Biochemical Pharmacology, Vol. 53, pp. 519–530, 1997.
Copyright © 1997 Elsevier Science Inc.

ISSN 0006-2952/97/$17.00 + 0.00
PII S0006-2952(96)00768-X

Mechanistic Model of G-Protein Signal Transduction

DETERMINANTS OF EFFICACY AND EFFECT OF PRECOUPLED RECEPTORS

*Lonnie Shea and Jennifer J. Linderman**

DEPARTMENT OF CHEMICAL ENGINEERING, THE UNIVERSITY OF MICHIGAN, ANN ARBOR, MI 48109-2136, U.S.A.

ABSTRACT. Tissue-specific characteristics (e.g. receptor number) and agonist-specific characteristics (e.g. agonist binding kinetics) play roles in determining cellular response. The roles that these characteristics play are quantified by models of signal transduction. We examined signal transduction through G-protein-linked receptors, using a model based on the collision coupling model but including interconverting receptor states and the precoupling of receptors with G-proteins prior to the addition of agonist. Reaction and diffusion of molecules within the plasma membrane were simulated using Monte Carlo techniques. The G-protein activation produced by our model was compared with that produced by the collision coupling model. We quantitatively examined how the parameters characteristic of the tissue and agonist determine the midpoint and maximal response of the dose–response curve. Activation through agonist binding to precoupled receptors can produce significantly higher activation rates than does collision coupling. Tissue and agonist characteristics have qualitatively similar effects but quantitatively distinct effects on activation for the two models. Using standard experimental techniques, it may be possible to exploit these differences to determine the mechanism of G-protein activation in a specific cell system. A quantitative comparison of model predictions with published data on the β-adrenergic receptor system (Stickle D and Barber R, *Mole Pharmacol* **40:** 276–288, 1991) also is presented. BIOCHEM PHARMACOL 53;4:519–530, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. efficacy; precoupling; mathematical model; diffusion; receptor states; switching

Two fundamental properties of an agonist are affinity and efficacy. Affinity determines the equilibrium number of receptors that the agonist occupies at a given concentration; efficacy reflects the power of the agonist to produce a response. Variations in agonist efficacy are used to explain the different behavior of agonists. For example, one agonist may require 50% receptor occupancy to produce a maximal response but a second agonist may require only 10% receptor occupancy. The separation between the midpoint of the dose–response curve and the midpoint of the agonist-binding curve has historically been quantified through the number of spare receptors. Differences in efficacy also explain why some agonists are incapable of producing a maximal response, a property that defines partial agonism.

Affinity andefficacy are used by Stephenson [1] to define a stimulus according to:

$$\text{Stimulus} = S = \frac{e \cdot [A]}{[A] + K_D} \tag{1}$$

where [A]† is the agonist concentration, K_D is the equilibrium dissociation constant, and e is the efficacy of the agonist. The fractional response is then defined as a function of the stimulus by:

$$\text{Fractional response} = \frac{E_A}{E_M} = f(S) = f\left(\frac{e \cdot [A]}{[A] + K_D}\right) \tag{2}$$

where E_A is the response produced by the agonist, E_M is the maximal response, and f is a monotonic, continuous function relating the response to the stimulus. This relationship between the agonist concentration and the response can explain many experimental phenomena seen in a dose–response curve, such as partial agonism and spare receptors. Two aspects of the model are responsible for explaining a diversity of responses: the function (f) and agonist efficacy (e). The function determines the shape of the dose–response curve, and the efficacy determines the location of the midpoint and maximal response of the dose–response curve.

Although the Stephenson model can produce the observed relationships between receptor occupancy and re-

* Corresponding author: Prof. Jennifer Linderman, University of Michigan, Department of Chemical Engineering, 3074 H.H. Dow Bldg., Ann Arbor, MI 48109-2136. Tel. (313) 763-0679; FAX (313) 763-0459.

Received 14 March 1996; accepted 4 October 1996.

† *Abbreviations:* A, agonist; $[A]_{50}$, agonist concentration producing a half-maximal response; e, agonist efficacy; f, function relating stimulus to response in Stephenson model; G, heterotrimeric guanine nucleotide binding protein; K_D, equilibrium dissociation constant; R, inactive (non-signaling) free receptor; RA, inactive (non-signaling) receptor–agonist complex; RA*, active (signaling) receptor–agonist complex; and RG, precoupled receptor–G-protein complex.

sponse, the characteristics that determine the functional dependence f and efficacy e are not specified; consequently, the quantitative relationship between these characteristics and the response is unknown. To determine this quantitative relationship, we used a mechanistic model of the signal transduction pathway.

The signal transduction pathway that we examined involves G-protein-linked receptors (reviewed in Refs. 2 and 3). Several models of G-protein activation have been proposed. The collision coupling model of Tolkovsky and Levitzki [4] hypothesizes that a receptor–agonist complex acts as a mobile catalyst for the activation of enzymes (e.g. G-proteins) in the plasma membrane. This model was the only model tested by Tolkovsky and Levitzki that qualitatively agreed with all of their experimental data. The collision coupling model has been an integral piece in understanding the basic mechanism of signal transduction and is used as the basis for many models of signal transduction through G-protein-linked receptors.

The encounter coupling model [5], which is based on the collision coupling model, was developed by Stickle and Barber to explain their experimental data showing that certain agonists have a demonstrable switching effect. Stickle and Barber [6] define switching to be the movement of agonist among the receptors. Their encounter coupling model modifies the collision coupling model to include agonist switching. They hypothesize that switching is important when the lifetime of the receptor–agonist complex is less than the encounter time of the receptor and G-protein, where the encounter time is defined as the length of time that a receptor and G-protein interact [5]. The encounter coupling model predicts shifts in the midpoint of the dose–response curve based on variations in the binding frequency; however, model predictions depend strongly on the encounter time, which is not readily measurable and is fit to the data.

Monte Carlo simulations by Mahama and Linderman [7], also based on the collision coupling model, include the collision of receptor–agonist complexes with inactive G-proteins, the dissociation of the activated G-protein into two subunits (α-GTP and $\beta\gamma$), the inactivation of α-GTP to produce α-GDP, and the recombination of α-GDP and $\beta\gamma$ to form an inactive G-protein. Their simulations demonstrate that switching aids in the activation of G-proteins by allowing receptor–agonist complexes to access G-proteins anywhere on the cell surface rather than just in the immediate vicinity of one receptor–agonist complex. An advantage of modeling using Monte Carlo techniques is that agonist switching is inherent in the simulations. Beyond specifying the agonist association and dissociation rate constants, no terms are added to ensure that switching occurs. Although their simulations demonstrate the effect of agonist switching, the simulations do not address how different agonists shift the dose–response curve.

Recent experimental evidence with constitutively active systems suggests a mechanism in which the receptors convert between an active (signaling) and an inactive (nonsignaling) conformation [8,9]. Although this mechanism has been proposed to explain some experimental data, it has not been incorporated into the models just described, and the effects on G-protein signal transduction have not been quantified.

Using Monte Carlo techniques, we examined a model of signal transduction through G-protein-linked receptors which utilizes the basic concepts of the collision coupling model and incorporates the concept of interconverting receptors states. Additionally, the model allows for the precoupling of receptors and G-proteins, which is thought to occur in a number of cell systems [10–13]. Using our mechanistic model, we describe specific characteristics of the tissue and agonist which partly determine the function (f) and efficacy (e) and the quantitative relationship between these characteristics and the intermediate tissue response of G-protein activation. Specifically, we compared our model to the standard collision coupling model and demonstrated the different quantitative effects that the agonist characteristics have on efficacy and that the tissue and agonist characteristics have on the dose–response curve.

