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Theoretical Effects of Single and Multiple Transducer Receptor Coupling Proteins on Estimates of the Relative Potency of Agonists

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SUMMARY

A mathematical model is presented that simulates the steady state kinetics of agonists interacting with a promiscuous receptor. The model system consists of a single receptor that forms a ternary complex with either of two transducer proteins (G proteins). At a given agonist concentration, the concentrations of the two ternary complexes are determined by the relative quantities of the two G proteins and the ratio of the dissociation constants for the two ternary complexes. Accordingly, the potency of an agonist is dependent upon the relative quantities of the G proteins. If receptors are truly promiscuous and if the distribution of G proteins varies with tissue type, then the agonist potency ratio would be tissue dependent as well as receptor dependent. Experimental data from literature studies are reviewed in the context of the promiscuous receptor model, and implications of the model regarding pharmacologic classification of receptors are discussed.

The classification of receptors is based on the quantification of their interaction with endogenous and foreign ligands. Two basic types of interaction are observed, namely, agonism and antagonism, and both of these can be studied in biochemical or functional (whole organ) systems. As data are accumulated on the mechanisms of receptor activation, it is apparent that the membrane-bound transducer proteins (G proteins) play a critical role in the translation of both the type and strength of the messages that agonists are able to impart to cells. What is less clear are the effects these coupling proteins have on the quantitative scales used by pharmacologists to classify drug receptors in functional organ systems.

The following is an analysis of the effects of qualitative and quantitative differences in coupling proteins on estimates of the relative efficacy of agonists in intact systems. Specifically, this paper aims to explore the consequences of permitting a single receptor to interact with two transducer proteins in the same membrane. A preliminary account of this work has been presented elsewhere (1).

Methods

System Components

The model consists of the following membrane and cellular components, as shown in Fig. 1A.

Receptor (R). Agonist A interacts with a transmembrane protein, R, which has at least two recognition domains; an extracellular domain for the binding of drugs and an intracellular (or perhaps intramembrane) domain for the binding of transducer proteins.

Stimulus transduction proteins (G_1, G_2). In light of abundant evidence that transducer proteins are GTP-dependent, these will be referred to as G proteins. There are two separate G proteins (designated G_1 and G_2), both membrane bound and each controlling separate cellular functions.

Intermediate stimuli (S_1, S_2). Ternary complexes, ARG_1, and ARG_2, initiate the production of intermediate stimuli, S_1 and S_2, respectively. Ultimately, S_1 and S_2 produce a cellular change of state, i.e., effect.

Summation stimulus (S_3). At some point in the cascade of biochemical reactions that control the cellular metabolic state, the intermediate stimuli are combined to yield a summation stimulus, S_3.

Cellular effect (E). The response, E, is postulated to be a nonlinear monotonically increasing function of S_3.

Model Assumptions

Excess agonist. It is assumed that total agonist concentration greatly exceeds total receptor concentration. Thus, the concentration of free agonist is equal to total agonist concentration.

Conservation of receptor. The concentration of free receptor, $[R]$, is given by:

$$[R] = [R_t] - [AR] - [ARG_1] - [ARG_2] \quad (1)$$

where $[R_t]$ = total receptor concentration; $[AR]$ = concentration of the agonist-receptor complex; $[ARG_1]$ = concentration of the G_1 ternary complex; and $[ARG_2]$ = concentration of the G_2 ternary complex.

Conservation of G proteins. For G proteins G_1 and G_2, the concentration of free G protein, $[G_i]$, is given by:

ABBREVIATIONS: G protein, guanine nucleotide-binding protein.

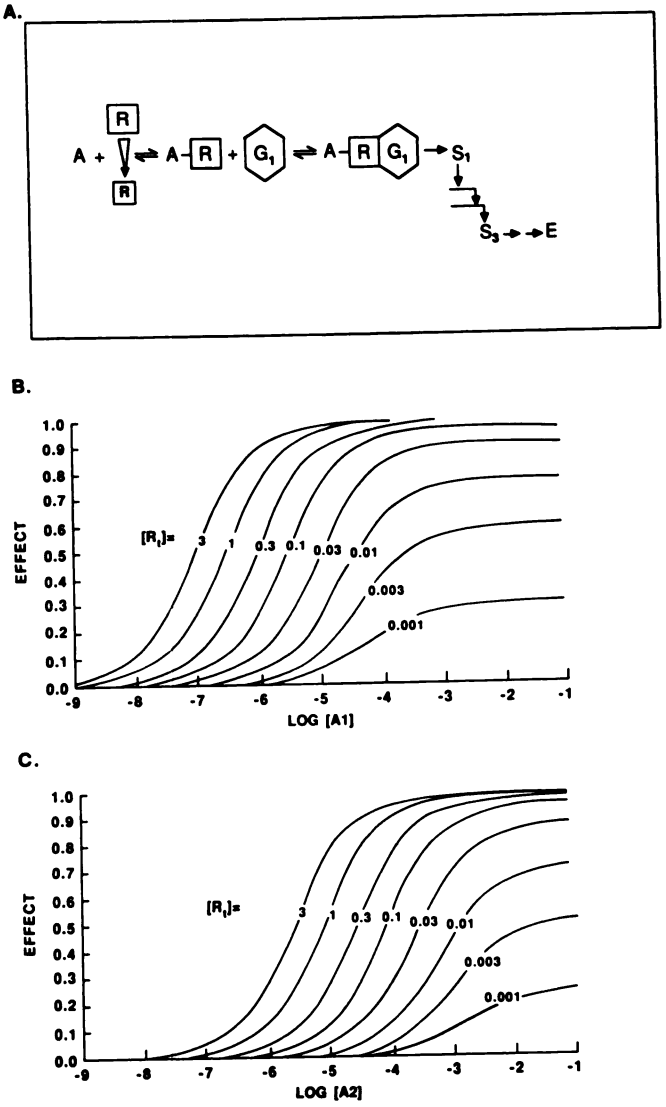

Fig. 1. Effects of the depletion of receptors (R), relative to the amount of transducer protein (G_1), on the effects of agonists. A shows a schematic representation of the molecular association of A, R, and G_1. The effects of receptor depletion on the responses to agonist *A1* and *A2* are shown in B and C, respectively. *Ordinates* are agonist effect; *abscissae* are logarithms of the molar concentrations of the agonists. The kinetic constants for the agonist interactions with the receptor and the resulting interaction with the coupling protein are given in Table 1. For these calculations, $[G_1] = 10$; $[R]$ varies from 3 to 0.001 as shown on the graph.

$$[G_i] = [G_{it}] - [ARG_i] \quad (2)$$

where $[G_{it}]$ = total concentration of the G_i protein; and $[ARG_i]$ = concentration of the G_i ternary complex.

Receptor equilibria. It is assumed that the law of mass action governs interactions involving receptor species:

$$K_a = [A]\ [R]/[AR] \quad (3)$$

where K_a is the dissociation constant for the AR complex.

G protein equilibria. It is assumed that the law of mass action governs interactions involving G proteins:

$$K_{gi} = [G_i]\ [AR]/[ARG_i] \quad (4)$$

where K_{gi} = the dissociation constant for ARG_i.

