}, K_G, and $[R]_t$. Experimental variations in K_A and K_{AG} generally are achieved by using a series of agonists, as illustrated in Figure 2 where the curves have been calculated at constant $[R]_t/K_G$ for different values of K_A/K_{AG} between 10^5 and 10^{-5}. Hill coefficients are listed in Table I and indicate that the binding sites appear homogeneous (n_H = 1) only when K_A equals K_{AG}. As the difference in the affinity of the agonist for the two forms of the receptor increases, the Hill coefficient decreases until a minimal value is attained (Figure 3, insets). Further changes in the ratio of K_A to K_{AG} may alter the position but not the shape of the binding profile. This dependence reflects the equality between K_A/K_{AG} and K_G/K_{GA}. K_G and $[G]_t$ are constant within any particular preparation of tissue; changes in the degree of association between G protein and receptor thus are determined exclusively by the value of K_{GA}, which in turn is determined by the agonist. Maximal perturbation of the system will occur when two conditions are met: first, K_G must be such that the system in the absence of an agonist exists predominantly in the state not favored by agonists; second, K_{GA} must be such that a sufficient concentration of agonist can preclude measurable quantities of uncoupled R and G ($K_A/K_{AG} > 1$) or the RG complex ($K_A/K_{AG} < 1$). Agonists that induce a value of K_{GA} either smaller ($K_A/K_{AG} > 1$) or larger ($K_A/K_{AG} < 1$) than is necessary to fulfill the second condition will be without further, discernible effect on the numbers of receptors present

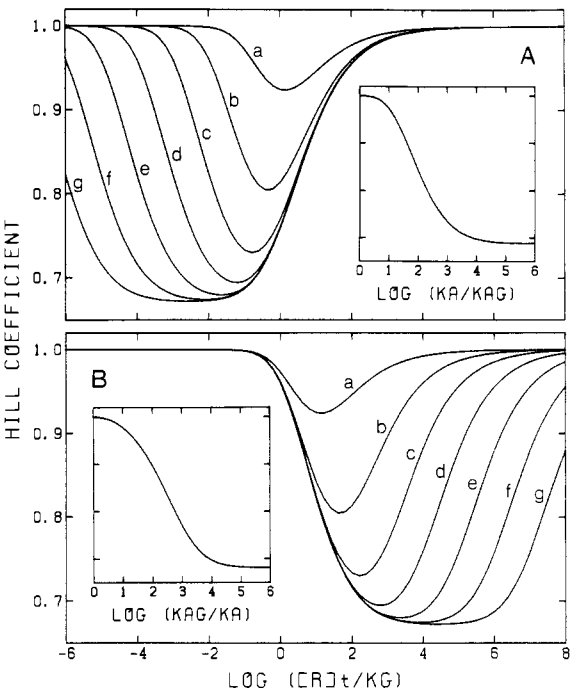


FIGURE 3: Effect of $[R]_t/K_G$ and K_A/K_{AG} on the Hill coefficient of binding curves simulated according to the ternary model. Data were simulated according to eq 4 and analyzed according to eq 6 (n = 1) to yield the Hill coefficients plotted on the ordinate. $[G]_t$ was set equal to $[R]_t$ throughout, and relative affinities of the agonist were as follows: log (K_A/K_{AG}) > 0 (A); log (K_A/K_{AG}) < 0 (B). Curves in the outer frames illustrate the dependence of n_H on log ($[R]_t/K_G$) at the following values of log (K_A/K_{AG}): a, ±1; b, ±2; c, ±3; d, ±4, e, ±5; f, ±6; and g, ±7. The curve in each inset illustrates the dependence of n_H on the absolute value of log (K_A/K_{AG}) when log ($[R]_t/K_G$) equals −1 (A) or +3 (B).

in one or the other form, and hence without further effect on the Hill coefficient.

In several neurohumoral systems, the binding of agonists is sensitive to magnesium, guanylyl nucleotides, ADP-ribosylation, sulfhydryl reagents, phosphorylation, and temperature in a manner that may reflect an effect on the interaction between the G protein and the receptor. Changes such as those induced by guanylyl nucleotides and magnesium often are ascribed, at lest implicitly, to an increase or decrease in the value of K_G. The predicted effect of K_G on the binding of an agonist is illustrated in Figure 4. Values selected for K_A and K_{AG} differ sufficiently to yield Hill coefficients near the lower limit that is possible at each value of $[R]_t/K_G$ when $[G]_t$ and $[R]_t$ are equal. If the agonist favors the association of G and R (Figure 4A), the Hill coefficient is essentially 1 when K_G is sufficiently small ($<10^{-2}[R]_t$) that G and R are fully coupled in the absence of agonist or sufficiently large

Table II: Parametric Values Obtained by Fitting Equations 5 and 6 to the Simulated Data Illustrated in Figure 4[a]

	eq 4		eq 6 (n = 1)		eq 5 (n = 2)			
figure and curve	log (K_A/K_{AG})	log ($[R]_t/K_G$)	n_H	−log IC_{50}	−log K_1	−log K_2	log (K_2/K_1)	F_2
4A, a	4	3	0.99	7.98				
4A, b	4	2	0.96	7.93	7.96	6.37	1.60	0.03
4A, c	4	1	0.88	7.79	7.88	6.27	1.62	0.08
4A, d	4	0	0.76	7.38	7.62	5.96	1.66	0.17
4A, e	4	−1	0.70	6.60	6.98	5.37	1.61	0.26
4A, f	4	−2	0.72	5.67	6.10	4.72	1.38	0.33
4A, g	4	−3	0.82	4.82	5.24	4.30	0.94	0.45
4A, h	4	−4	0.96	4.25	4.50	4.09	0.41	0.61
4A, i	4	−5	1.00	4.04				
4A, j	4	−6	1.00	4.00				
4B, a	−4	7	0.99	4.02				
4B, b	−4	6	0.96	4.07	5.67	4.04	1.63	0.97
4B, c	−4	5	0.88	4.21	5.77	4.12	1.65	0.92
4B, d	−4	4	0.76	4.62	6.05	4.38	1.67	0.83
4B, e	−4	3	0.70	5.40	6.67	5.03	1.64	0.75
4B, f	−4	2	0.72	6.33	7.29	5.90	1.39	0.68
4B, g	−4	1	0.82	7.18	7.70	6.77	0.94	0.56
4B, h	−4	0	0.96	7.75	7.91	7.50	0.41	0.40
4B, i	−4	−1	1.00	7.96				
4B, j	−4	−2	1.00	8.00				

[a] Simulations were carried out according to the ternary model (eq 4) with $[G]_t = [R]_t$; other parametric values were as listed in the table and in the legend to Figure 4.