METHODS

Our model, which utilizes the concepts of interconverting receptor states, collision coupling, and precoupling of receptors with G-proteins, is shown schematically in Fig. 1. Monte Carlo techniques were used to simulate the reaction and diffusion of molecules in the plasma membrane. Simu-

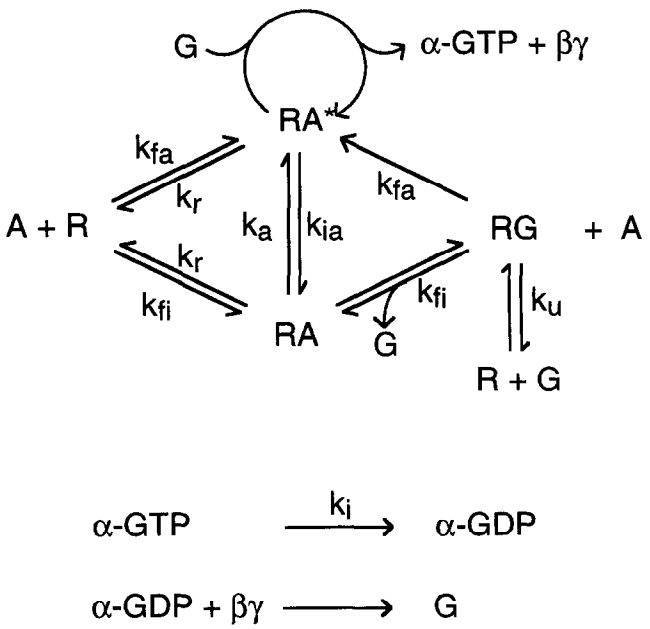

FIG. 1. Model of G-protein signal transduction mechanism. The model incorporates the concepts of interconverting receptor states, collision coupling, and the precoupling of receptors and G-proteins.

lation of these events allows us to follow the spatial position and distribution of molecules in the plasma membrane as well as to incorporate agonist switching. A triangular lattice is used to simulate a section of the plasma membrane. Particles are randomly placed on the lattice and undergo a random walk. Reactions between species in the membrane may occur only as the result of a collision, or multiple collisions, between species. Monte Carlo simulations were performed using the procedure described by Mahama and Linderman [7].

Interconverting Receptor States

Agonist (A) binds to a free receptor (R), with an overall rate constant k_f (M^{-1} sec^{-1}), and forms either an inactive (non-signaling) receptor–agonist complex (RA) or an active (signaling) receptor–agonist complex (RA*). Agonist dissociates from RA and RA* with rate constant k_r (sec^{-1}). We assume that the number of unbound receptors in the active conformation (R*) is small enough (0.01 to 1% of the total receptor number, depending on tissue characteristics) that activation through R* can be neglected. Thus, the model in its present form is not applied to constitutively active systems or to inverse agonists.

Receptor–agonist complexes interconvert between the two conformations with rate constants k_a (sec^{-1}) and k_{ia} (sec^{-1}). These two rate constants are assumed to be dependent upon the agonist and are used to define an agonist efficiency according to:

$$\text{Agonist efficiency} = \frac{k_a}{k_a + k_{ia}} \tag{3}$$

The agonist efficiency represents the fraction of time that a receptor–agonist complex is in the active conformation. An agonist efficiency of 1 indicates that the receptor–agonist complex is always in an active (signaling) conformation, whereas an agonist efficiency of 0 indicates that the receptor–agonist complex is always in an inactive (non-signaling) conformation. The agonist efficiency is a way of formally allowing an agonist to induce an active conformation on a receptor for a fraction of the time that the receptor is bound. We assume that k_a and k_{ia} are large and thus the steady-state distribution between RA and RA* is established rapidly; consequently, any collision between any receptor–agonist complex and a G-protein produces an activated complex with a probability equal to the agonist efficiency. Rapid interconversion between RA and RA* does not prohibit inactivation through other mechanisms, such as lateral segretation or receptor phosphorylation.

Collision Coupling

The interaction of RA* with an inactive G-protein (G) through collisions in the plasma membrane activates the G-protein, causing it to dissociate into an α-GTP subunit and a βγ subunit, and leave RA* intact. It is assumed that the exchange of GDP for GTP and the subunit dissociation from the receptor occur on the order of 1 sec or less [14] and thus are neglected in the simulation. α-GTP is hydrolyzed to α-GDP with rate constant k_i (sec^{-1}). The interaction of α-GDP with a βγ subunit, through collisions in the plasma membrane, forms G.

In the reaction schematic shown in Fig. 1, rate constants are not specified for reactions involving two species in the plasma membrane, such as RA* with G, because the reactions occur only after a collision between the two reacting species. Therefore, the reaction rates are determined by the collision frequency of the reacting molecules. The collision frequency between two species depends on the diffusivity of each species, the concentration of each species, and the area to which they are confined.

Precoupling

Precoupling of receptors and G-proteins is modeled as a dynamic process in which R couples with G to form a precoupled complex (RG) and RG uncouples into R and G with rate constant k_u (sec^{-1}). Receptor–agonist complexes are produced by the binding of agonist to RG. The binding of agonist to RG results in either: (1) RA* which activates the coupled G-protein, thus producing α-GTP and βγ, or (2) RA which does not activate the G-protein and causes the inactive G-protein to dissociate from the receptor.

The agonist efficiency determines the fraction of agonist binding events that result in the formation of RA*. We assume that agonist binding to RG has the same association rate constant as agonist binding to R [14]. k_f represents the overall rate constant for the binding of agonist to a receptor to form a receptor-agonist complex; however, the rate constants for the formation of RA* and RA are calculated according to:

$$k_{fa} = k_f \cdot \frac{k_a}{k_a + k_{ia}} = k_f \cdot \text{agonist efficiency} \tag{4}$$

$$k_{fi} = k_f \cdot \frac{k_{ia}}{k_a + k_{ia}} = k_f \cdot (1 - \text{agonist efficiency}) \tag{5}$$

where k_{fa} (M^{-1} sec^{-1}) is the rate constant for agonist binding to a receptor to form RA* and k_{fi} (M^{-1} sec^{-1}) is the rate constant for agonist binding to a receptor to form RA.

The association of R and G is dependent upon collisions between R and G. The maximum association rate occurs when every collision between R and G results in the formation of a precoupled complex. Association rates less than the maximum are produced in the model by incorporating a *precoupling efficiency*, which we define as the probability that a collision between R and G results in the formation of RG. For a precoupling efficiency of less than 1, multiple interactions between R and G are required, on average, to produce a precoupled complex. Thus, the association rate of R and G is given by [15]:

$$\text{Association rate} = \eta_{pc} \frac{4(D_R + D_G)}{S} [R][G] \quad (6)$$

where D_R and D_G are the diffusivities of R and G, [R] and [G] are the concentrations of R and G, S is the cell surface area, and η_{pc} is the precoupling efficiency.

All of the parameters governing the association rate of R and G are known or assumed; however, the parameters governing the dissociation rate of RG into R and G are unknown. The dissociation rate of RG is given by:

$$\text{Dissociation rate} = k_u[RG] \quad (7)$$

The number of precoupled complexes prior to agonist addition is assumed. Assuming that the precoupling reactions are initially at a steady state in the absence of agonist, the association rate equals the dissociation rate and k_u can be calculated from:

$$k_u = \eta_{pc} \frac{4(D_R + D_G)}{S} \frac{[R][G]}{[RG]} \quad (8)$$

Simulation Parameters

Parameter values used in the simulations, listed in Table 1, are representative of those reported by Stickle and Barber [6] for the β-adrenergic receptor system on S49 cells. The number of G-proteins on the cell surface is not known for the S49 cell system. Control simulations are run in which the number of G-proteins in the plasma membrane is varied between 500 and 20,000 per cell. All other simulations are performed with 2000 G-proteins per cell, which is equal to the receptor number.

A 1000 × 1000 site triangular lattice with a lattice spacing of 7 nm, approximately a protein diameter, is used to simulate an area of 49 μm^2, which is approximately one-fifth of the total surface area of a 9 μm diameter cell. Approximately 0.1% of all lattice sites are occupied with particles, and all species are given the same diffusivity. Multiple simulations are run for each case to obtain a mean result. Except for simulations with low α-GTP production, simulations are repeated until a 95% confidence interval is obtained, such that the actual mean of the steady-state level of α-GTP is within 5% of the mean of the simulations. Simulations with low α-GTP production are stopped after 30 simulations in order to keep computer time reasonable.