Intermediate stimuli. It is assumed each ternary complex, ARG_i, leads to the production of an intermediate stimulus, S_i, in accordance with a hyperbolic function:

$$S_i = [ARG_i]/([ARG_i] + B_i) \quad (5)$$

where B_i is a chain constant. Selection of a rectangular hyperbola to model the terminal stages of the stimulus-response cascade is based on the observation that many experimental data appear to fit a hyperbolic relationship (2–5).

Summation stimulus. The summation stimulus, S_3, is assumed to be the arithmetic sum of intermediate stimuli S_1 and S_2:

$$S_3 = S_1 + S_2 \quad (6)$$

Cellular response. The final stimulus-response coupling function is given by:

$$E = S_3/(S_3 + B_c) \quad (7)$$

where E = cellular response; S_3 = summation stimulus; and B_c = cellular chain constant.

This function models the frequently observed finding that cells amplify receptor stimuli and that diminution of receptor number does not lead necessarily to depression of the tissue maximal response.

Stimulus-response models consist of stages that can be broadly categorized as related to either receptor equilibria or signal amplification. Assumptions pertaining to receptor equilibria are critical for the promiscuous receptor model because they specify the partitioning of signal transduction along parallel paths. Assumptions relating to signal amplification, i.e., the mathematical approximations for S_1, S_2, S_3 and E, are included primarily to provide the model with an output response. Consequently, the general predictions of the promiscuous receptor model are not dependent on the specific mathematical formulations employed at the signal amplification stage.

Computer Simulation

Simulation of the promiscuous receptor model was implemented using RS/1, a scientific data analysis package from BBN Software Products (Cambridge, MA). The simulation system, designated G PROTEIN, consists of a table of parameters and functions that return values of the components of the model.

In describing the simulation, it is convenient to distinguish three classes of quantities: 1) input parameters; 2) $[AR]$-dependent concentrations; and 3) terminal variables.

Input parameters are known quantities. These include the following: $[A]$, $[R_t]$, $[G_{1t}]$, $[G_{2t}]$, K_a, K_{g1}, K_{g2}, and B_c.

$[AR]$-dependent quantities are concentrations whose calculation depends on knowledge of the input parameters and $[AR]$. These include the following: $[R]$, $[ARG_1]$, $[ARG_2]$, $[G_1]$, and $[G_2]$. The equation for $[ARG_i]$ is obtained by substituting for $[G_i]$ from Eq. 2 into Eq. 4:

$$[ARG_i] = [G_{it}]\ [AR]/(K_{gi} + [AR]) \quad (8)$$

Given $[ARG_i]$, $[G_i]$ can be calculated from Eq. 2. $[R]$ is obtained from Eq. 3.

Terminal variables include S_i, S_3, and E. These quantities are calculated from $[ARG_i]$ and the input parameters by chaining Eq. 5, 6, and 7.

It is apparent that stimulation of the promiscuous receptor model depends on estimating equilibrium concentrations of $[AR]$. To this end, the expression for $[R]$ from Eq. 1 is substituted into Eq. 3:

$$[AR] = ([R_t] - [ARG_1] - [ARG_2])/(K_a/[A]+1) \quad (9)$$

Substituting in Eq. 9 for $[ARG_1]$ and $[ARG_2]$ from Eq. 8 yields an expression for $[AR]$ that is dependent only on the input parameters. Because this equation cannot be solved explicitly, the Newton-Raphson method (6) was used to obtain numerical estimates for $[AR]$.

Parameter Values

The following parameters have been chosen to illustrate the properties of this model. The effects of two agonists, *A1*, and *A2*, will be

calculated. For simplicity, both will have the same equilibrium dissociation constant for the receptor (10^{-2} M). In accordance with Black and Leff (7), intrinsic efficacy, described by Furchgott (8) as the quantal unit of stimulus imparted to the receptor by a given agonist, will be defined as the reciprocal of the equilibrium dissociation constant of the activated receptor-transducer protein complex. Thus, an agonist that produces an agonist-receptor complex (or activated receptor) with a high affinity for the transducer protein will be regarded as having a high intrinsic efficacy. Under these circumstances, the equilibrium dissociation constant of the agonist-receptor-transducer complex will be a direct measure of the intrinsic efficacy of the agonist.

For these calculations, *A1* will be G_1 protein selective, in that the affinity of the receptor, when occupied (or activated) by agonist *A1*, will be higher for transducer protein G_1 than for G_2. Similarly, agonist *A2* will be G_2 protein selective. The respective equilibrium dissociation constants of the agonist-activated G protein complexes for each agonist-transducer protein pair are given in Table 1.

These constants permit comparison of the effects of two agonists that differ in intrinsic efficacy but not in receptor affinity. For simplicity, the nonlinear chain constants were assumed to be equal ($B_1 = B_2 = 0.3$) and chosen to model a nonlinear relationship between receptor occupancy and intermediate receptor stimulus. The summation stimulus, B_c, was assigned a value of 0.01 to prevent diminutions in receptor number from reducing maximal tissue response (as has been found in experimental studies on the relationship between irreversible receptor inactivation and maximal tissue response (9).

Results

One receptor and one transducer protein: effects of receptor depletion. Initially, the most simple model was to assume one receptor (R) interacting with one G protein (designated G_1); this is shown schematically in Fig. 1A. The calculated dose-response curves to agonists *A1* and *A2* (according to the equilibrium dissociation constants given in Table 1) were calculated for varying concentrations of receptor (R_t). The effects of serial decreases in the amounts of R_t (relative to G_1) on the dose-response curves to *A1* and *A2* are shown in Fig. 1, B and C, respectively. The molecular constants used in the calculations were chosen to mimic the dextral displacement and depression of maximal response to high efficacy agonists with receptor depletion observed experimentally with receptor-alkylating agents (8, 9).

A curve-by-curve comparison of the relative responses to *A1* and *A2* at each receptor concentration indicates that the relative potencies of these agonists, as expected from classical receptor theory, does not change with decreasing receptor number. The graphical and numerical demonstrations are shown in Fig. 8 in the Appendix. Similarly, the relative intrinsic efficacy of the two agonists, as calculated by the method of Furchgott (8), does not change with changes in the relative concentrations of R and G_1.

One receptor and one transducer protein: effects of transducer protein depletion. The effects of depletion of the transducer protein in the presence of a constant amount of receptor are shown in Fig. 2 (schematic shown in Fig. 2A). The calculations were done for agonists *A1* and *A2* (Table 1) and, as with the depletion of receptor number, the dose-response curves to the agonists were shifted to the right and the maximal responses were depressed (Fig. 2, B and C). The relevant feature of this calculation, as that for Fig. 1, is that a comparison of the relative dose-response curves to agonists *A1* and *A2*, with a given ratio of amounts of receptors to transducer proteins, indicates no differences in the relative potency or efficacy (as predicted by classical receptor theory; see Appendix, Fig. 9).

Relative potency of agonists: different receptor G protein pairs. As expected, the model predicts that totally different estimates of the relative potency of agonists *A1* and *A2* for the same receptor would be obtained in different organs, if they were coupled to different G proteins in these organs. Fig. 3A shows the relative potency of *A1* and *A2* when receptor R is

TABLE 1

Parameters for simulation of dose-response data

K_1 and K_2 refer to the equilibrium dissociation constants of the receptor/G protein complexes when the receptor is activated by agonists; subscripts 1 and 2 designate coupling proteins G_1 and G_2, respectively.