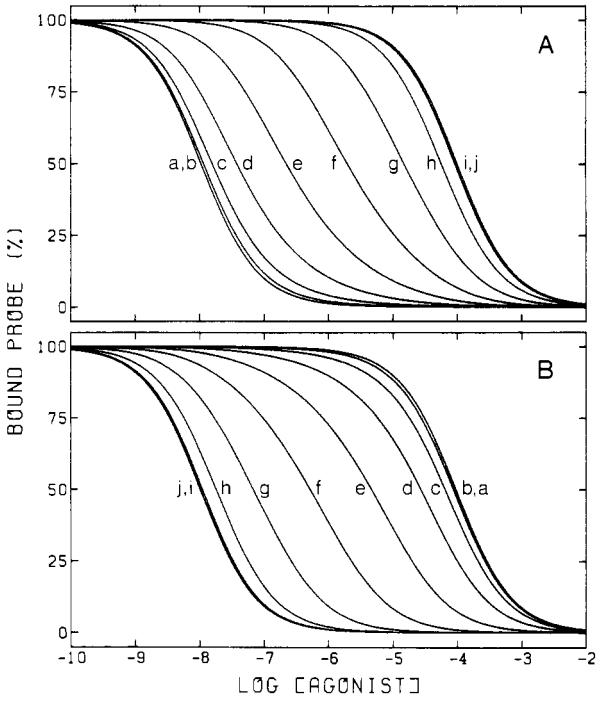


FIGURE 4: Effect of $[R]_t/K_G$ on the behavior of the ternary model. Lines were simulated according to eq 4 with the affinities of the agonist set as follows: (A) log K_A = −4 and log K_{AG} = −8; (B) log K_A = −8 and log K_{AG} = −4. The value of log ($[R]_t/K_G$) was decremented by 1 from +3 (curve a) to −6 (curve j) in (A) and from +7 (curve a) to −2 (curve j) in (B). $[G]_t$ was set equal to $[R]_t$ throughout. Parametric values derived from best fits of the Hill equation (eq 6, n = 1) and eq 5 (n = 2) to the simulated data are listed in Table II (fitted curves not shown).

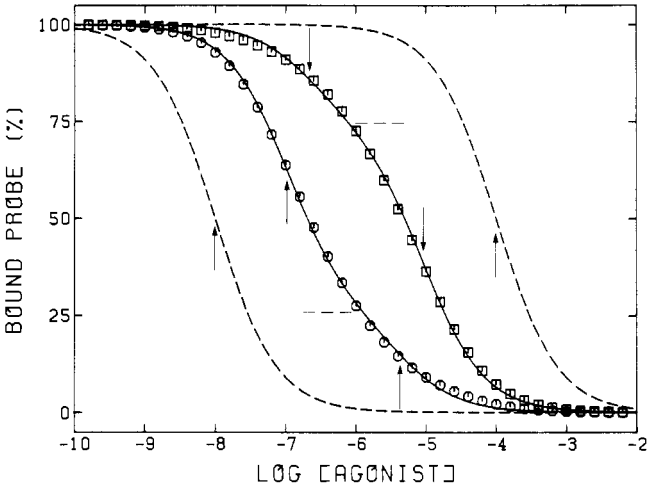


FIGURE 5: Two-site analysis of binding data simulated according to the ternary model with $[G]_t$ set equal to $[R]_t$. Points were simulated according to eq 4 with parametric values set as follows: (○) log K_A = −4, log K_{AG} = −8, and log ($[R]_t/K_G$) = −1; (□) log K_A = −8, log K_{AG} = −4, and log ($[R]_t/K_G$) = +3. The solid lines represent the best fit of eq 5 (n = 2) to the simulated data; parametric values obtained by regression are as follows: (○) log K_1 = −6.98, log K_2 = −5.37, and F_2 = 0.26; (□) log K_1 = −6.65, log K_2 = −5.03, and F_2 = 0.74. Values of log K_S and F_2 are indicated by arrows and by the horizontal dashed lines, respectively. The dashed curves indicate the patterns expected for a uniform population of sites with log K = −8 or −4; the values of log K are indicated by arrows.

(>$10^4[R]_t$ when $K_A/K_{AG} = 10^4$) that coupling remains negligible in the presence of agonist (Table II). If the agonist disfavors the association of G and R (Figure 4B), the Hill coefficient is essentially 1 when K_G is sufficiently large (>$[R]_t$) that R and G are fully uncoupled in the absence of agonist or sufficiently small (<$10^{-6}[R]_t$ when $K_A/K_{AG} = 10^{-4}$) that coupling remains virtually complete in the presence of agonist (Table II). The Hill coefficient passes through a minimum at intermediate values of K_G (Table II, Figure 4). Since the agonist promotes association of G and R when K_A/K_{AG} exceeds 1 and dissociation when K_A/K_{AG} is less than 1, the apparent affinity of the drug (−log IC_{50}) decreases with increasing K_G on the one hand and increases with increasing K_G on the other hand (Table II). The limiting values of IC_{50} yield estimates of K_A and K_{AG}.

The data in the insets to Figure 3 indicate that no agonist, irrespective of its relative affinities for R and RG, can exhibit a Hill coefficient less than a minimum determined by the ratio of $[R]_t$ to K_G. Similarly, no change in $[R]_t/K_G$ can reduce the Hill coefficient of an agonist below a minimum determined by K_A/K_{AG} (Figure 4, Table II). The combination of these restrictions is summarized in the outer frames of Figure 3, where the relationship between n_H and log ($[R]_t/K_G$) is shown for a series of agonists differing in their relative affinities for RG and R. A Hill coefficient of 0.67 is the smallest obtainable

when $[G]_t$ and $[R]_t$ are equal.

Data on the binding of agonists generally are analyzed by assuming a mixture of independent sites (eq 5). It therefore is of interest to consider the results of such an analysis when the data are generated according to the ternary model. The points in Figure 5 were calculated with values of K_A, K_{AG}, and K_G that yield a Hill coefficient of 0.70; the deviation from 1 thus is close to the maximum obtainable when $[G]_t$ and $[R]_t$ are equal. The solid lines represent the best fit of eq 5 with two classes of sites ($n = 2$). Since the two expressions are not equivalent, the fitted curves are seen to deviate from the simulated data. The residuals are within typical, experimental error, however, and it seems unlikely that the deviation would be detected in most investigations.