We generated a dose–response curve from our simulations. This curve is given in terms of a steady-state number of α-GTP as a function of a normalized agonist concentration ($[A]/K_D$). We assume that the steady-state number of α-GTP can be related to responses that are measured experimentally.

RESULTS

Using our mechanistic model of signal transduction through G-protein-linked receptors, we determined the quantitative effect that the characteristics of the tissue and the agonist have on the midpoint and maximal response of the dose–response curves. The values for the parameters characteristic of the tissue and agonist are based on the experimental data collected for various agonists binding to the β-adrenergic receptor system on S49 cells [16]. Experimentally determined dose–response curves for various agonists binding to the β-adrenergic receptor on S49 cells are shown in Fig. 2.

Dose–response curves generated by the model were compared to the experimental data. We focused on two aspects of the dose–response curve: the maximal response and the midpoint. We measured the midpoint of the response in terms of a normalized EC_{50} ($[A]_{50}/K_D$, the agonist concentration that produces a half-maximal response ($[A]_{50}$) divided by the equilibrium dissociation constant (K_D)). The parameter $[A]_{50}/K_D$ was used to represent the EC_{50} of the response because it is a measure of the number of occupied receptors required to produce a half-maximal response and has been used to quantify the number of sparse receptors [17].

Tissue-Specific Properties

Model parameters that are characteristic of the tissue are the receptor number, the G-protein number, the diffusivity, the number of precoupled receptors, and the kinetics for the formation of precoupled receptors. To examine only the tissue-specific parameters, we chose one set of parameters for the agonist and kept them constant. The agonist was

TABLE 1. Parameter values for the β-adrenergic receptor system on S49 cells as reported by Stickle and Barber [5, 16]

Parameter	Definition	Value
$[R_{tot}]$	Total receptor number	2000/cell
k_f	Agonist–receptor association rate constant	6.67×10^6 M^{-1} sec^{-1}
k_r	Agonist–receptor dissociation rate constant	0.2–200 sec^{-1}
k_i	Inactivation rate constant for α-GTP	0.023 sec^{-1}
D	Diffusion coefficient	8×10^{-11} cm^2/sec
d	Diameter of the cell	9 μm
$[G_{tot}]$	Total G-protein number	Not known
k_u	Dissociation rate constant for RG	Not known

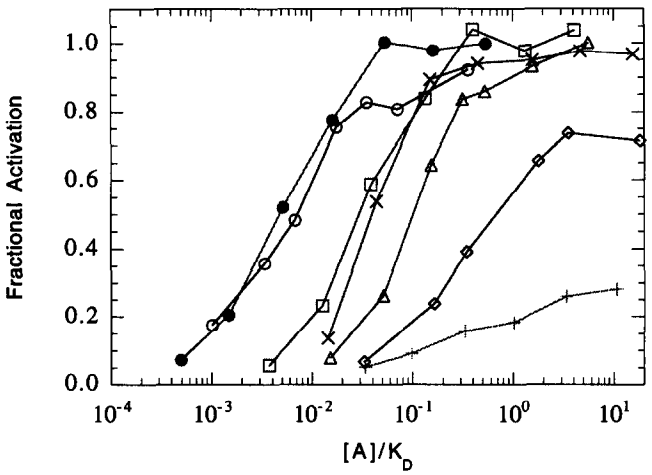

FIG. 2. Dose–response curves for seven different agonists [epinephrine (●), isoproterenol (○), salbutamol (□), metaproterenol (×), zinterol (△), dobutamine (◇), and ephedrine (+)] binding to the β-adrenergic receptor on S49 cells. Fractional activation represents 10min cAMP accumulation normalized by the maximum response obtained using epinephrine. Reproduced with permission from *Mol Pharmacol* 40: 276–288, 1991. Copyright (1991) American Society for Pharmacology and Experimental Therapeutics. [Ref. 16].

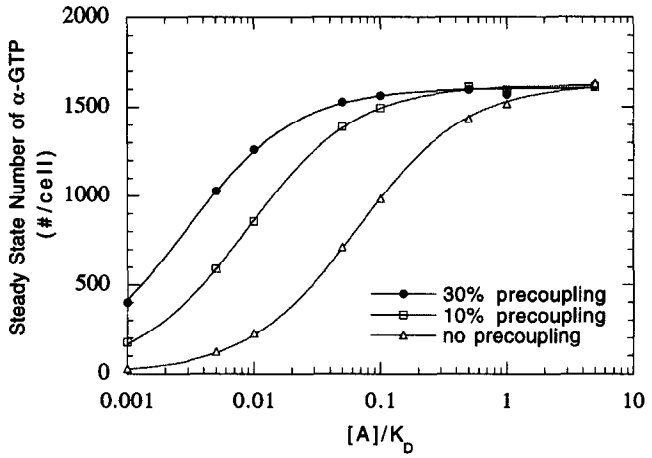

FIG. 3. Predicted dose-response curves for different percentages of precoupled receptors. Simulations were performed with a precoupling efficiency (η_{pc}) of 1.0. The curves represent different percentages of precoupled receptors prior to agonist addition. For all simulations, R_{tot} = 2000/cell, G_{tot} = 2000/cell, k_i = 0.023 sec^{-1}, k_f = 6.67 × 10^6 M^{-1} sec^{-1}, k_r = 20 sec^{-1}, D = 10^{-10} cm^2 sec^{-1} and agonist efficiency = 1.0. The rate constant k_u, calculated as described in Methods, is equal to ∞, 5, and 1 sec^{-1} for no precoupling, 10% precoupling, and 30% precoupling, respectively. In this and other figures, simulation results are shown with a curve fit through the data points.

given a dissociation rate constant (k_r) of 20 sec^{-1} and an association rate constant (k_f) of 6.67 × 10^6 M^{-1} sec^{-1}, values typical of the agonists binding to the β-adrenergic receptor on S49 cells [16]. Additionally, we assumed an agonist efficiency of unity, which means that a receptor–agonist complex is always in the active conformation.

The collision coupling model is a standard mechanism for G-protein activation used in many models. To demonstrate the quantitatively different effects of including precoupling, we compared activation solely through collision coupling with activation through collision coupling and precoupling. We began by illustrating the effect of the parameters that are specific to precoupling: the number of precoupled receptors and the kinetics of precoupling. Figure 3 shows predicted dose–response curves with the initial percentage of precoupled receptors varying from 0% (no precoupling allowed) to 30%. In these and later simulations, the percentage precoupling refers to a steady-state percentage of precoupled receptors found prior to the addition of agonist. Note that increasing the percentage of precoupled receptors decreased the value for the normalized EC_{50} but did not affect the value of the maximal response. The maximal response was unaffected by the inclusion of precoupling because the dominant activation pathway at high receptor occupancies is collision coupling; however, precoupling affected the normalized EC_{50} because activation through precoupled receptors is significant at low receptor occupancies. The inclusion of precoupling can produce substantially higher activation rates than does collision coupling alone because the receptor–agonist complex does not always have to "search" for a G-protein following agonist binding.