Agonist	K_A	K_1	K_2	K_1/K_2
A1	0.01	0.1	1	0.1
A2	0.01	3	0.1	30

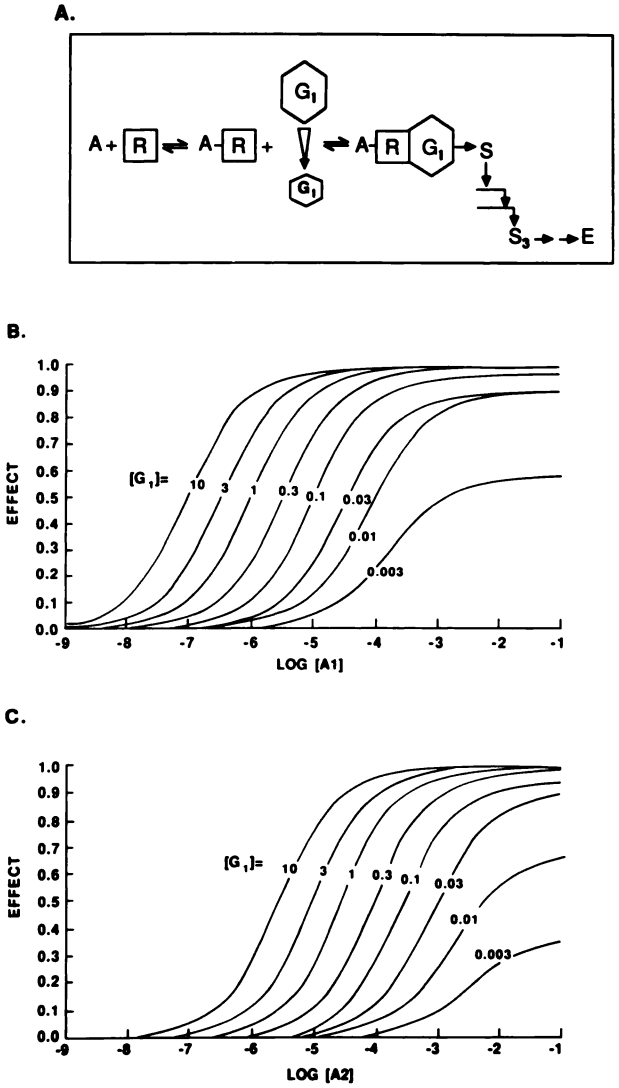

Fig. 2. Effects of the depletion of transducer protein (G_1), relative to the concentration of receptors, on the responses to *A1* and *A2*. A shows a schematic representation of the molecular association of *A*, *R*, and G_1. The effects of transducer protein (G_1) depletion on the responses to agonist *A1* and *A2* are shown in B and C, respectively; *axes* are as for Fig. 1. The kinetic constants for the agonist interactions with the receptor and the resulting interaction with the transducer protein are given in Table 1. For these calculations, $[R] = 3$; $[G_1]$ varies from 10 to 0.003 as shown on the graph.

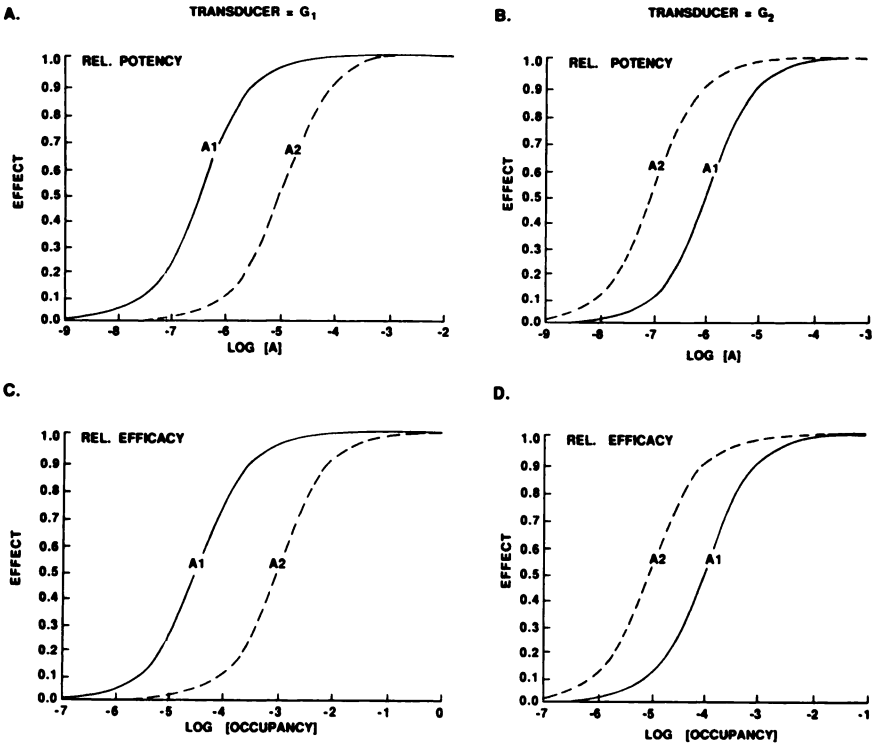

Fig. 3. The relative potency of agonists *A1* and *A2* when receptor *R* is coupled to two different coupling proteins in two different tissues; *axes* for *A* and *B* are as for Fig. 1. A, Receptor *R* is coupled to G_1; $[R] = 3$, $[G_1] = 3$, kinetic constants according to Table 1. B, Receptor is coupled to G_2; $[R] = 3$, $[G_2] = 10$. *Ordinate axes* for C and D are effect; *abscissae* are the logarithms of the fractional receptor occupancy [defined as $([AR] + [ARG_1] + [ARG_2])/[R_t]$]. C, The relative efficacy of *A1/A2* when the receptor is coupled to G_1 (from Fig. 3A). D, The relative efficacy of *A1/A2* when the receptor is coupled to G_2 (from Fig. 3B).

coupled to G_1; the relative potency of *A1/A2* is 30. Fig. 3B shows the relative potency of the agonists in a tissue in which the receptor is coupled to G_2; the relative potency of *A1/A2* in this case is 0.1. Assuming no change in the affinity of the agonists for the receptor between the two tissues, these differences in the relative potency also translate into differences in the relative efficacy of the two agonists. Thus, in the tissue possessing coupling protein G_1, agonist *A1* is 30 times more efficacious than agonist *A2* (Fig. 3C). In the tissue with coupling protein G_2, agonist *A1* has only one tenth the efficacy of agonist *A2* (Fig. 3D).

One receptor and two G proteins: effects of receptor depletion. The relative potency of agonists *A1/A2* then was calculated for receptor *R* when it is present in a membrane containing both G_1 and G_2; this is shown schematically in Fig. 4A. For these calculations, it was assumed that $[G_1] = 10$ and $[G_2] = 1$ and that receptor *R* is promiscuous with respect to interaction with either. Under these circumstances, the relative potency of *A1* and *A2* depends upon the relative quantities of G_1 and G_2 and the relative magnitudes of the equilibrium dissociation constants of the agonist/receptor/G protein complexes.

Intuitively, it would not be predicted that the relative quantity of receptor would affect the relative potency of the agonists. Fig. 4B shows the effects of receptor depletion on the potency of *A1* in a one receptor/two G protein system. Similarly, the potency of *A2* is shown in Fig. 4C. The potency ratios of these agonists with different quantities of $[R]$ are shown in the Appendix. These calculations indicate that the potency ratio and relative efficacy of *A1/A2* does not change as the receptor number, relative to the quantities of G protein, is altered. This assumes that the relative quantities of G protein do not change with respect to each other (see Appendix, Fig. 10).