The failure of eq 5 faithfully to describe the simulated data illustrates an important distinction between binding patterns arising from the two models. For a mixture of independent and dissimilar sites (eq 5), points of inflection measured on the abscissa of a semilogarithmic plot can be related directly to the equilibrium dissociation constants of the ligand for the different classes of sites. With the ternary model, however, Hill coefficients less than 1 arise from the fixed ratio of $[G]_t$ to $[R]_t$ and the mutual depletion of both. As pointed out by Boeynaems and Dumont (1980) and described above, increases in the concentration of agonist are accompanied by changes in the concentration of uncoupled G protein; accordingly, the apparent affinity of an agonist (K_A', eq 1) varies with its concentration. At no point on the two curves shown in Figure 5 is there a simple relationship between the concentration of the drug and its equilibrium dissociation constant for R (K_A) or for the RG complex (K_{AG}). Estimates of K_1 and K_2 obtained by fitting eq 5 ($n = 2$) to the simulated data are shown by the arrows corresponding to the solid lines; the values of K_A and K_{AG} used in the simulation are shown by the arrows corresponding to the dashed lines. The comparison in Figure 5 illustrates that values of K_S derived from eq 5 always are less than the larger of K_A or K_{AG} and exceed the smaller. For systems in which the pools of interacting G proteins and receptors are of equal size, K_1 or K_2 will approach K_A or K_{AG} only as the value of n_H approaches 1 (Table I); that is, the apparent dissociation constants (eq 5) are relevant to the model only when $[R]_t/K_G$ and K_A/K_{AG} are such that the agonist has little effect on the number of receptors coupled to G proteins. The quantity F_2 is not relevant to the ternary model when $[G]_t$ equals $[R]_t$ and does not represent the distribution of receptors between the coupled and uncoupled states; indeed, this distribution is determined by the concentration of the agonist and varies along the curve.

While mechanistically inappropriate, there is a diagnostic advantage to be gained from the interpretation of putative, ternary systems as a heterogeneous mixture of sites. The data in Figure 6 summarize the results obtained when data simulated according to eq 4 are analyzed in terms of eq 5 with two classes of sites. For each fit, the value of log (K_2/K_1) is plotted vs. the fraction of sites (F_2) ostensibly of lower affinity (K_2) for the agonist. Binding studies usually involve several agonists and a preparation of homogenized tissue in which $[R]_t/K_G$ presumably is constant. Accordingly, the solid lines indicate the relationship for different values of K_A/K_{AG} at the same value of $[R]_t/K_G$; successive increases in the absolute value of log (K_A/K_{AG}) yield corresponding but successively smaller increases in the value of log (K_2/K_1) (Table I). The dashed lines illustrate the upper limit on the value of log (K_2/K_1) beyond which the shape of the binding profile is independent of the relative affinity of the agonist for R and for RG. An

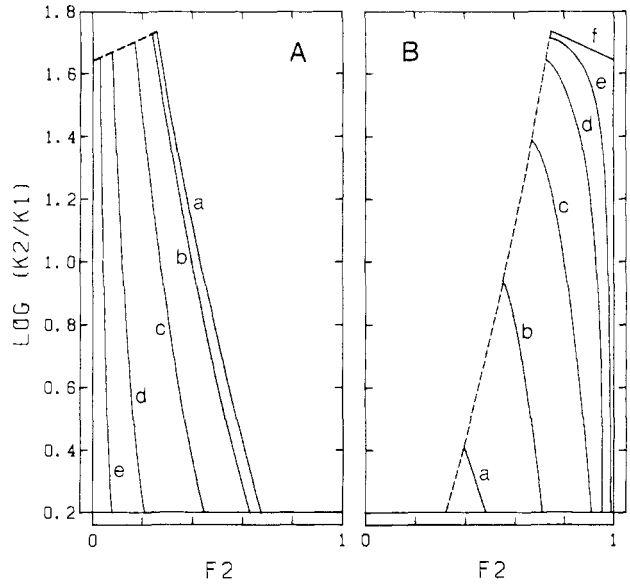


FIGURE 6: Limitations on the apparent heterogeneity possible with the ternary model when $[G]_t$ equals $[R]_t$. Each solid line represents the relationship between log (K_2/K_1) and F_2 for best fits of eq 5 ($n = 2$) to data simulated according to eq 4 with a particular value of log $([R]_t/K_G)$ and values of log (K_A/K_{AG}) between 0 and either +9 (A) or −9 (B). The dashed lines represent the asymptotes at which log (K_2/K_1) and F_2 are independent of further increases in the absolute value of log (K_A/K_{AG}). Values of log $([R]_t/K_G)$ for log $(K_A/K_{AG}) > 0$ (A) are as follows: a, <−2; b, −1; c, 0; d, +1; and e, +2. Values of log $([R]_t/K_G)$ for log $(K_A/K_{AG}) < 0$ (B) are as follows: a, 0; b, +1; c, +2; d, +3; e, +4; and f, >+7.

inspection of Figure 6 reveals that there are restricted domains for the relationship between log (K_2/K_1) and F_2. It is impossible, for example, for the value of log (K_2/K_1) to exceed 0.75 with any agonist that reveals a 50:50 mixture of sites, regardless of whether the value of K_A/K_{AG} exceeds 1 (Figure 6A) or is less than 1 (Figure 6B). Moreover, there is an upper limit of about 1.74 on the value of log (K_2/K_1) obtainable at any value of $[R]_t/K_G$ or at any ratio of K_A to K_{AG}; this follows from the same considerations that account for the lower limit on the value of n_H (Figure 3). Finally, both forms of the model predict a negative correlation between log (K_2/K_1) and F_2 for a series of agonists in a preparation where $[R]_t/K_G$ is constant.

The patterns in Figure 6 have been drawn to describe the consequences of changing K_A/K_{AG} at constant $[R]_t/K_G$. Analogous patterns can be drawn to describe the consequences of changing $[R]_t/K_G$ at constant K_A/K_{AG}. As noted above, the latter approach would correspond to an experiment in which factors known or believed to control the affinity of G for R were studied for their effects on the binding profile of a single agonist. The parametric values listed in Table II are derived from best fits of eq 5 ($n = 2$) to data simulated according to eq 4 and illustrate such a situation under optimal conditions. Since the difference in the affinity of A for R and for RG is large [log $(K_A/K_{AG}) = \pm 4$], the agonist is able to exert a major effect on the equilibrium between G and R at appropriate values of $[R]_t/K_G$; the Hill coefficient thus approaches the lower limit for any system in which $[G]_t$ and $[R]_t$ are equal. With both forms of the model, the value of log (K_2/K_1) first increases slightly and then decreases as K_G is increased from values where coupling of G and R in the absence of agonist is virtually complete to values at which coupling is substantially less. Both forms also predict that a rightward shift in the binding curve is accompanied by an increase in F_2, although this occurs with increasing K_G when K_A/K_{AG} exceeds 1 and with decreasing K_G when K_A/K_{AG} is less than 1.