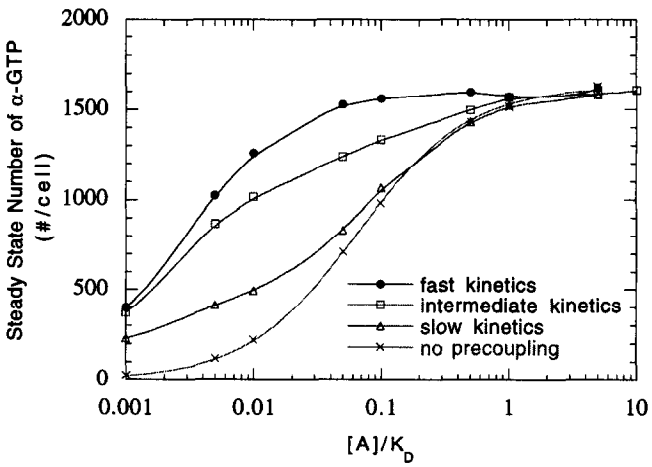

FIG. 4. Predicted dose–response curves for varying kinetics in the association and dissociation of precoupled receptors. The association and dissociation kinetics are varied through the precoupling efficiency (η_{pc}). For fast precoupling kinetics, η_{pc} = 1.0. For intermediate precoupling kinetics, η_{pc} = 0.1. For slow precoupling kinetics, η_{pc} = 0.01. For no precoupling, η_{pc} = 0.0. For simulations with precoupling, 30% of receptors are coupled to G-proteins prior to the addition of agonist. For all simulations, R_{tot} = 2000/cell, G_{tot} = 2000/cell, k_i = 0.023 sec^{-1}, k_f = 6.67 × 10^6 M^{-1} sec^{-1}, k_r = 20 sec^{-1}, D = 10^{-10} cm^2 sec^{-1} and agonist efficiency = 1.0. The rate constant k_u is equal to 0.0, 0.01, 0.1, and 1.0 sec^{-1} for no precoupling, slow precoupling kinetics, intermediate precoupling kinetics, and fast precoupling kinetics, respectively.

Figure 4 shows predicted dose–response curves for varying kinetics in the association and dissociation of precoupled complexes. These association and dissociation rates of precoupled complexes are varied through changes in the precoupling efficiency η_{pc}, the probability that a collision between a receptor and G-protein produces a precoupled complex. Smaller values for the precoupling efficiency correspond to lower rates of axxocation and dissociation. In Fig. 4, the precoupling efficiency for receptor and G-protein coupling is varied from 0 (no precoupling) to 1; for simulations with precoupling, 30% of the receptors are precoupled with G-proteins prior to agonist addition. Figure 4 shows that the precoupling kinetics did not affect significantly the maximal response but did alter the value for the normalized EC_{50}. Varying the precoupling kinetics affected the steady-state level of precoupled complexes in the plasma membrane following agonist addition. Slow precoupling kinetics produced relatively low levels of precoupled complexes at steady state and therefore activation through precoupling was low; conversely, fast precoupling kinetics resulted in relatively high levels of precoupled complexes at steady state, and activation through this pathway was significant.

We now focus on the other parameters characteristic of the tissue: G-protein number, receptor number, and diffusivity. For simulations with precoupling, 30% of the receptors are precoupled prior to agonist addition and the precoupling efficiency is equal to 0.1.

Predicted dose–response curves in which the G-protein number was varied are given in Fig. 5a for activation through collision coupling alone and in Fig. 5b for activation through collision coupling and precoupling. To better demonstrate variations in the normalized EC_{50}, the fractional activation as a function of the normalized agonist concentration is provided in the inset to each figure. These simulations were performed with a total receptor number of 2000 per cell and a diffusivity of 10^{-10} cm^2 sec^{-1}, values representative of that reported for the S49 cell system. Variations in the G-protein number affected the maximal response in both cases; however, variations in the G-protein number only significantly affected the normalized EC_{50} for simulations with precoupling.

Predicted dose–response curves in which the receptor number was varied are given in Fig. 6a for activation through collision coupling alone and in Fig. 6b for activation through collision coupling and precoupling. As seen in both panels, increasing the total receptor number shifted the response upward and to the left, thereby decreasing the value for the normalized EC_{50} and increasing the maximal response. The prediction that receptor number affects the normalized EC_{50} and maximal response is consistent with experimental results in which differential expression of the β-adrenergic receptor on L cells affects the response [18]. Note that the magnitude of the change in the normalized EC_{50} was different for the two mechanisms. Variations in the receptor number had a larger effect on activation when precoupling was part of the activation mechanism. For the parameters used here, changing the receptor number from 500 to 2000 per cell for activation through collision coupling changed the normalized EC_{50} by a factor of 3, and for activation through collision coupling and precoupling the normalized EC_{50} changed by a factor of 8.

Predicted dose-response curves in which the diffusivity was varied are given in Fig. 7a for activation through collision coupling alone and in Fig. 7b for activation through collision coupling and precoupling. For both mechanisms of activation, increasing the diffusivity increased the maximal response and decreased the normalized EC_{50} of the predicted dose–response curve. The prediction that diffusivity affects the response is consistent with experimental results in which altering the rate of diffusion of molecules in the plasma membrane affects the response [19, 20]. The magnitude of the change in the normalized EC_{50} was different for the two mechanisms. For the parameters used here, changing the diffusivity from 10^{-11} to 10^{-10} cm^2 sec^{-1} changed the normalized EC_{50} by a factor of 4 for activation by collision coupling; however, for activation by collision coupling and precoupling, the normalized EC_{50} changed by a factor of 25.

Agonist-Specific Properties

Model parameters characteristic of the agonist are the agonist binding kinetics and the agonist efficiency. To examine only the agonist-specific properties, we hold the tissue-specific properties constant. We use a G-protein number of 2000 per cell, a receptor number of 2000 per cell, and a diffusivity of 10^{-10} cm^2 sec^{-1}. For simulations with precoupling, 30% of the receptors are precoupled prior to agonist addition and the precoupling efficiency is equal to 0.1.

Predicted dose–response curves for varying agonist dissociation kinetics are given in Fig. 8a for activation through collision coupling alone and in Fig. 8b for activation through collision coupling and precoupling. All agonists were given the same association rate. The dissociation rate was varied from 0.2 to 200 sec^{-1}, consistent with the agonists used in Fig. 2 [16]. The agonist efficiency was assumed to be 1. In both panels of Fig. 8, increasing the agonist dissociation rate constant affected the value of the normalized EC_{50} but not the maximal response. These changes in the normalized EC_{50} are the result of different amounts of switching. Note that switching, which increases with increased values of k_r, had a significantly greater effect in the presence of precoupled receptors. For the parameters used here, varying k_r from 0.2 to 200 sec^{-1} changed the normalized EC_{50} by a factor of approximately 2 for activation through collision coupling; however, for activation through collision coupling and precoupling, the normalized EC_{50} changed by a factor of more than 100.

Predicted dose–response curves in which the agonist efficiency was varied from 0.01 to 1.0 are given in Fig. 9 for activation through collision coupling alone. Results including precoupling are similar (data not shown). Changing the