One receptor and two G proteins: effects varying relative quantities of G proteins. The relative potency of *A1* versus *A2* then was calculated for systems with varying relative quantities of G_1 and G_2. Initially, the potency of the agonists was calculated for an essentially pure G_1 system (i.e., $[G_1] = 1$, $[G_2] = 0.001$). This is shown schematically in Fig. 5A, and the effects on the potency of *A1* and *A2* is shown in Fig. 5, B and C, respectively. These figures show that, as the relative quantity of G_1 diminishes with respect to G_2, the potency of the predominantly G_1-dependent agonist *A1* is affected more than the potency of the predominantly G_2-dependent agonist *A2*.

A curve-by-curve comparison of the effects of *A1* and *A2* in systems of varying relative ratios of G_1 and G_2 is shown in Fig. 6. In a G_1-dominant organ ($[G_1] = 1$, $[G_2] = 0.001$), *A1* is 30-fold more potent (Fig. 6A) and efficacious (Fig. 6B) than *A2*. This calculation was repeated for selected increases in the quantity of G_2 with a constant amount of G_1 ($[G_1] = 1$). The resulting dose-response curves are shown in Fig. 6, C, E, G, and I and the relative efficacy curves are shown in Fig. 6, D, F, and H. The interesting feature of these curves is the change in the relative potency and the relative efficacy of agonists *A1* and *A2*. Thus, in a tissue with predominantly G_2 ($[G_1] = 1$, $[G_2] = 100$; Fig. 6, I and J) agonist *A1* is now 10-fold *less* potent than *A2*. This occurs with no change in the affinities of *A1* and *A2* for the receptor.

The change in the relative efficacy of *A1* and *A2* with changes in the relative quantities of G_1 and G_2 is shown in Fig. 7. It can be seen from this figure that, if the receptor is promiscuous with respect to the transducers that it utilizes to produce response, the relative efficacy is subject to the relative quantities of coupling proteins.

Discussion

The relative efficacy of agonists as a drug receptor-related quantity has been a precept of classical receptor pharmacology

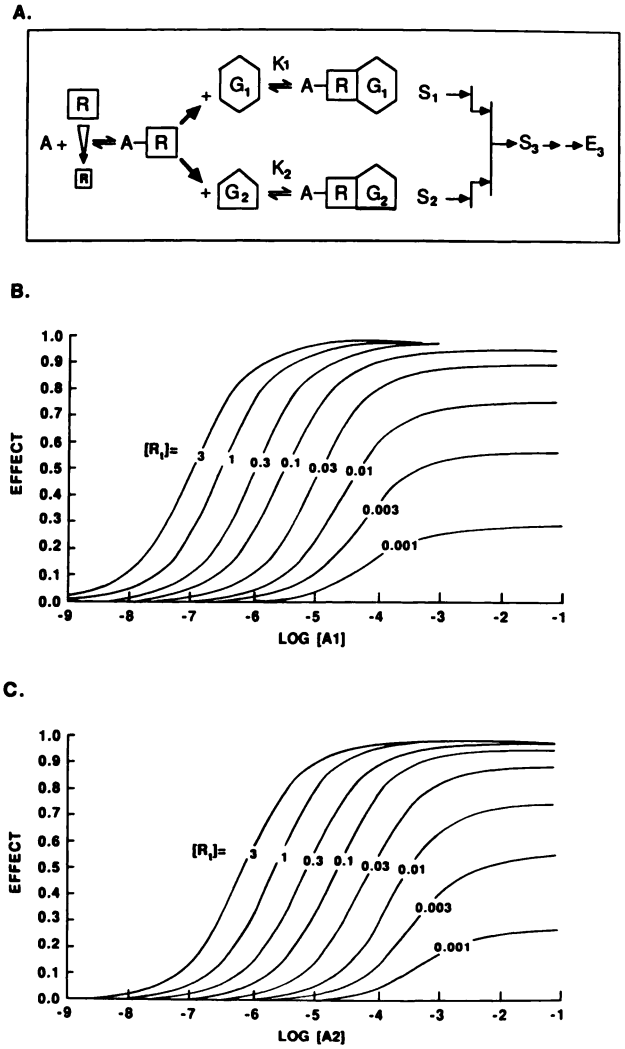

Fig. 4. The effects of receptor depletion on the potency of agonists *A1* and *A2* in a membrane containing both G_1 and G_2. A, This shows a schematic of the molecular associations between receptor *R* and coupling proteins G_1 and G_2. *Axes* for B and C are as for Fig. 1. B, The effects of reducing [*R*] from 3 to 0.001 on the potency of *A1*. C, The effects of the same reduction in receptor number on the potency of agonist *A1*. For these calculations, $[G_1] = 10$, $[G_2] = 1$ and the kinetic constants are from Table 1.

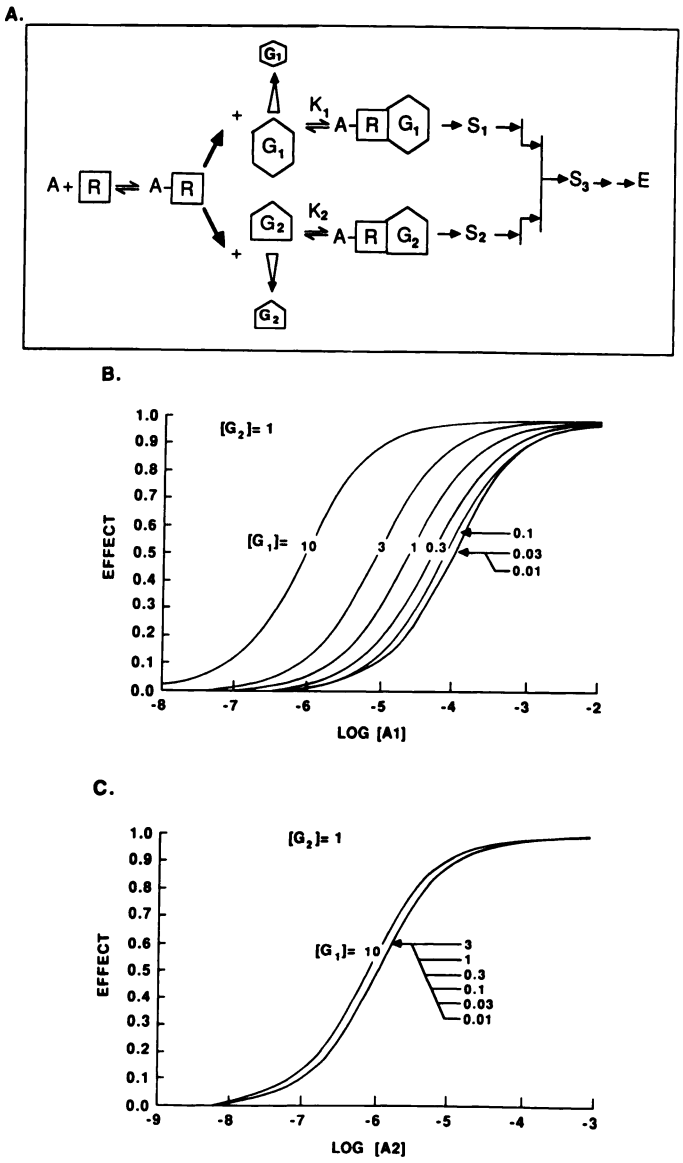

Fig. 5. The effects of reducing the relative concentration of $[G_1]$, with respect to $[G_2]$, in a membrane containing both G_1 and G_2 and a promiscuous receptor that can interact with both. A, Schematic diagram of the membrane components. B and C, *Ordinates*, agonist effect; *abscissae*, logarithms of the molar concentrations of the agonists. B, Effects, on the potency of *A1*, of reducing $[G_1]$ from 10 to 0.001 while $[G_2]$ is kept at $[G_2] = 1$. C, Effects of the above relative changes in $[G_1]$ and $[G_2]$ on the potency of *A2*.