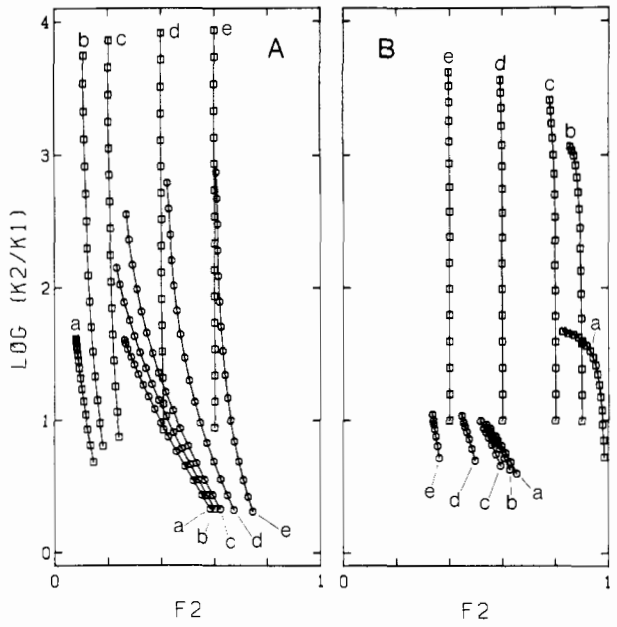


FIGURE 7: Limitations on the apparent heterogeneity possible with the ternary model when $[G]_t$ is less than $[R]_t$. The points represent values of log (K_2/K_1) and F_2 obtained from best fits of eq 5 ($n = 2$) to data simulated according to eq 4. Points lying along the same line reflect simulations carried out at the same values of $[G]_t/[R]_t$ (a, 1.0; b, 0.9; c, 0.8; d, 0.6; and e, 0.4) and log $([R]_t/K_G)$ [(A) +1 (□) and −1 (○); (B) +4 (□) and +1 (○)]. The value of log (K_A/K_{AG}) was incremented by 0.2 log unit from +1 to + 4 in (A) and from −1 to −4 in (B). The simulated data for examples in which $[G]_t/[R]_t$ equals 0.6 are illustrated in supplementary material Figures 2 and 3; parametric values derived from eq 5 ($n = 2$) for representative examples at different values of $[G]_t/[R]_t$ are listed in supplementary material Tables I and II.

$[G]_t$ Greater Than $[R]_t$. When $[G]_t$ exceeds $[R]_t$, formation of the RG complex depletes the pool of unbound G proteins proportionately less than when $[G]_t$ and $[R]_t$ are equal; accordingly, values of n_H (eq 6, $n = 1$) increase toward 1 as $[G]_t/[R]_t$ exceeds 1 (Figure 1, curves g–j). With a 1.4-fold excess of G proteins, calculations analogous to those summarized in Figure 3 indicate a lower limit of 0.85 on the Hill coefficient; a 2-fold excess results in a lower limit of 0.91. Values of log (K_2/K_1) and F_2 that can be obtained from eq 5 ($n = 2$) likewise are more restricted when G proteins outnumber receptors. The permitted domains shrink with increasing $[G]_t/[R]_t$, thereby constituting a progressively smaller portion of those obtained when $[G]_t$ and $[R]_t$ are equal as illustrated in Figure 6. The permitted domains for a 1.4-fold excess of G proteins are illustrated in supplementary material Figure 1 (see paragraph at end of paper regarding supplementary material); the upper limit on log (K_2/K_1) is about 1, and log (K_2/K_1) continues to correlate negatively with F_2 for a series of agonists at any particular value of $[R]_t/K_G$.

$[G]_t$ Less Than $[R]_t$. As the ratio of $[G]_t$ to $[R]_t$ decreases from 1, the simulated binding curves can become discernibly biphasic (Figure 1, curves b–e). Although the computed data are not necessarily congruent with the best fit of a two-site model (Figure 1, curve d), the residuals are sufficiently small that the nonrandom nature of the distribution is likely to be overlooked with experimental data. If the assumption of two classes of sites is maintained (eq 5), such a system can yield values of log (K_2/K_1) and F_2 that fall outside the domains permitted when $[G]_t$ and $[R]_t$ are equal.

The relationships between log (K_2/K_1) and F_2 for a series of agonists in systems with less G protein than receptor are illustrated in Figure 7. Two possibilities are considered for each form of the model: when the agonist favors the RG complex ($K_A/K_{AG} > 1$), the available G protein may be partially coupled to receptor ($[R]_t/K_G = 10$) or predominantly uncoupled ($[R]_t/K_G = 0.1$) in the absence of agonist; when the agonist favors free R ($K_A/K_{AG} < 1$), the available G protein may be fully coupled to receptor ($[R]_t/K_G = 10\,000$) or partially coupled ($[R]_t/K_G = 10$) in the absence of agonist. For each form of the model, agonists with a 10 000-fold difference between K_A and K_{AG} will have little or no effect on the number of coupled G proteins in the first possibility and a substantial effect in the second. Five different values of $[G]_t/[R]_t$ are considered for each of the four conditions presented in Figure 7. Data for equal quantities of $[G]_t$ and $[R]_t$ (curves a) are included to facilitate comparisons with Figure 6. For values of $[G]_t/[R]_t$ less than 1 (curves b–e), a rough approximation of the permitted domain for log (K_2/K_1) and F_2 at any value of $[R]_t/K_G$ can be obtained by comparing the lines for the two values of $[R]_t/K_G$ presented in the figure. It can be seen that the domains differ markedly from those presented in Figure 6 but nevertheless are highly restrictive. Values of log (K_2/K_1) can be much larger when $[G]_t$ is less than $[R]_t$, but the range of possible values of F_2 is narrower.

The data illustrated in Figure 7 indicate that log (K_2/K_1) approaches the absolute value of log (K_A/K_{AG}) only when $[R]_t/K_G$ is sufficiently large for the agonist to have little effect on the total concentration of G-coupled receptors (i.e., [RG] + [ARG] + [PRG]). In the limit of no change, the multisite and ternary models are indistinguishable. Values of K_1 and K_2 derived from eq 5 ($n = 2$) equal either K_{AG} and K_A ($K_A/K_{AG} > 1$) or K_A and K_{AG} ($K_A/K_{AG} < 1$), respectively. Also, the value of F_2 equals either $1 - [G]_t/[R]_t$ ($K_A/K_{AG} > 1$) or $[G]_t/[R]_t$ ($K_A/K_{AG} < 1$); all agonists thus are expected to reveal the same number of sites in one or the other state of affinity.