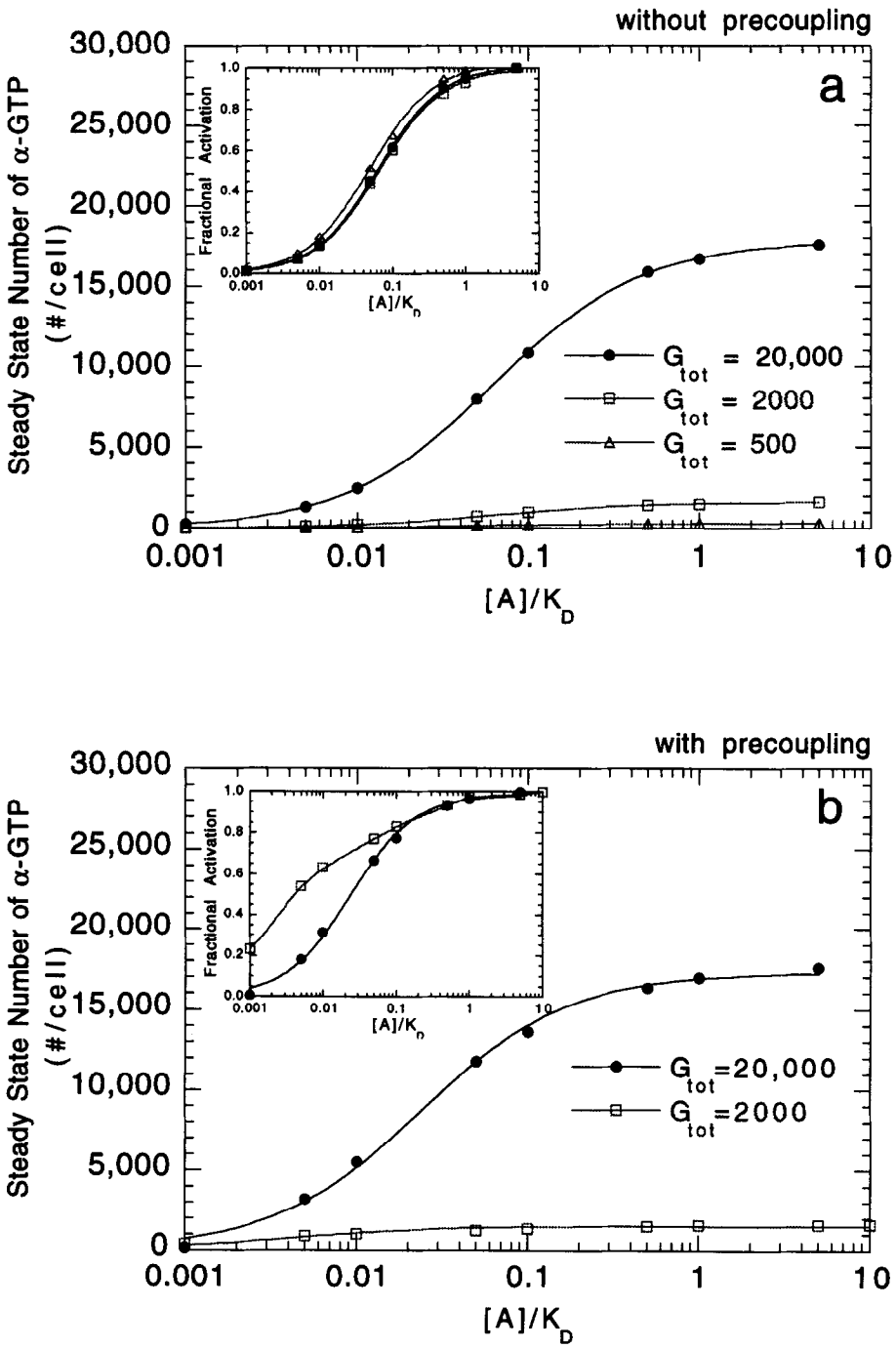

FIG. 5. Predicted dose–response curves giving the steady-state activation of G-proteins for varying numbers of G-proteins (G_{tot}) in the plasma membrane. (a) Activation through collision coupling alone, and (b) activation through collision coupling and precoupling. Insets: Dose–response curves giving the fractional activation of G-proteins. Fractional activation is the ratio of the steady-state number of α-GTP to the maximal number of α-GTP produced for the same dose–response curve. For (a) and (b), R_{tot} = 2000/cell, k_r = 20 sec^{-1}, k_i = 0.023 sec^{-1}, k_f = 6.67 × 10^6 M^{-1} sec^{-1}, D = 10^{-10} cm^2 sec^{-1}, and agonist efficiency = 1.0. For (b), η_{pc} = 0.1, k_u = 1.4 or 0.1 for G_{tot} = 20,000 or 2000, and 30% of the receptors are precoupled with G-proteins prior to agonist addition.

agonist efficiency affected the two reactions in the model that produce α-GTP: the collision of a receptor–agonist complex with an inactive G-protein and the binding of agonist to RG. All previous simulations have assumed that the agonist efficiency for these reactions is 1, i.e. the receptor–agonist complex is always in the active conformation. Variations in the agonist efficiency affected both the normalized EC_{50} and the maximal response of a dose–response curve.

Note that by varying only the properties of the agonist, i.e. agonist dissociation constant and the agonist efficiency, a broader range of values can be produced for the maximal response and the normalized EC_{50} when precoupling is present than when it is not. For the parameter ranges examined here, values for the normalized EC_{50} ranged from less than 0.001 to 0.5 for our model but only from 0.05 to 0.5 for the collision coupling model. The maximal response produced by the two models, which is determined by the value of the agonist efficiency, was the same for both models.

DISCUSSION

Monte Carlo techniques are used to simulate the reaction and diffusion of molecules in the plasma membrane. Simu-

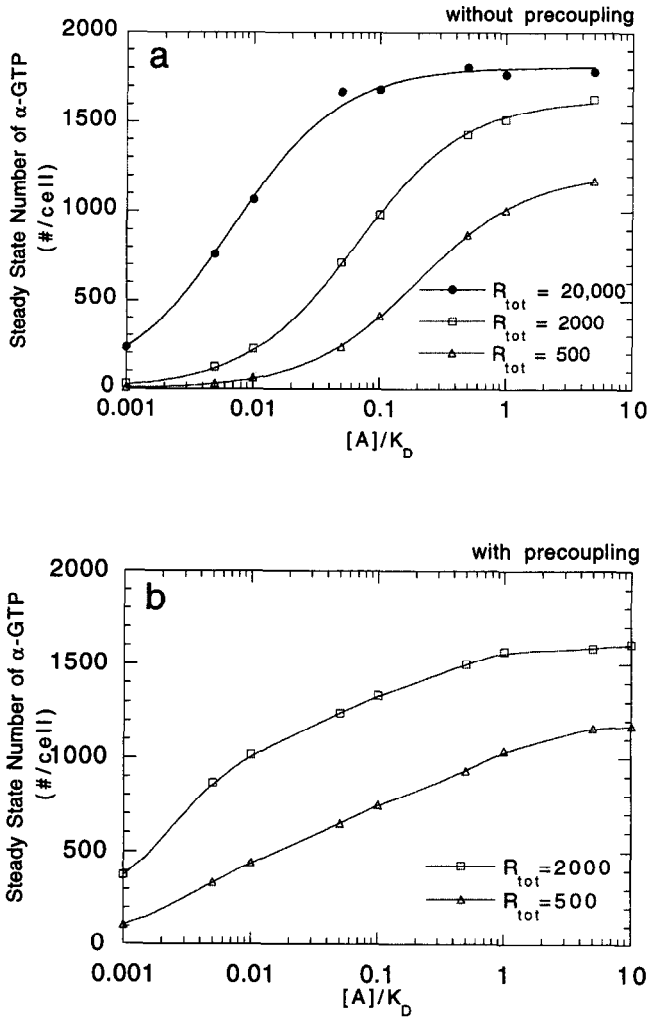

FIG. 6. **Predicted dose–response curves for varying numbers of receptors (R_{tot}) in the plasma membrane. (a) Activation through collision coupling alone, and (b) activation through collision coupling and precoupling. For (a) and (b), G_{tot} = 2000/cell, k_r = 20 sec^{-1}, k_i = 0.023 sec^{-1}, k_f = 6.67 × 10^6 M^{-1} sec^{-1}, D = 10^{-10} cm^2 sec^{-1}, and agonist efficiency = 1.0. For (a), R_{tot} = 20,000/cell, 2000/cell, or 500/cell. For (b), η_{pc} = 0.1, k_u = 0.1 or 0.136 for R_{tot} = 2000 or 500, respectively, and 30% of the receptors are precoupled with G-proteins prior to agonist addition.**