since the introduction of the concept by Ariens (10). The relative potency and efficacy of agonists subsequently has been a very valuable tool in the classification of drugs and drug receptors (8, 11). Implicit in the use of relative efficacy as a quantitative scale for receptor classification has been the idea that the nature of the receptor-coupling mechanism does not affect the relative efficacy of agonists, i.e., the agonist-specific step in the stimulus-response chain is related only to the drug receptor.

The idea that the drug receptor forms a complex with the agonist, is qualitatively altered by the interaction, and, subsequently, combines with another membrane-bound protein to produce cellular response fundamentally has altered the concept of drug action (12, 13). There is abundant experimental evidence to support this model of agonism and it has important implications for the use of intrinsic efficacy as a pharmacological scale for receptor classification. In this scheme, the receptor has two recognition domains, namely, the recognition domain for the agonist and a recognition domain for the transducing protein. Therefore, changes in the tertiary structure of either of these domains would constitute a change in what an agonist could detect as the tertiary conformation of the receptor. In contrast, a change only in the extracellular domain for the recognition of drugs would be detected by an antagonist. Under these circumstances, a different index of classification could be observed if the classification were accomplished by agonists versus antagonists. If verified experimentally, this would constitute a significant change in the way that pharmacologists classify drug receptors and exploit pharmacologic data therapeutically. This paper is a presentation of the theoretical pre-

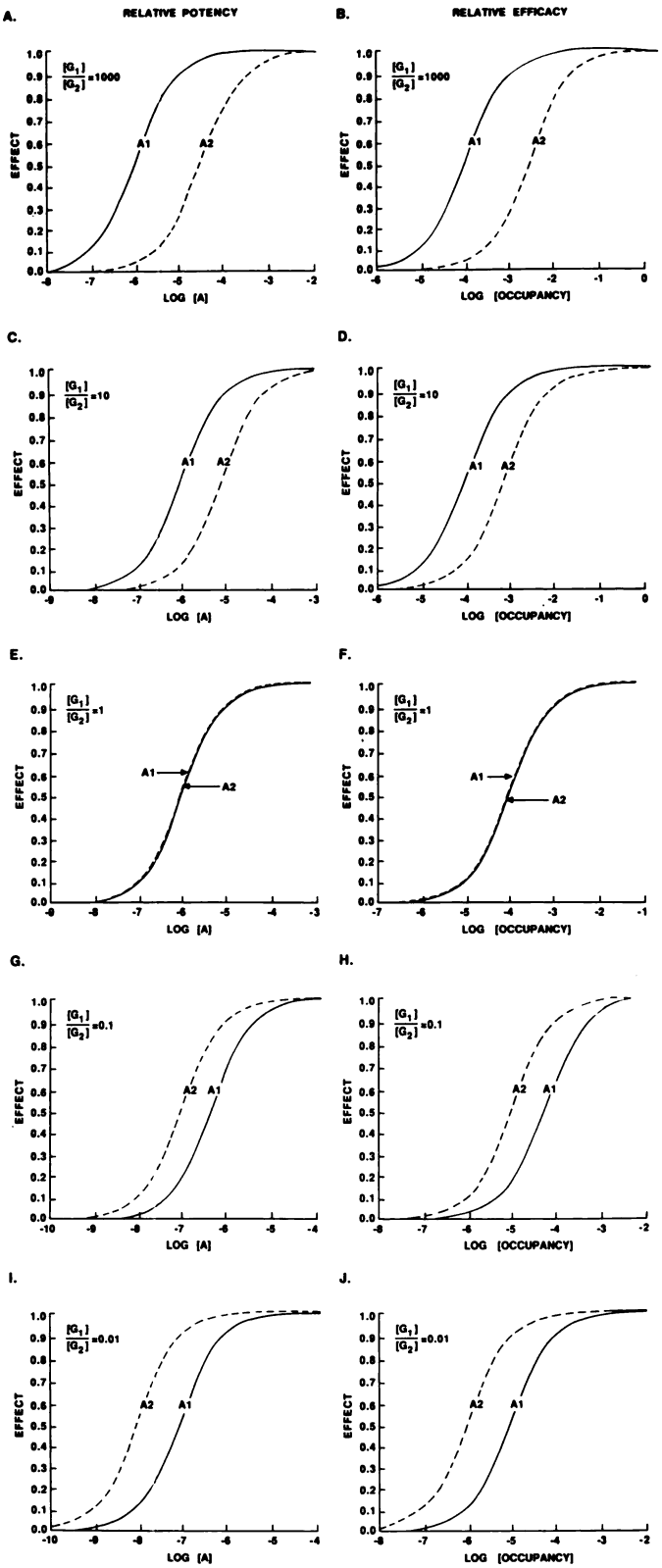

Fig. 6. The relative potency (A, C, E, G, I, and I) and efficacy (B, D, F, H, and J) of agonists *A1* and *A2* in systems with differing relative quantities of G_1 and G_2. *Ordinates*, effect; *abscissae* for relative potency, logarithms of the molar concentrations of *A1* and *A2*; for relative efficacy, logarithms of the calculated fractional receptor occupancy for *A1* and *A2*. For A and B: $[G_1] = 1$, $[G_2] = 0.001$; for C and D: $[G_1] = 1$, $[G_2] = 0.1$; for E and F: $[G_1] = 1$, $[G_2] = 1$; for G and H: $[G_1] = 1$, $[G_2] = 10$; for I and J: $[G_1] = 1$, $[G_2] = 100$.

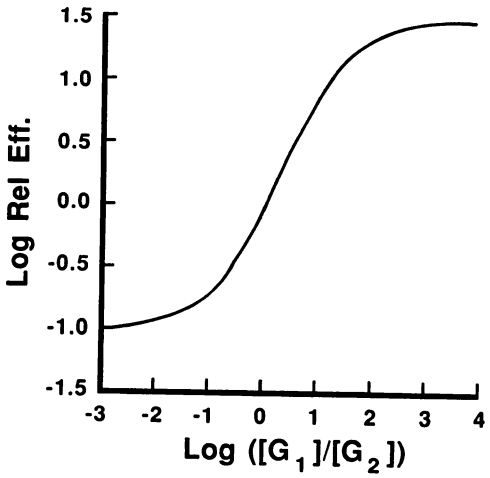

Fig. 7. The effects of the relative concentrations of G_1 and G_2 on the relative efficacy of *A1* and *A2*. *Ordinates*, the logarithms of the relative efficacy of *A1* and *A2*; *abscissae*, the logarithms of the relative amounts of $[G_1]$ and $[G_2]$. As the relative amount of G_1 increases, with respect to G_2, the relative efficacy of *A1* versus *A2* changes from 0.1 to 30.

dictions of the effects of agonists in one receptor/two G protein systems. Fundamental to this analysis is the assumption that different agonists do not produce identically activated receptor states for subsequent combination with transducer proteins to produce ternary complexes, i.e., the nature of the agonist/receptor complex constitutes the molecular basis of intrinsic efficacy as a drug receptor-related property.