The absolute value of log (K_A/K_{AG}) exceeds log (K_2/K_1) whenever $[R]_t/K_G$ and K_A/K_{AG} are such that the agonist can foster an appreciable change in the total concentration of coupled receptors (Figure 7). Agonists which differ in the magnitude of their effect reveal different values of F_2, and the negative correlation that exists between log (K_2/K_1) and F_2 recalls that illustrated in Figure 6 for systems in which $[G]_t$ and $[R]_t$ are equal. If K_2/K_1 is less than K_A/K_{AG} or K_{AG}/K_A, either K_1 or K_2 must differ from K_{AG}, as appropriate; similarly, F_2 is not necessarily an approximation of $1 - [G]_t/[R]_t$ or $[G]_t/[R]_t$. Parametric values corresponding to examples from Figure 7 are listed in supplementary material Tables I ($K_A/K_{AG} > 1$) and II ($K_A/K_{AG} < 1$); the simulated data for examples in which $[G]_t/[R]_t$ equals 0.6 are illustrated in supplementary material Figures 2 and 3. When the agonist favors RG over R ($K_{AG} < K_A$), uncoupled receptors are reflected in the sites of lower affinity ($K_{AG} << K_1$ and $K_2 \leq K_A$). Differences between K_2 and K_A tend to be small and often are negligible; K_1 thus exceeds K_{AG} by an amount that largely accounts for the difference between K_2/K_1 and K_A/K_{AG}. When the agonist favors R over RG ($K_A < K_{AG}$), uncoupled receptors are reflected in the sites of higher affinity ($K_A \leq K_1$ and $K_2 << K_{AG}$); K_1 approximates K_A, and the difference between K_2 and K_{AG} thus accounts for the difference between K_2/K_1 and K_{AG}/K_A.

When $[R]_t$ exceeds $[G]_t$, either the upper ($K_A/K_{AG} > 1$) or the lower ($K_A/K_{AG} < 1$) inflection of the binding curve reflects the ternary interaction among agonist, receptor, and G protein. When K_{AG} differs appreciably from K_1 or K_2 (eq 5, $n = 2$) as described above, that portion of the curve will deviate from a rectangular hyperbola. The deviations might

be discernible with experimental data if K_A were to differ sufficiently from K_{AG} and if $[G]_t/[R]_t$ were not so small that only a minor fraction of specific binding reflected the ternary interaction. The parametric values listed in supplementary material Table III reflect best fits of eq 6 ($n = 2$) and eq 5 ($n = 3$) to data simulated according to the ternary model with $[G]_t$ less than $[R]_t$; values of K_A/K_{AG} and $[R]_t/K_G$ were selected to obtain a maximal, agonist-mediated change in the number of G-coupled receptors. Both analyses yield fitted curves that are virtually superimposable with the simulated data. In the first analysis, two classes of sites are assumed to reveal a microheterogeneity that is reflected in the corresponding Hill coefficient; in the second, there are assumed to be three classes of distinct and noninteracting sites.

The analysis in terms of eq 6 illustrates that one component of the inhibition arises from the excess of receptors over G proteins and reflects the interaction of the agonist with free R: The Hill coefficient is near or equal to 1; the corresponding value of K_S closely approximates K_A; and the corresponding value of F_S effectively equals $1 - [G]_t/[R]_t$. The second component reflects the effect of the agonist within the ternary system: the Hill coefficient is less than 1, and the corresponding value of K_S differs from K_{AG}. It is noteworthy that the Hill coefficient corresponding to the ternary interaction (n_{H1} when $K_A/K_{AG} > 1$, n_{H2} when $K_A/K_{AG} < 1$) is controlled by K_A/K_{AG} and $[R]_t/K_G$ in a manner similar to that shown in Figure 3. The minimum obtainable with any combination of K_A/K_{AG} and $[R]_t/K_G$ reflects the potential for a ternary interaction and hence increases with decreasing $[G]_t/[R]_t$; it always exceeds the minimum of 0.67 obtainable when $[G]_t$ and $[R]_t$ are equal.

A similar pattern emerges from an analysis of the simulated data in terms of eq 5 with three classes of sites. The sites either of lowest affinity ($K_A/K_{AG} > 1$) or of highest affinity ($K_A/K_{AG} < 1$) represent the excess of R over G; K_S for those sites equals or closely approximates K_A, and F_S equals $1 - [G]_t/[R]_t$. Sites of the other two classes reflect the ternary interaction among agonist, receptor, and G protein. The relationship between log (K_2/K_1) and $F_2/(F_1 + F_2)$ ($K_A/K_{AG} > 1$), or between log (K_3/K_2) and $F_3/(F_2 + F_3)$ ($K_A/K_{AG} < 1$), is similar to that illustrated in Figure 6; a negative correlation exists at all values of $[R]_t/K_G$, and the domain of possible relationships lies within that found when $[G]_t$ and $[R]_t$ are equal. As $[G]_t/[R]_t$ decreases from 1, the permitted domain shrinks in a manner similar to that observed when $[G]_t/[R]_t$ exceeds 1 (cf. supplementary material Figure 1).

Discussion

The nature of the ternary model narrowly restricts the parametric values that are possible when binding data obtained from such a system are analyzed in terms of the Hill equation (eq 6) or as a mixture of noninterconverting sites (eq 5). Although the present investigation has dealt exclusively with systems in which the radioligand is indifferent to the presence of the G protein ($K_P = K_{PG}$), similar restrictions occur when the radioligand differentiates between free receptor and the RG complex. Also, restrictions analogous to those observed with the present scheme can be expected for any system in which an apparent dispersion of affinities is attributed to random and freely reversible interactions between the receptor and other macromolecules within the membrane. Both the stimulatory and the inhibitory G proteins are oligomeric complexes consisting of α, β, and γ subunits (Northup et al., 1983a,b; Bokoch et al., 1984; Katada et al., 1984a,b,c). It is assumed in the present report that the receptor interacts with the G protein only in its oligomeric form and that the agonist

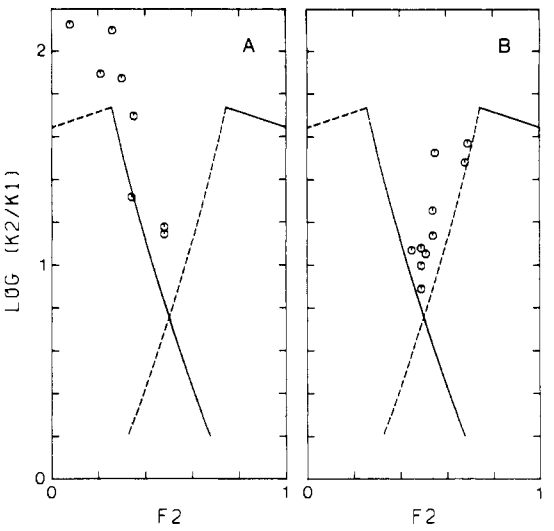


FIGURE 8: Comparison of parametric values derived from two-site analysis of experimental and simulated data. Points represent the parametric values reported for best fits of eq 5 ($n = 2$) to data on the binding of agonists to β-adrenergic receptors from frog erythrocytes (panel A; Kent et al., 1980) and D_2 dopaminergic receptors from bovine pituitary glands (panel B; Sibley & Creese, 1983b). The lines are replotted from Figure 6 and indicate the limits on parametric values obtained from best fits of eq 5 ($n = 2$) to data simulated according to the ternary model with $[G]_t$ set equal to $[R]_t$.