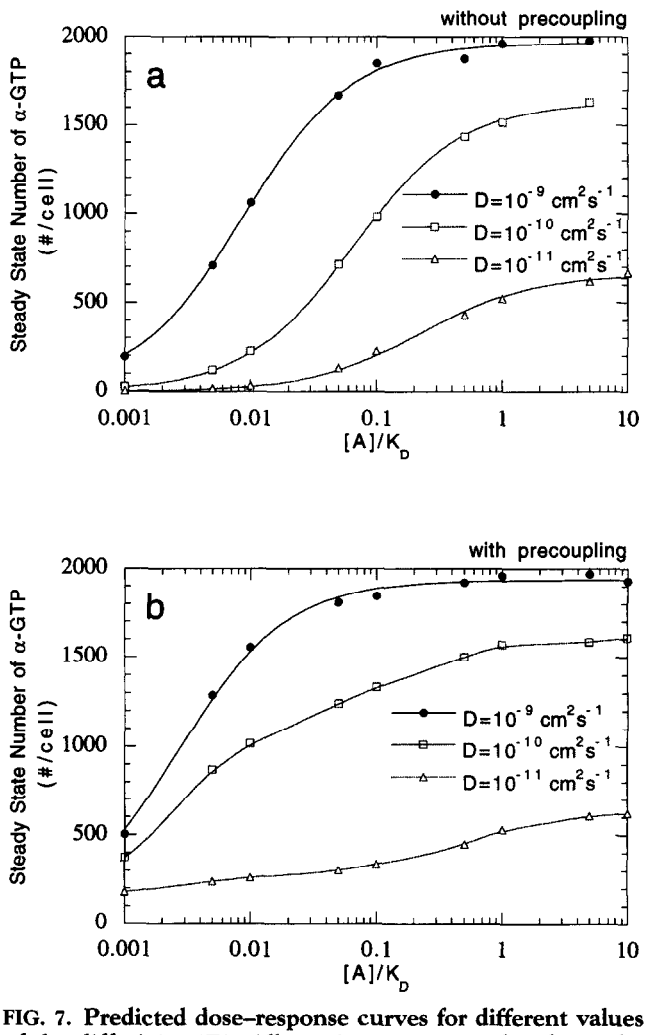

FIG. 7. **Predicted dose–response curves for different values of the diffusivity (D). All species are assumed to have the same diffusivity. (a) Activation through collision coupling alone, and (b) activation through collision coupling and precoupling. For (a) and (b), R_{tot} = 2000/cell, G_{tot} = 2000/cell, k_i = 0.023 sec^{-1}, k_f = 6.67 × 10^6 M^{-1} sec^{-1}, k_r = 20 sec^{-1} and agonist efficiency = 1.0. For (b), η_{pc} = 0.1, k_u = 0.01, 0.1, or 1.0 for D = 10^{-11}, 10^{-10}, or 10^{-9} cm^2 sec^{-1}, respectively, and 30% of the receptors are precoupled with G-proteins prior to agonist addition.**

lations are based on a model which includes interconverting receptor states, collision coupling, and the precoupling of receptors with G-proteins. We examined how G-protein number, receptor number, diffusivity, number of precoupled receptors, kinetics in the formation of precoupled receptors, agonist binding kinetics, and agonist efficiency affect the normalized EC_{50} and maximal response of the predicted dose–response curve for G protein activation. A comparison was made between our model and a model of activation solely through collision coupling.

Tissue-specific properties (number of precoupled receptors, kinetics of precoupling, G-protein number, receptor number, and diffusivity) partly determine the function (f) in the Stephenson model (equations 1 and 2). The tissue-specific parameters have different quantitative effects on the predicted dose–response curve depending on the mechanism of G-protein activation. For example, variations in the G-protein number did not affect significantly the normalized EC_{50} when activation occurred only through collision coupling; however, the G-protein number did affect the normalized EC_{50} when activation occurred through collision coupling and precoupling. The quantitative effects of receptor number and diffusivity were also different for the two mechanisms of activation. These quantitative differences may be exploited to determine the mechanism of activation in an experimental system. Varying the receptor number, such as through the use of antagonists or by altering receptor expression, and determining the effect on the normalized EC_{50} may provide some insight into the activa-

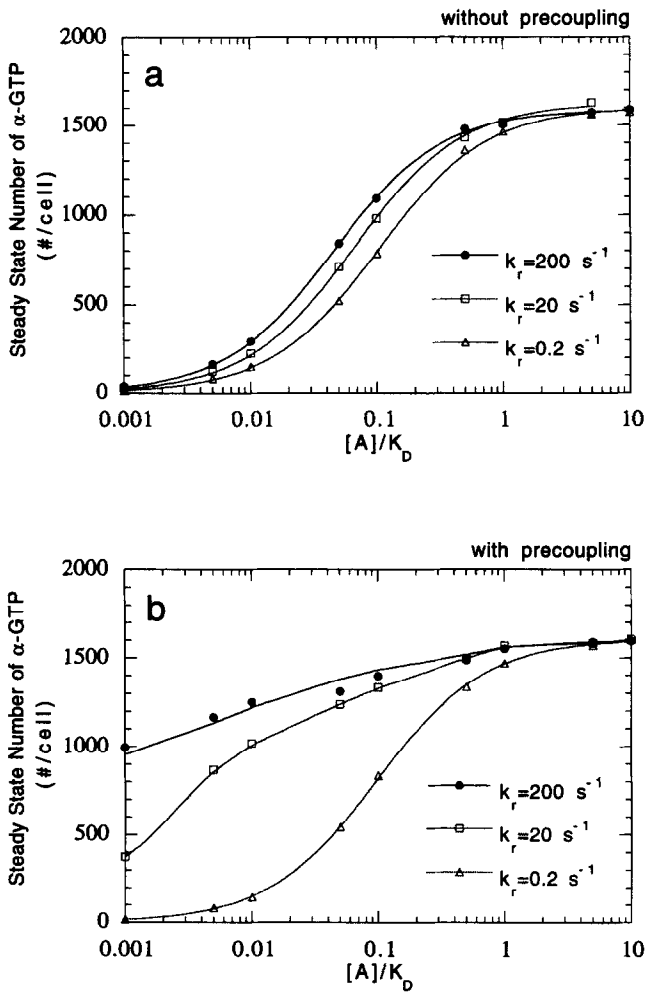

FIG. 8. Predicted dose–response curves for different values of the dissociation rate constant (k_r) for agonist from a receptor. Activation occurs through (a) collision coupling alone, and (b) collision coupling and precoupling. For (a) and (b), R_{tot} = 2000/cell, G_{tot} = 2000/cell, k_i = 0.023 sec^{-1}, k_f = 6.67 × 10^6 M^{-1} sec^{-1}, D = 10^{-10} cm^2 sec^{-1}, agonist efficiency = 1.0, and k_r = 200, 20, and 0.2 sec^{-1}. For (b), η_{pc} = 0.1, k_u equals 0.1 sec^{-1}, and 30% of the receptors are precoupled with G-proteins prior to agonist addition.

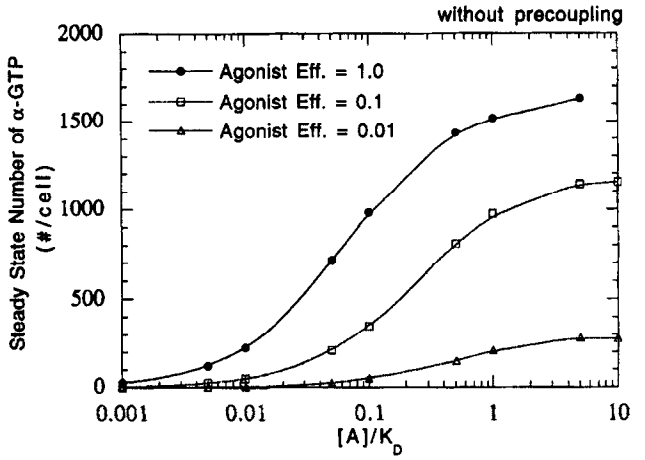

FIG. 9. Predicted dose–response curves for different values for the agonist efficiency. Activation occurs through collision coupling alone. Curves represent results for agonists with agonist efficiencies that vary from 0.01 to 1.0. Parameters used are: R_{tot} = 2000/cell, G_{tot} = 2000/cell, k_i = 0.023 sec^{-1}, k_f = 6.67 × 10^6 M^{-1} sec^{-1}, k_r = 20 sec^{-1}, D = 10^{-10} cm^2 sec^{-1}.

tion mechanism. Similarly, the G-protein number could be varied, such as through graded pertussis toxin treatment [21].