These predictions were illustrated with two agonists, *A1*, which produced an activated receptor that preferred binding to transducer protein G_1, and *A2*, which produced a G_2 transducer protein-preferred complex. With respect to the relative applicability of this model to pharmacologic systems, comments on the assumptions made in terms of the relative efficiency of receptor coupling are relevant. First, it should be noted that the kinetic equations used in this model assume free diffusion, whereas the physical system described is one of membrane-bound proteins with two-dimensionally constrained mobility. Therefore, differences between the actual magnitude of the parameters calculated by this model and those in real cell systems would be expected (14). Second, these calculations were carried out for additive stimuli that produce qualitatively similar responses. Equally plausible would be the calculation of the effects in a system in which the stimuli opposed each other (e.g., contraction versus relaxation). However, the major prediction of the promiscuous receptor hypothesis is that agonist potency ratios will be tissue (i.e., cell type) dependent and not only receptor dependent; therefore, the relative orientation of the stimuli (whether additive or subtracting) does not affect this prediction. For simplicity, a single scenario of additive stimuli was chosen. The use of the general logistic function in the coupling of receptor stimulus to tissue response should not be regarded as significant with respect to the important predictions of this model. The relative efficiency of coupling of the stimulus to response, as manipulated with this function, only changes the quantitative aspects of the calculations, i.e., at which point, with respect to $[G_1]/[G_2]$, the relative efficacy of the agonists becomes altered. The strength of such an approach is the flexibility in the modeling of differing efficiencies of coupling of various receptor-coupling mechanisms in cells.

An important feature of this model, and the reason why a

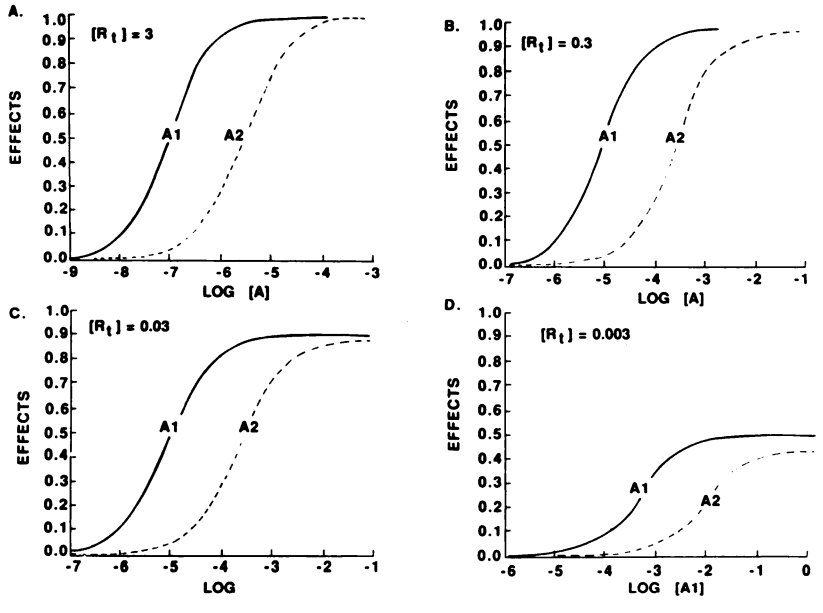

Fig. 8. Potency ratios of *A1* and *A2* for a one receptor/one G protein system in which the relative receptor density is reduced from $[R] = 3$ to 0.01. *Ordinates*, effect as a fraction of the maximal effect to the agonist; *abscissae*, logarithms of the molar concentrations of agonist. Values for the calculations are as given in Table 1 and, for A, $[R] = 3$; B, $[R] = 0.3$; C, $[R] = 0.03$; and D, $[R] = 0.003$.

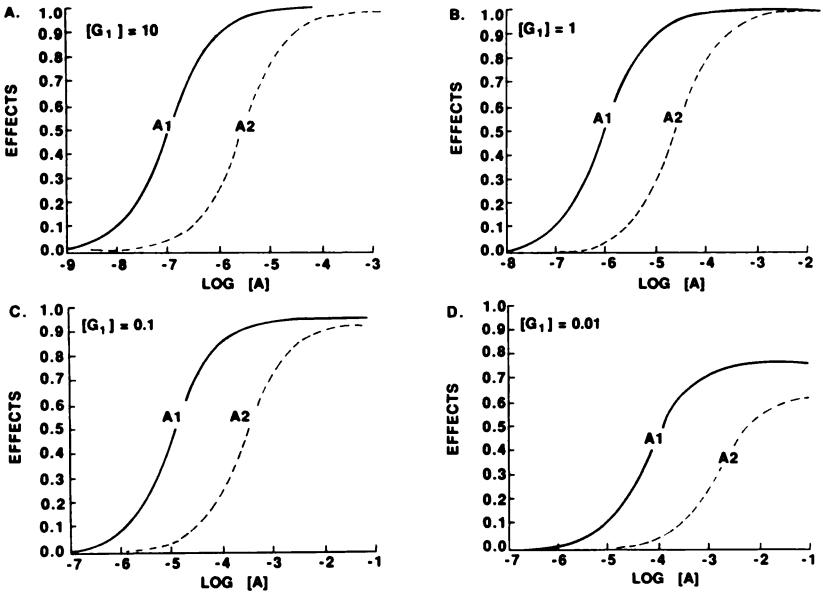

Fig. 9. Potency ratios for *A1* and *A2* for a one receptor/one G protein system in which the relative G protein density is reduced from $[G_1] = 10$ to $[G_1] = 0.01$. *Ordinates* and *abscissae* as for Fig. 8. Values for the calculations as given in Table 1 and, for A, $[G_1] = 10$; B, $[G_1] = 1$; C, $[G_1] = 0.1$; and D, $[G_1] = 0.01$.

computer iterative technique was required to solve for the relative quantities of ternary complex, is the fact that the relative amount of $[R]$, $[G_1]$, and $[G_2]$ are conserved with respect to each other. This more closely models physiologic systems. Current biochemical data suggest that, in terms of relative quantity, the amount of G protein significantly exceeds the amount of receptor protein. If the receptor can interact with more than one G protein within the cell membrane, then the relative amounts of the G proteins and the equilibrium dissociation constants of the receptor/G protein complexes (upon activation by agonist) become critical in determining the relative quantities of ternary complexes and subsequently the magnitude of the agonist response.

There are two important aspects to this hypothesis. The first relates to the equilibrium dissociation constant of the agonist-activated receptor/G protein complex. It is assumed that different agonists do not produce the same change in the receptor and, thus, the same dissociation constant for the ternary complex. This is the basis for the differences in intrinsic efficacy of agonists, i.e., why some drugs are partial and some full agonists. Previously, the magnitude of the equilibrium dissociation constant of the receptor/G protein complex has been described as a molecular basis of intrinsic efficacy (see Ref. 7). What this means is a promiscuous receptor/multiple G protein system is that different agonists would not rely upon the same G proteins for the production of response.