alters the kinetics but not the mechanism of that interaction. Any tendency for the oligomer itself to dissociate while not engaged with the receptor is consistent with the model and would be reflected in the value of K_G. It is noteworthy, however, that the oligomeric nature of the G protein suggests a number of alternatives that may not be consistent with the model. The RG complex may form sequentially, for example, with the α and β subunits associating with the receptor in a stepwise manner; moreover, the ratio of $[G]_t$ to $[R]_t$ may be determined in part by the ratio of $[\alpha]_t$ to $[\beta]_t$. A further complication relates to the effects of magnesium and guanylyl nucleotides on the tendency of the G protein to dissociate (Smigel et al., 1985). The model therefore may be inadequate to describe the molecular events involved, and conclusions related to its applicability are qualified accordingly.

Kent et al. (1980) have reported that agonists discern two classes of sites while antagonists discern one class among β-adrenergic receptors in membranes from frog erythrocytes. Their comparison of the relative capacities derived from a model analogous to eq 5 indicates that F_2 varies significantly from agonist to agonist; moreover, values of log (K_2/K_1) and F_2 correlate negatively ($P = 0.0016$) as predicted by the ternary model. Their data are compared in Figure 8A with the patterns expected from the model when the pools of interacting receptor and G protein are of equal size. The comparison indicates that the binding patterns are consistent with a ternary system in which agonists promote formation of the RG complex ($K_A/K_{AG} > 1$). Furthermore, the data approximate the line that reflects the asymptotic condition in which $[R]_t/K_G$ is less than 0.01; that is, there is virtually no coupled receptor in the absence of agonist. Discrepancies between the theoretical relationship and the data could reflect the error associated with a quantity such as K_2/K_1, particularly when the sites are predominantly in one or the other state of affinity (De Lean et al., 1982a); alternatively, there may have been a small excess of receptors over G proteins (cf. Figure 7).

At least two receptors that attenuate the activity of adenylate cyclase are reported to bind agonists in a manner qualitatively similar to that described by Kent et al. (1980)

for the β-adrenergic receptor. Sibley and Creese (1983b) have studied the binding properties of ergoline agonists at D_2 dopaminergic receptors labeled by [^{3}H]spiroperidol in bovine anterior pituitary. The data were analyzed for two classes of sites to yield values of F_2 that probably are indistinguishable from 0.5 for most of the drugs studied but nevertheless vary from 0.45 to 0.69. Similar behavior has been observed for cardiac muscarinic receptors from Syrian hamsters, where agonists appear to control the distribution of receptors among at least three states of affinity (Wong et al., 1986). The comparison presented in Figure 8B illustrates, however, that the parametric values reported by Sibley and Creese (1983b) for dopaminergic agonists are correlated in a manner that is not consistent with the model. Values of log (K_2/K_1) and F_2 lie outside the permitted domain irrespective of whether the agonist is thought to favor or to disfavor the association between receptor and G protein. Moreover, the value of log (K_2/K_1) appears to increase with the fraction of receptors ostensibly of lower affinity ($P < 0.00001$), and a similar observation has been reported more recently by Wreggett and De Lean (1984). Parametric values reported for agonists at muscarinic receptors are similarly at odds with a ternary system: estimates of relative affinity and fractional capacity lie outside the permitted domains, and there is a general tendency for the former to increase with the latter [see Figure 5 in Wong et al. (1986)]. As illustrated in Figures 6 and 7, the ternary model predicts values of F_2 either to correlate negatively with log (K_2/K_1) or to be the same for all agonists, depending upon the ratio of $[G]_t$ to $[R]_t$; under no circumstance is a series of agonists expected to yield a positive correlation.

The relationships between log (K_2/K_1) and F_2 suggest that the ternary model may describe the binding of agonists to β-adrenergic receptors but not to D_2 dopaminergic or muscarinic cholinergic receptors. Since the activity of adenylate cyclase is stimulated by the former and inhibited by the latter, the tendency for agonists to reveal a negative correlation on the one hand and a positive correlation on the other may reflect mechanistic differences in the interaction of stimulatory and inhibitory receptors with their respective G proteins. It is noteworthy, however, that agreement between the model and the binding patterns of β-adrenergic agonists is not found under all conditions; as pointed out below, the model fails to predict the effects of other agents known or believed to perturb the interaction between β-adrenergic receptors and the stimulatory G protein. Also, Ehlert (1985) recently has provided evidence that the model is in good agreement with the binding properties of muscarinic receptors in rabbit heart; while an analysis in terms of eq 5 ($n = 2$) or an equivalent expression was not reported, it seems likely that the data would yield a negative correlation between log (K_2/K_1) and F_2. It thus is unclear whether or not any mechanistic significance can be ascribed to the comparisons in Figure 8.

The relevance of the ternary model to neurohumoral receptors depends in part upon the relationship between the apparent affinities of eq 5 (K_S) and the intrinsic affinities of eq 2 and 3 (K_A and K_{AG}). For ternary systems with equal quantities of receptor and G protein, at least one of the former must differ from the latter when two classes of sites are required with the multisite model. When the Hill coefficient approaches the lower of 0.67, neither apparent affinity is likely to provide even a first approximation of either K_A or K_{AG} (Figure 5). For systems in which there is an excess of receptors, either K_1 or K_2 is expected to approximate K_A, but the other will agree with K_{AG} only under the extreme condition that virtually all of the available G protein is complexed with receptor at any concentration of agonist. Such a condition can be excluded for any system in which the value of F_2 varies significantly from agonist to agonist, since the agonist is not expected to induce a net change in the number of receptors exhibiting one or the other affinity. It is consistent with systems in which F_2 is found to be constant but implies that the reversible association between receptor and G protein does not lie in the mechanistic pathway leading to a response. If the interaction is slow on the time scale of a pharmacological measurement, the exchange per se is unlikely to be of mechanistic importance; alternatively, a rapid exchange would suggest that the response is unrelated to the net distribution of receptors between coupled and uncoupled states. Both alternatives are difficult to reconcile with the actions of magnesium, guanylyl nucleotides, and similar agents on the binding of agonists; moreover, parameters that reflect the apparent dispersion of affinities within a binding curve are known to reflect correlates of pharmacological response such as efficacy (Birdsall et al., 1977; Ehlert, 1985) and intrinsic activity (Kent et al., 1980). It therefore seems reasonable to conclude that K_1 or K_2 or both (eq 5, $n = 2$) must differ from the corresponding intrinsic affinity (K_A or K_{AG}) if the ternary model is to be considered a tenable hypothesis.[2]