The agonist-specific properties that determine the efficacy (e) in the Stephenson model are the agonist efficiency and the agonist binding kinetics. The agonist efficiency (equation 3), defined as the fraction of time that a receptor–agonist complex is in the active conformation, represents the ability of the receptor–agonist complex to couple with and activate a G-protein. Variations in this coupling between receptors and G-proteins as a function of the agonist have been reported [22, 23]. Large values for the agonist efficiency reflect an effective agonist because the receptor–agonist complex is in the active conformation much of the time. Variations in the agonist efficiency affect the maximal response and the normalized EC_{50}. Values for the agonist efficiency of less than 1 effectively slow the rate of activation of G-proteins by requiring multiple interactions to produce a reaction; thus, the activation of G-proteins by receptor–agonist complexes becomes limited not only by the rate of diffusion but also by the intrinsic kinetics of the interaction. Although we define the agonist efficiency according to the concept of interconverting receptor states, our mathematical implementation of agonist efficiency is also consistent with the more conventional idea that a receptor–agonist complex attains a fixed configuration that is able to activate G-proteins to an extent that may be less than optimal compared with other agonist-induced conformations.

As first demonstrated by Stickle and Barber [5, 6], agonist binding kinetics also influence efficacy. Variations in the agonist dissociation rate constant, which reflect changes in the average lifetime of a receptor–agonist complex, affect the normalized EC_{50} but not the maximal response. The binding kinetics affect the normalized EC_{50} of the predicted dose–response curve through agonist switching. Agonist switching is defined as the movement of agonist among receptors [6]. Agonists that exhibit significant switching increase G-protein activation because agonists can "find" a G-protein faster by moving among different receptors than by diffusing as a receptor–agonist complex in the plasma membrane. For activation through collision coupling, switching acts to "mix" the system, thereby improving the access of receptor–agonist complexes to inactive G-proteins [7]. For activation through precoupling, switching allows agonists to "find" a G-protein instantly by binding to a precoupled receptor. Agonist switching affects both mechanisms of activation; however, as seen in Fig. 8, the magnitude of the effect is significantly different for the two mechanisms.

Our model and Stickle and Barber's encounter coupling model [5] both utilize an "association" between receptors and G-proteins to account for the large changes that result from agonist switching. Nevertheless, the implementation of this "association" is different as is the explanation of why switching affects the response. The encounter coupling model defines an encounter as the period of time during which a receptor and a G-protein "see" each other and finds that the switching effect is significant when the length of the encounter is longer than the lifetime of the receptor–agonist complex. An agonist exhibiting switching does not "waste" time by remaining in an encounter in which the G-protein has already been activated [5]. Our model utilizes precoupled receptors that result from the collision of receptors with G-proteins. In our model, switching enhances the response because an agonist can "find" a G-protein faster by binding to a different receptor than by diffusing in the membrane as a receptor–agonist complex.

Enhancement of activation due to precoupled receptors depends on both tissue-specific and agonist-specific properties. The effect of precoupling is most pronounced at small ratios of G:R, small diffusivities, large agonist dissociation rate constants (k_r), large agonist efficiencies, and fast precoupling kinetics. Small ratios of G:R ($\approx$1:1) can more easily produce low values for the normalized EC_{50} because the percentage of total G-proteins involved in precoupled complexes is relatively large; however, an effect of precoupling is possible even when the ratio of G:R is as large as 100:1. Precoupling may be favored in the cases of low diffusivity because it is much faster for an agonist to "find" a G-protein by binding to another receptor than by diffusing in the plasma membrane; nevertheless, an effect at large diffusivities can be seen in some situations. The agonist dissociation rate constant and the agonist efficiency affect the usefulness of precoupling because they determine the amount of switching and the effectiveness of that switching. The precoupling kinetics determine the rate at which precoupled complexes form; therefore, they determine the supply of precoupled complexes. Fast precoupling kinetics ($\eta_{pc} = 1$) allow precoupled receptors to be replenished rapidly once the G-proteins have been activated by agonist. Cell systems and agonists that have an appropriate combination of the above properties could show significantly higher activation rates in the presence of precoupled receptors.

The quantitative effects of the agonist-specific properties on the predicted dose–response curve depend on the mechanism of activation. For an activation mechanism that includes precoupling, the binding kinetics and the agonist efficiency have relatively large effects on the normalized EC_{50}; however, for activation without precoupling, the binding kinetics and agonist efficiency have a relatively small effect on the normalized EC_{50}. The effect of these parameters on the maximal response is the same for activation with and without precoupling. Perhaps the mechanism of activation can be determined in a particular experimental system by exploiting the different effects of these parameters on the normalized EC_{50}.

Having qualitatively identified parameters that affect cellular responses, we next quantitatively compared the predictions of the model with experimental data. Our simulations are based on the parameters characteristic of the β-adrenergic receptor system on S49 cells for which experimental data is shown in Fig. 2. To make this comparison, we make a simplifying assumption that the fractional activation measured by Stickle and Barber (cyclic AMP accumulation) can be directly compared with the fractional activation obtained from our simulation (G-protein activation). By making this assumption, we can demonstrate how variations in only the properties of the agonist, that is, the agonist efficiency and the agonist binding kinetics, can produce the range of values for the normalized EC_{50} and the maximal response seen experimentally. The experimental data in Fig. 2 show values for the maximal response ranging from 0.3 (ephedrine) to 1.0 (epinephrine, isoproterenol, salbutamol, metaproterenol, zinterol) and values for the normalized EC_{50} ($[A]_{50}/K_D$) ranging from 0.005 (epinephrine) to 0.67 (ephedrine). In our model, a range of values for the maximal response was produced by variations in the agonist efficiency. As seen in Fig. 9, changing the agonist efficiency from 1.0 to 0.01 caused the maximal response to drop from 100% of maximum to 22% of maximum. A range of values for the normalized EC_{50} was produced by variations in the agonist efficiency and the agonist binding kinetics. Figures 8a and 9, which demonstrate how the agonist efficiency and the agonist binding kinetics affect the predicted dose–response curve for activation through collision coupling alone, show values for the normalized EC_{50} ranging from 0.05 to 0.5, a range significantly smaller than seen experimentally. By decreasing the agonist efficiency, the collision coupling model can produce values for the normalized EC_{50} as large as 0.67, the largest value seen in Fig. 2; however, the collision coupling model used with parameter values of the β-adrenergic receptor system (Table 1) does not produce values for the normalized EC_{50} as small as 0.005, the smallest value seen in Fig. 2. The collision coupling model is limited in its ability to produce low values for the normalized EC_{50} by the collision frequency of receptor–agonist complexes and G-proteins in the plasma membrane. The faster G-protein activation rates produced by precoupling are essential to producing values for the normalized EC_{50} lower than 0.05. Simulation results for activation through collision coupling and precoupling, shown in Fig. 8b, show that values for the normalized EC_{50} range from less than 0.001 to approximately 0.5, consistent with the values seen in Fig. 2.

The magnitude of the switching effect in the simulation of our model is consistent with values measured by Stickle and Barber [16]. In our model, the agonist-specific properties, agonist dissociation kinetics and agonist efficiency, determine the effect due to switching. Stickle and Barber [16] found that inhibiting switching for agonists with high binding frequency and high efficiency, epinephrine ($k_r \approx 13$

sec^{-1}) and isoproterenol ($k_r \approx 2\ sec^{-1}$), could increase the EC_{50} by a factor of 6 and 3, respectively. According to our model, these agonists can rapidly move among receptors, some of which are precoupled, and activate G-proteins, thus producing a large shift in the EC_{50} when switching is reduced. Inhibiting switching for an agonist with a high binding frequency and an intermediate efficiency, such as metaproterenol ($k_r \approx 53\ sec^{-1}$), had only a marginal effect on the EC_{50}. Our model accounts for this result because even though the agonist exhibits rapid switching among receptors, it activates only a fraction of the G-proteins that it encounters; therefore, the EC_{50} shifts, but the shift is not as large as with an efficient agonist. For agonists with low efficiency, dobutamine ($k_r \approx 19\ sec^{-1}$) and zinterol ($k_r \approx 0.1\ sec^{-1}$), inhibiting switching has no effect on the EC_{50}. In our model, an agonist with a high binding frequency and low efficiency moves rapidly among receptors but activates few of the precoupled G-proteins and, therefore, switching has little or no effect. An agonist with a low binding frequency does not move rapidly among receptors and therefore activation occurs primarily through collision coupling.