A second aspect of this model relates to the differing dependence of agonists on different transducer proteins. Intuitively, it would not be expected that various organs would have identical relative quantities of receptor and coupling proteins. In the cases of multiple transducer proteins, this would mean that the relative efficacy of agonists, being dependent upon the ratio of different G proteins, would change with different organs. This is a fundamentally different view of the relative potency of agonists, which always has been assumed to be constant for the same receptor, irrespective of the organ in which that receptor

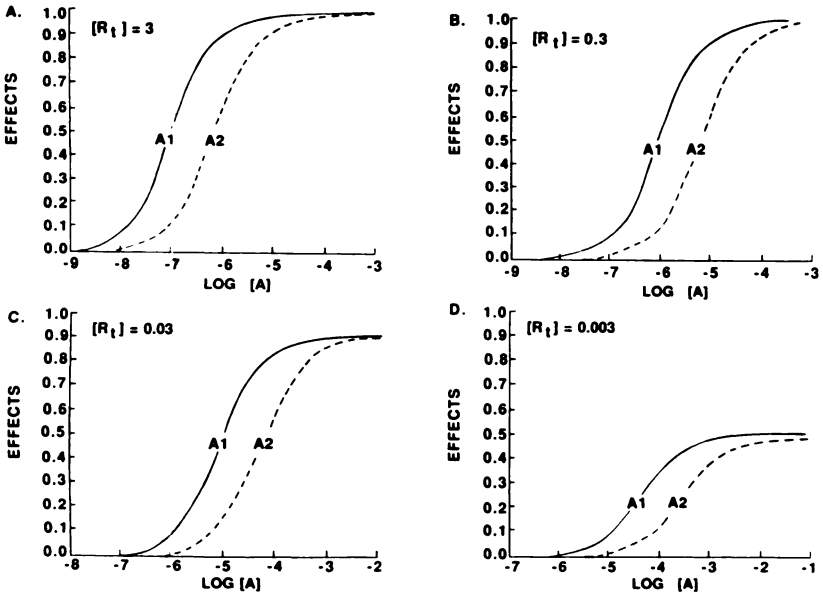

Fig. 10. Potency ratios of *A1* and *A2* for one receptor/two G protein system in which the relative receptor density is reduced from $[R] = 3$ to $[R] = 0.001$. *Ordinates*, effect as a fraction of the maximal effect to the agonist; *abscissae*, logarithms of the molar concentration of agonist. Values for the calculations are as given in Table 1 and, for A, $[R] = 3$; B, $[R] = 0.03$; C, $[R] = 0.03$; and D, $[R] = 0.003$. As given in the text, $[G_1] = 10$ and $[G_2] = 1$.

is operative. Thus, depending upon the relative equilibrium dissociation constants of the various receptor/G protein complexes produced by each agonist and the relative quantities of multiple G proteins with which the receptor can interact, a spectrum of relative potencies (and relative intrinsic efficacies) for agonists can be predicted (i.e., Fig. 7).

There is much experimental evidence to show that agonists are pleiotropic, i.e., that they can elicit multiple cellular signals. For example, muscarinic agonists in cardiac tissue are known to affect potassium ion fluxes (15), phosphinositol metabolism, and intracellular levels of cyclic AMP (16). Presently, it is not known whether these multiple biochemical signals emanate from three separate cardiac muscarinic receptors, from divergent biochemical cascades within the cell emanating from a single agonist-receptor interaction, or from a receptor that can interact with multiple membrane-bound G proteins. Experiments in which one or more of these signals is biochemically altered suggest that multiple G proteins may be responsible, but it is premature to ascribe the agonist-selective triggering event to a single receptor.

There is, however, evidence that a single receptor may interact with multiple G proteins in the same cell membrane in the case of muscarinic receptors in cardiac muscle (17, 18), angiotensin in a range of tissues (19), thrombin (20) and phosphatidic acid (21) in 3T3 fibroblasts, muscarinic agonists in rat striatum (22), prostaglandins in NG 108-15 cells (23), carbachol in neuroblastoma cells (24), and glucagon in rat hepatocytes (25) (for references see Ref. 26). It is clear from reconstitution studies that receptors are capable of interacting with multiple G proteins (27, 28). For example, biochemical studies utilizing receptor/G protein reconstitution indicate that a single type of muscarinic receptor is capable of interacting with G_i and G_o (29). Muscarinic receptors purified from porcine cerebrum and reconstituted in phospholipid vesicles have been shown to interact with G_i, G_o, and G_n (30). Recently, Ashkanazi *et al.* (31) demonstrated that recombinant muscarininc M2 receptors expressed in transfected CHO cells are capable of coupling to separate transducers that mediate inhibition of cyclic AMP and phosphoinositide turnover. It is difficult to confirm whether these types of interactions occur physiologically in cells because the reconstitution data may reflect the behaviour of these systems at abnormally high relative concentrations of receptor and coupling protein. However, the fact that these interactions can occur opens the possibility for physiologically relevant receptor promiscuity.

The differentiation of these mechanisms from those relating to a single agonist-receptor interaction is critical to the use of the relative potency of agonists for the classification of receptors. Agonist potency ratios have been utilized for many years in the process of receptor classification. Thus, if two agonists were found to have different potency ratios in two different tissues, it was assumed that those tissues possessed qualitatively different receptors. This present model suggests that the same receptors in different tissues can produce different relative potencies of agonists if the transducing proteins with which they interact in the membrane are different. These alternatives can be further differentiated with analytical techniques to study receptors, such as Schild analysis, which presumably defines only the extracellular recognition domain of receptors.

This model also predicts ways in which promiscuous receptor systems can be identified. Specifically, in tissues in which changes in the stimulus response-coupling characteristic alter the relative potency of agonists, this model suggests that one receptor/two G protein interactions could be operative. As these calculations demonstrate, the relative potency of agonists may vary from tissue to tissue if the receptors in these tissues depend upon more than one coupling protein for transduction of stimulus. However, differences in agonist potency ratios in single transduction molecule systems would indicate differences in receptors. This latter condition could be confirmed by experiments with antagonists that measure only the affinity for the receptor and not signal transduction. Therefore, in systems in which differences in agonist potency ratios can be demonstrated but not confirmed by analysis with antagonists, receptor promiscuity with respect to coupling proteins could be operative. In organs in which promiscuous receptors can be identified, it would be predicted that pathogenic-selective, or more organ-selective, agonists could be designed, i.e., those that utilized the dominant transducer protein. This has potentially broad implication for drug development.