As illustrated in Figure 5, parameters that characterize the multisite and ternary models are expected to exhibit the rank order $K_{AG} < K_1 < K_2 < K_A$ for a ternary system in which agonists favor RG over R. Any shift in the equilibrium between uncoupled and G-coupled receptors is expected to be reflected in the values of K_1 and K_2 (eq 5, $n = 2$), which may increase or decrease within the limits defined by K_{AG} and K_A. Such effects would be noncompetitive with respect to the binding of agonists or antagonists and could arise from changes in either K_G or $[G]_t/[R]_t$. It therefore is of interest to identify conditions that might alter either property and to consider whether or not parametric values derived from eq 5 have been found to change in an appropriate manner. The noncompetitive effects of guanylyl nucleotides on the binding of agonists generally are taken to reflect dissociation of the RG complex. Since the effect occurs at relatively high concentrations of the nucleotide and does not appear to be irreversible, it seems likely to arise from an increase in K_G rather than from any change in $[G]_t/[R]_t$. Conversely, the noncompetitive effects of magnesium could be rationalized, at least in part, as a decrease in the value of K_G.

GTP and its nonhydrolyzable analogues typically are reported to alter the binding of agonists through a reduction in the relative capacity corresponding to the sites of higher affinity and a concomitant increase in that corresponding to the sites of lower affinity (eq 5, $n = 2$); there generally is little or no change in the apparent affinity of the agonist for the sites of either class [see, for example, Kent et al. (1980), Sibley & Creese (1983b), and Wong et al. (1986) and references cited therein]. According to the ternary model, however, the values of K_1 and K_2 found in the presence of the nucleotide are expected to exceed those found in its absence if one assumes that association of R and G is favored by the agonist and disfavored by the nucleotide. For the system illustrated in Figure 5, a nucleotide that increases K_G sufficiently to preclude any interaction between R and G would reduce F_1 to zero and

[2] It has been pointed out above that systems with a small excess of receptors could reveal three classes of sites with eq 5 (supplementary material Table III). While the sites of lowest affinity (K_3, eq 5) correspond to uncoupled receptors (K_A, eq 4; $K_A/K_{AG} > 1$), the fact that three classes can be resolved in itself suggests that K_1 exceeds K_{AG}.

would increase K_2 23-fold from 4.3 μM (log K_2 = −5.37) to 100 μM (log K_2 = −4.00). The increase in K_2 would be smaller with an agonist for which K_A/K_{AG} is relatively small, but the binding curve would be much steeper in the absence of nucleotide (Figure 2) and the value of F_2 much larger. In the report of Kent et al. (1980), the inhibition of (−)-[^{3}H]dihydroalprenolol by (−)-isoproterenol is characterized by values of 1.89 and 0.21 for log (K_2/K_1) and F_2, respectively; their data thus correspond closely to the simulated example of Figure 5. The values reported for individual parameters can be substituted in eq 3 of Wong et al. (1986) to compute the corresponding binding curve (log K_1 = −7.50, log K_2 = −5.60, log K_P = −8.70, F_2 = 0.21, $[P]_t$ = 2 nM, $[R]_t$ = 465 pM, and C = 0.01); an analysis of the simulated data in terms of the ternary model with $[G]_t$ set equal to $[R]_t$ yields the following results: log K_A = −4.5, log K_{AG} < −8.6, and log K_G > −8.4.[3] Guanylyl nucleotides thus would be expected to cause a marked, rightward shift in the position of the binding curve; in contrast, graded concentrations of GMP-PNP were found to be without discernible effect on either K_1 or K_2 (Kent et al., 1980).

Magnesium appears to promote the interconversion of receptors from a state of lower affinity for agonists to a state of higher affinity. Heidenreich et al. (1982) have reported, for example, that magnesium increases the number of β-adrenergic receptors labeled by the agonist [^{3}H]hydroxybenzylisoproterenol in membranes from rat lung but has no effect on the affinity. Similar results have been reported by Hulme et al. (1983) for binding of the agonist [^{3}H]oxotremorine M to muscarinic receptors in ethylenediaminetetraacetic acid (EDTA)-washed preparations from rat myocardium and hippocampus. The failure of magnesium to increase the apparent affinity is difficult to reconcile with the ternary model if one assumes that the cation acts to decrease the value of K_G, thereby favoring the association of R and G. The absence of an effect is particularly noteworthy in view of the relatively large increase in capacity (approximately 85%, Heidenreich et al., 1982; 40–90%, Hulme et al., 1983). If $[G]_t$ equals $[R]_t$ and the agonist favors RG over free R, a decrease in K_G is expected to have little or no effect on K_1 (eq 5) only when most of the receptors are in the coupled state in the absence of magnesium (Figure 4); in contrast, both β-adrenergic and muscarinic receptors appear to be predominantly in a state of lower affinity in the absence of magnesium (Bird & Maguire, 1978; Cech et al., 1980; Hulme et al., 1983). Magnesium-induced changes in K_1 and K_2 have been observed by Sibley and Creese (1983a) for the inhibitory effect of apomorphine on the binding of [^{3}H]spiroperidol to D_2 dopaminergic receptors; in their report, however, the relationships between log (K_2/K_1) and F_2 differ from those expected from a ternary system.

When membranes are pretreated with the sulfhydryl reagent *N*-ethylmaleimide, the binding of agonists generally is altered in a manner similar to that achieved by guanylyl nucleotides and magnesium. With the β-adrenergic receptor from rat lung, the number of sites labeled by [^{3}H]hydroxybenzylisoproterenol is increased with no change in their affinity for the agonist (Heidenreich et al., 1982). With the D_2 dopaminergic receptor from bovine pituitary, the number of sites ostensibly of higher affinity for apomorphine is reduced with a concomitant increase in the number of sites of lower affinity; there is no change in apparent affinity at the sites of either class (Sibley & Creese, 1983a). With the muscarinic cholinergic receptor from rat heart, Harden et al. (1983) have reported that *N*-ethylmaleimide reduces the capacity for [^{3}H]oxotremorine M with little or no change in the affinity. Other conditions that appear to alter the binding of neurohumoral agonists via a selective effect on relative capacity (F_2) rather than affinity (K_1 and K_2) include pretreatment of the β-adrenergic (De Lean et al., 1980) or D_2 dopaminergic receptor (Sibley & Creese, 1983a) with the sulfhydryl reagent *p*-(hydroxymercuri)-benzoate, incubation of the α_1-adrenergic receptor at low temperature (Lynch et al., 1985), and incubation of the D_2 dopaminergic receptor at higher temperature (Kilpatrick et al., 1982).