Many of the predicted dose–response curves with precoupling do not exhibit the standard rectangular hyperbola shape. The biphasic nature of the curves (e.g. Fig. 6b) is the result of two phenomena. First, the mechanism of activation that dominates G-protein activation varies from activation through precoupled receptors to activation by collision coupling as the agonist concentration increases. As the agonist concentration increases, the steady-state number of precoupled complexes progressively decreases, causing a shift in the dominant activation pathway. A second phenomenon that causes the curves to appear biphasic is the inhomogeneous spatial distribution of molecules that arises as the agonist concentration increases [7]. The inhomogeneous spatial distribution alters the collision frequency between species and therefore the reaction rates.

The spatial organization or distribution of molecules in the plasma may be an important aspect of the signal transduction mechanism. The precoupling of receptors with G-proteins, which was simulated in this work, is just one example of membrane organization that increases the rate of interaction for receptor, agonist, and G-protein. Recent experimental evidence suggests that molecules may be organized in the plasma membrane [24]. Organizing molecules in the plasma membrane into higher order structures may enable them to interact faster, thereby producing higher activation rates. As another example, the membrane could be composed of more than one domain, with G-proteins having access to only one domain [24, 25]. Confining receptors and G-proteins to a small fraction of the plasma membrane reduces the distance between molecules and increases the collision frequency. Additionally, the spatial distribution may change with time, which affects the reaction rates between species [7]. Monte Carlo simulations provide a method with which to follow how the concentration and distribution of a species evole over time.

Our simulations have only examined agonists with positive efficacy; however, by incorporating an active form of a free receptor, one could examine agonists with negative efficacy or constitutively active systems. Additionally, the simulations only addressed the G-protein signaling mechanism up to G-protein activation. Examining adenylate cyclase activation or steps further downstream may be important. Other aspects of the G-protein signal transduction mechanism could also be incorporated, such as desensitization, up-regulation, or internalization. Finally, results presented here report only steady-state values; however, characterizing the transient response could be important since many cellular functions do not occur at a steady-state level of G protein activation. Monte Carlo techniques offer an excellent tool with which to study these types of phenomena.

We thank Dr. Richard R. Neubig and Dr. Geneva Omann for their critical reading of this manuscript. This work was supported by an NSF Presidential Young Investigator award and NSF award BES-9410403 to J.J.L. This work was also supported by an NSF graduate fellowship to L.D.S. Some computing services were provided by the University of Michigan Center for Parallel Computing which is partially funded by NSF Grant CDA-92-14296.

References

1. Stephenson RP, A modification of receptor theory. *Br J Pharmacol* **11:** 379–393, 1956.
2. Taylor CW, The role of G proteins in transmembrane signaling. *Biochem J* **272:** 1–13, 1990.
3. Birnbaumer L, Abramowitz J and Brown AM, Receptor–effector coupling by G proteins. *Biochim Biophys Acta* **1031:** 163–224, 1990.
4. Tolkovsky AM and Levitzki A, Mole of coupling between the β-adrenergic receptor and adenylate cyclase in turkey erythrocytes. *Biochemistry* **17:** 3795–3810, 1978.
5. Stickle D and Barber R, The encounter coupling model for β-adrenergic receptor/GTP-binding protein interaction in the S49 cell. Calculation of the encounter frequency. *Biochem Pharmacol* **43:** 2015–2028, 1992.
6. Stickle D and Barber R, Evidence for the role of epinephrine binding frequency in activation of adenylate cyclase. *Mol Pharmacol* **36:** 437–445, 1989.
7. Mahama PA and Linderman JJ, A Monte Carlo study of the dynamics of G protein activation. *Biophys J* **67:** 1345–1357, 1994.
8. Samama P, Cotecchia S, Costa T and Lefkowitz RJ, A mutation-induced activated state of the β_2-adrenergic receptor: Extending the ternary complex model. *J Biol Chem* **268:** 4625–4636, 1993.
9. Costa T, Ogino Y, Munson PJ, Onaran HO and Rodbard D, Drug efficacy at guanine nucleotide-binding regulatory protein-linked receptors: Thermodynamic interpretation of negative antagonism and of receptor activity in the absence of ligand. *Mol Pharmacol* **41:** 549–560, 1992.
10. Neubig RR, Gantzos RD and Thomsen WJ, Mechanism of agonist and antagonist binding to α_2-adrenergic receptors: Evidence for a precoupled receptor–guanine nucleotide protein complex. *Biochemistry* **27:** 2374–2384, 1988.
11. Tian W, Duzic E, Lanier SM and Deth RC, Determinants of α_2-adrenergic receptor activation of G-proteins: Evidence for a precoupled receptor/G-protein state. *Mol Pharmacol* **45:** 524–531, 1994.
12. Polastron J, Jauzac P and Meunier JC, The delta-opiod receptor in neuroblastoma × glioma NG 108-15 hybrid cells is

strongly precoupled to a G-protein. *Eur J Pharmacol* **226:** 133–139, 1992.
13. Fay SP, Posner RG, Swann WN and Sklar LA, Real-time analysis of the assembly of ligand, receptor, and G protein by quantitative fluorescence flow cytometry. *Biochemistry* **30:** 5066–5075, 1991.
14. Posner RG, Fay SP, Domalewski MD and Sklar LA, Continuous spectrofluorometric analysis of formyl peptide receptor ternary complex interactions. *Mol Pharmacol* **45:** 65–73, 1994.
15. Torney DC and McConnell HM, Diffusion-limited reaction rate theory for two-dimensional systems. *Proc R Soc Lond A* **387:** 147–170, 1983.
16. Stickle D and Barber R, Comparisons of the combined contributions of agonist binding frequency and intrinsic efficiency to receptor-mediated activation of adenylate cyclase. *Mol Pharmacol* **40:** 276–288, 1991.
17. Kenakin T, *Pharmacologic Analysis of Drug–Receptor Interaction*, 2nd Ed. Raven Press, New York, 1993.
18. Whaley BS, Yuan N, Clark RB and Barber R, Differential expression of the β-adrenergic receptor modifies agonist stimulation of adenylyl cyclase: A quantitative evaluation. *Mol Pharmacol* **45:** 481–489, 1994.
19. Hanski E, Rimon G and Levitzki A, Adenylate cyclase activation by the β-adrenergic receptors as a diffusion controlled process. *Biochemistry* **18:** 846–853, 1979.
20. Bakardjieva A, Galla HJ and Helmreich EJM, Modulation of the β-receptor adenylate cyclase interactions in cultured Chang liver cells by phospholipid enrichment. *Biochemistry* 18: 3016–3023, 1979.
21. Omann GM and Porasik-Lowes MM, Graded G-protein uncoupling by pertussis toxin treatment of human polymorphonuclear leukocytes. *J Immunol* **146:** 1303–1308, 1991.
22. Eason MG, Jacinto MT and Liggett SB, Contribution of ligand structure to activation of α_2-adrenergic receptor subtype coupling to G_s. *Mol Pharmacol* **45:** 696–702, 1994.
23. Chabre O, Conklin BR, Brandon S, Bourne HR and Limbird LE, Coupling of the α_{2A}-adrenergic receptor to multiple G-proteins. A simple approach for estimating receptor-G-protein coupling efficiency in a transient expression system. *J Biol Chem* **269:** 5730–5734, 1994.
24. Neubig RR, Membrane organization in G-protein mechanisms. *FASEB J* **8:** 939–946, 1994.
25. Klotz KN and Jesaitis AJ, Neutrophil chemoattractant receptors and the membrane skeleton. *Bioessays* **16:** 193–198, 1994.