References

1. Morgan, P. H., and T. P. Kenakin. *FASEB J.* **2**:A782 (1988).
2. Ruffolo, R. R., Jr. Review: Important concepts of receptor theory. *J. Autonomic Pharmacol.* **2**:277–295 (1982).
3. Kenakin, T. P. The classification of drugs and drug receptors in isolated tissues. *Pharmacol. Rev.* **36**:165–222 (1984).
4. Kenakin, T. P. *The Pharmacologic Analysis of Drug Receptor Interaction.* Raven Press, New York, 31–51 (1987).
5. Kenakin, T. P., and D. Beek. Is prenalterol (H133/80) really a selective *beta*-1 adrenoreptor agonist? Tissue selectivity resulting from differences in stimulus-response relationships. *J. Pharmacol. Exp. Ther.* **213**:406–412 (1980).
6. Burden, R. L., J. D. Faires, and A. C. Reynolds. In *Numerical Analysis.* Prindle, Weber and Schmidt, Boston, 34–44 (1981).
7. Black, J. W., and P. Leff. Operational models of pharmacological agonism. *Proc. R. Soc. Lond. Biol. Sci.* **220**:141–162 (1983).
8. Furchgott, R. F. The use of *beta*-haloalykylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complexes. *Adv. Drug Res.* **3**:21–55 (1966).
9. Nickerson, M. Receptor occupancy and tissue response. *Nature (Lond.)* **178**:697–698 (1956).
10. Ariens, E. J. Affinity and intrinsic activity in the theory of competitive inhibition. *Arch. Int. Pharmacodyn. Ther.* **99**:32–49 (1954).
11. Kenakin, T. P. The quantification of the relative efficacy of agonists. *J. Pharmacol. Methods* **13**:281–308 (1985).
12. Cuatrecasas, P. Membrane receptors. *Annu. Rev. Biochem.* **43**:169–214 (1974).
13. Jacobs, S., and P. Cuatrecasas. The mobile receptor hypothesis and "cooperativity" of hormone binding: application to insulin. *Biochim. Biophys. Acta.* **433**:482–495 (1976).
14. Abbott, A. J., and G. L. Nelsestuen. The collisional limit: an important consideration for membrane associated enzymes and receptors. *FASEB J.* **2**:2858–2866 (1988).
15. Pfaffinger, P. J., J. M. Martin, D. D. Hunter, N. M. Nathanson, and B. Hille. GTP-binding proteins couple cardiac muscarinic receptors to a K channel. *Nature (Lond.)* **317**:536–538 (1985).
16. Brown, J. H., and S. L. Brown. Agonists differentiate muscarinic receptors that inhibit cyclic AMP formation from those that stimulate phosphoinositide metabolism. *J. Biol. Chem.* **259**:377–3781 (1984).
17. Endoh, M., M. Murayama, and T. Ijima. Further evidence that muscarinic cholinergic receptors of 1321N1 astrocytoma cells couple to a guanine nucleotide regulatory protein that is not N_i. *Biochem. J.* **229**:539–544 (1985).
18. Brown, J. H., and D. Goldstein. Differences in muscarinic receptor reserve for inhibition of adenylate cyclase and stimulation of phosphoinositide hydrolysis in chick hearts. *Mol. Pharmacol.* **30**:566–570 (1986).
19. Garcia-Sainz, J. Angiotensin II receptors: one type coupled to two signals or receptor subtypes? *Trends Pharmacol. Sci.* **8**:48–49 (1987).
20. Murayama, T., and M. Ui. Receptor-mediated inhibition of adenylate cyclase and stimulation of arachidonic acid release in 3T3 fibroblasts: selective susceptibility to islet activating protein, pertussis toxin. *J. Biol. Chem.* **260**:7226–7233 (1985).
21. Murayama, T., and M. Ui. Phosphatidic acid may stimulate membrane receptors mediating adenylate cyclase inhibition and phospholipid breakdown in 3T3 fibroblasts. *J. Biol. Chem.* **262**:5522–5529 (1987).
22. Kelly, E., T. A. Rooney, and S. R. Nahorski. Pertussis toxin separates two muscarinic-effector mechansims in the striatum. *Eur. J. Pharmacol.* **119**:129–130 (1985).
23. Kurose, H., and M. Ui. Functional coupling of muscarinic receptors from adenylate cyclase in rat cardiac membranes by the active component of islet activating protein, pertussis toxin. *J. Cyclic Nucleotide Protein Phosphorylation Res.* **9**:305–318 (1983).
24. Bruni, P., D. L. Burns, E. L. Hewlett, and J. Moss. Effects of pertussis toxin on cAMP and cGMP responses to carbamylcholine in N1E-115 neuroblastoma cells. *Mol. Pharmacol.* **28**:229–234 (1983).
25. Wakelam, M. J., G. J. Murphy, V. J. Hruby, and M. D. Houslay. Activation of two signal-transduction systems in hepatocytes by glucagon. *Nature (Lond.)* **323**:68–71 (1986).
26. Kenakin, T. P. Are receptors promiscuous? Intrinsic efficacy as a transduction phenomenom. *Life Sci.* **43**:1095–1101 (1988).
27. Asano, T., T. Katada, A. G. Gilman, and E. M. Ross. Activation of the inhibitory GTP-binding protein of adenylate s/cyclase, G_i, by *beta*-adrenergic receptors in reconstituted phospholipid vesicles. *J. Biol. Chem.* **259**:9351–9354 (1984).
28. Cerione, R. A., J. W. Regan, H. Nakata, J. Codina, J. L. Benovic, P. Gierschik, R. L. Somers, A. Spiegel, L. Birnbaumer, R. J. Lefkovitz, and M. G. Caron. Functional reconstitution of $alpha_2$-adrenergic receptor with guanine nucleotide regulatory proteins in phospholipid vesicles. *J. Biol. Chem.* **261**:3901–3909 (1986).
29. Haga, K., T. Haga, and A. Ichiyama. Reconstitution of the muscarinic acetylcholine receptor. *J. Biol. Chem.* **262**:10133–10140 (1986).
30. Haga, T., K. Haga, G. Berstein, T. Nishiyama, H. Uchiyama, and A. Ichyama. Molecular properties of muscarinic receptors. *Trends. Pharmacol. Sci.* (Suppl. 1) 12–18 (1988).
31. Ashkanazi, A., J. W. Winslow, E. G. Peralta, G. L. Peterson, M. I. Schimerlik, D. J. Capon, and J. Ramachandran. An M2 muscarinic receptor subtype coupled to both adenylyl cyclase and phosphoinositide turnover. *Science (Wash. D. C.)* **238**:672–675 (1987).

Appendix

One receptor and one G protein: effects of receptor number on potency ratios. Fig. 8 shows the dose-response curves for *A1* and *A2* in a system composed of one receptor and one G protein ($[G_1] = 10$); $[R]$ varies from 3 to 0.003. It can be seen that, whereas depletion of receptor density shifts both curves to the right and eventually depresses the maximal responses, the relative location parameters of the curves to the two agonists (i.e., the relative potency) does not change.

One receptor and one G protein: effects of the quantity of G protein on potency ratios. Fig. 9 shows dose-response curves for *A1* and *A2* in the same system shown in Fig. 8 except, in this case, the quantity of receptor remains constant ($[R] = 3$) and the relative amount of G protein varies from 10 to 0.001. As with changes in receptor number, no effect is observed on the relative potency of the two agonists.

One receptor and two G proteins: effects of receptor number on potency ratios. Fig. 10 shows dose-response curves for *A1* and *A2* in a system composed of one receptor that can interact with either of two G proteins. The ratio of the two coupling proteins is fixed ($[G_1] = 10$, $[G_2] = 1$) and the receptor number varies from 3 to 0.003. Although depletion of receptors produces dextral displacements and eventual depressions of the maximal responses, no change is observed in the potency ratio of the two agonists. This is in contrast to the effects of differing relative amounts of coupling proteins, as shown in Fig. 6.

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