The various agents that alter the binding of agonists in a noncompetitive manner all appear to do so via an effect on the interaction between the G protein and the receptor. At least some mechanistic features are common to all, since it has been demonstrated in several systems that one agent can reduce or potentiate the effect of another [see, for example, Harden et al. (1982, 1983), Hulme et al. (1983), Lynch et al. (1985), and Steinberg et al. (1985)]. The widespread observation that such agents effect little or no change in the apparent affinities of agonists (eq 5) argues against the notion of a change in K_G within a ternary system. Ehlert (1985) recently has reported, however, that an increase in K_G can account for the effect of GTP on cardiac muscarinic receptors if the ratio of $[G]_t$ to $[R]_t$ is taken as 0.81. In that event, the limb of the binding curve defined by high concentrations of agonists reflects uncoupled receptors as in the examples of supplementary material Table III. An excess of receptors over G proteins is not supported by the report that phenoxybenzamine can be used to inactivate 63% of the D_2 dopaminergic receptors in bovine anterior pituitary with no discernible effect on the inhibition of [^{3}H]spiroperidol by apomorphine (Sibley & Creese, 1983a); a similar result has been obtained recently in our own laboratory, where propylbenzilylcholine mustard has been used to alkylate 70% of the muscarinic receptors in a preparation from hamster heart.[4] Also, the effect of GTP in homogenates of rabbit heart was virtually complete at the concentration used (Ehlert, 1985); agonists bound in an essentially monophasic manner, and in terms of the model, only uncoupled receptor was present in appreciable quantities. The incomplete effect of guanylyl nucleotides found by many investigators [see Wong et al. (1986) and references cited therein] and the graded effects studied by others indicate that values of K_S (eq 5) remain unchanged, in contrast to the prediction of the ternary model. A second, potential source of noncompetitive effects in a ternary system lies in the relative quantities of G protein and receptor; indeed, it has been reported that a change in the value of $[G]_t/[R]_t$ is consistent with the effects of *p*-(hydroxymercuri)benzoate and GMP-PNP on the β-adrenergic receptor (De Lean et al., 1980) and the effects of GMP-PNP on the D_2 dopaminergic receptor (Wreggett & De Lean, 1985). Whereas the effects of sulfhydryl reagents are complex (Birdsall et al., 1983a,b) and presumably irreversible, it is not clear that a similar action can be attributed to GMP-PNP.

The foregoing considerations suggest that the ternary model explored in the present report fails to describe the binding patterns of agonists at neurohumoral receptors associated with a G protein. The mechanistic basis of the model lies in the notion of a reversible equilibrium between two, membrane-

[3] The mathematical representation of the ternary model used for this analysis differs from eq 2–4 in that both ligands (A and P) enter into the equations as total rather than free concentration. The numerical solution will be described elsewhere.

[4] M. A. Green and J. W. Wells, unpublished observations.

bound proteins that are presumed to associate and to dissociate in random fashion such that all members of one pool potentially can interact with all members of the other. A word therefore is in order concerning the possibility that one component of the system is sequestered or compartmentalized in a region of the membrane that is inaccessible to the other. As pointed out by Neubig et al. (1985), the large excess of G proteins over α_2-adrenergic receptors in human platelets indicates that most of the former are not free to interact with the latter if the ternary model is to account for the behavior of agonists. Receptors localized in a compartment effectively devoid of G protein would appear as a subclass of independent sites and ought to be detectable in binding studies with several agonists. The patterns might be difficult to resolve, however, if there were additional complications such as an excess of receptors over G proteins within the ternary system. Further experiments designed to test such possibilities would be helpful.

It is of interest to consider the possibility that the ternary model is conceptually accurate but lacking in detail. Extensions of the model can be envisaged that would accommodate such factors as the oligomeric nature of the G protein or the effect of other receptors with a propensity to interact with the G protein or a subunit thereof. It seems unlikely, however, that such extensions would alter the basic premise of the model; namely, that the low Hill coefficients of agonists arise at least in part from changes in the free concentrations of one or more interacting proteins. Accordingly, there will continue to be limits on the shape of the binding patterns, and estimates of affinity derived from conic expressions can be expected to differ from the dissociation constants for the interactions involved. It therefore becomes relevant to consider whether or not the estimates of affinity derived from eq 5 or related expressions behave as one might expect for equilibrium dissociation constants. The evidence considered in the present report suggests that they do. Although the agreement is not universal, many investigators report that guanylyl nucleotides, magnesium, sulfhydryl reagents, and even temperature alter relative capacity with little effect on apparent affinity. Moreover, solubilized receptors have been found recently to bind agonists in a multiphasic manner, and estimates of affinity for some classes of sites approximate those measured in suspension [see, for example, Berrie et al. (1984), Hulme et al. (1983), and Horodeckyj & Wells (1985)]. As described in the present report, the low Hill coefficients predicted by the ternary model when $[G]_t$ equals $[R]_t$ occur only for values of $[R]_t/K_G$ within a narrow range; it seems unlikely that such conditions are maintained upon dissolution of the membrane. Finally, the model predicts that any change in the relative numbers of receptors and G proteins would alter the binding patterns of agonists. The failure of investigators to observe such changes with either the D_2 dopaminergic (Sibley & Creese, 1983a) or the muscarinic receptor (Birdsall et al., 1978) is contrary to the basic premise that the interaction is freely reversible on the time scale of a binding assay or a response.

The inadequacies of the ternary model leave open questions related to the nature of the interaction between the receptor and the G protein. Recent data on solubilized and reconstituted preparations indicate, however, that sites of higher affinity for agonists appear spontaneously in the presence of both constituents (Cerione et al., 1984; Shreeve et al., 1984; Florio & Sternweis, 1985; Haga et al., 1985). Membrane-bound receptor and G protein thus may form an oligomeric complex within which the interactions between agonists and guanylyl nucleotides are mediated allosterically in a manner analogous to that described by Koshland et al. (1966). Different states of affinity would arise not from collision coupling but rather from changes in the energies of interaction among the constituents of the complex. The tendency for such a complex to retain its quaternary integrity upon solubilization may vary from receptor to receptor and depend upon experimental conditions; its ultimate dissociation may account, however, for the observation that agonists bind to a fraction of solubilized muscarinic receptors from heart with affinities substantially weaker than those measured in membranes (Berrie et al., 1984; Horodeckyj & Wells, 1985). It remains unclear how such an oligomeric complex might give rise to the multiphasic binding patterns revealed by agonists and particularly to the differences observed among agonists in the number of sites ostensibly of one or other affinity.

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SUPPLEMENTARY MATERIAL AVAILABLE

Three tables and three figures depicting the behavior of the ternary model when $[G]_t/[R]_t$ is not equal to 1 (7 pages). Ordering information is given on any current masthead page.

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