A Monte Carlo Study of the Dynamics of G-Protein Activation

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ABSTRACT To link quantitatively the cell surface binding of ligand to receptor with the production of cellular responses, it may be necessary to explore early events in signal transduction such as G-protein activation. Two different model frameworks relating receptor/ligand binding to G-protein activation are examined. In the first framework, a simple ordinary differential equation model is used to describe receptor/ligand binding and G-protein activation. In the second framework, the events leading to G-protein activation are simulated using a dynamic Monte Carlo model. In both models, reactions between ligand-bound receptors and G-proteins are assumed to be diffusion-limited.

The Monte Carlo model predicts two regimes of G-protein activation, depending upon whether the lifetime of a receptor/ligand complex is long or short compared with the time needed for diffusional encounters of complexes and G-proteins. When the lifetime of a complex is relatively short compared with the diffusion time, the movement of ligand among free receptors by binding and unbinding ("switching") significantly enhances G-protein activation. Receptor antagonists dramatically reduce G-protein activation and, thus, signal transduction in this case, and significant clustering of active G-proteins near receptor/ligand complexes results. The simple ordinary differential equation model poorly predicts G-protein activation for this situation. In the alternative case, when diffusion is relatively fast, ligand movement among receptors is less important and the simple ordinary differential equation model and Monte Carlo model results are similar. In this case, there is little clustering of active G-proteins near receptor/ligand complexes.

Results also indicate that as the GTPase activity of the α-subunit decreases, the steady-state level of α-GTP increases, although temporal sensitivity is compromised.

INTRODUCTION

Cells have evolved elaborate strategies for sensing, responding to, and interacting with their environment. Binding of ligand to cell surface receptors is one way in which cells sample molecules in their surroundings. Receptor/ligand complexes on the cell surface may be the first signal in a cascade that translates the presence of extracellular ligand into a cellular response such as secretion, chemotaxis, or proliferation. In many systems, receptor/ligand complexes stimulate the activity of membrane-associated guanine nucleotide binding proteins or G-proteins, which transduce the ligand binding signal across the cell membrane (Taylor, 1990; Birnbaumer et al., 1990). Activated G-proteins increase the activity of other membrane enzymes, such as phospholipase C or adenylate cyclase, propagating the signal throughout the cell. Increases in intracellular calcium or cAMP that follow enzyme activation may then contribute to the observed cellular response.

Interaction of a ligand-bound receptor and an inactive G-protein results in G-protein activation. The G-protein activation cycle is illustrated in Fig. 1 and is discussed in recent reviews (Taylor, 1990; Birnbaumer et al., 1990). G-proteins are heterotrimeric proteins consisting of α, β, and γ subunits, with GDP bound to the α subunit when in its inactive conformation. Interaction of a receptor/ligand complex (C) with an inactive G-protein ($\beta\gamma\alpha$-GDP) results in the transient formation of a receptor/ligand/G-protein complex. The C-$\beta\gamma\alpha$-GDP complex is stabilized by the dissociation of the bound GDP and remains in the C-$\beta\gamma\alpha$- conformation until the association of a guanine nucleotide. C-$\beta\gamma\alpha$- has an enhanced affinity for GTP and a diminished affinity for GDP. In vivo, the combined effects of the differences in guanine nucleotide concentration and the association/dissociation kinetics of GTP and GDP for the α subunit are such that the C-$\beta\gamma\alpha$- conformation is short-lived (several ms), and its formation is likely to lead to the activation of the G-protein by the association of GTP. Binding of GTP to the α-subunit results in a conformational change which leads to the separation of the C-$\beta\gamma\alpha$-GTP complex into C, $\beta\gamma$, and α-GTP components. The receptor/ligand complex thus acts as a catalyst in G-protein activation, remaining unchanged by the activation and separation of the G-protein subunits. α-GTP activates other membrane enzymes that in turn propagate the receptor/ligand binding signal by interacting with other downstream components in the signaling cascade. The lifetime of α-GTP is dictated by the intrinsic GTPase activity of the α-subunit, which acts to hydrolyze the bound GTP to GDP. Although α-GTP has traditionally been considered the sole signaling molecule from G-protein activation, recent evidence suggests that $\beta\gamma$ subunits also regulate enzyme activity (Katada et al., 1984; Kamayema et al., 1993; Koch et al., 1993; Clatham and Neer, 1993). The inactive α-GDP subunit can collide and associate with a $\beta\gamma$ subunit, reforming the initial inactive G-protein, $\beta\gamma\alpha$-GDP.

The rate and extent of G-protein activation, and ultimately the cell response, are directly affected by receptor/ligand

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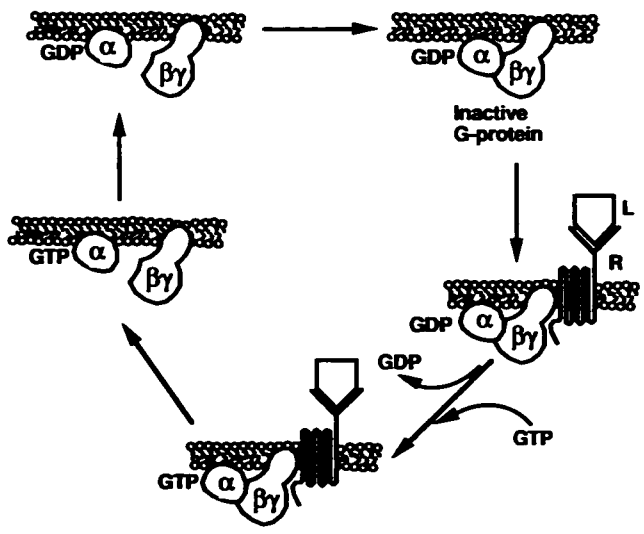

FIGURE 1 G-protein activation cycle. Receptor/ligand complexes catalyze the activation of G-protein by increasing the rate of exchange of GDP for GTP on the G-protein. Binding of GTP by the G-protein results in its separation into $\beta\gamma$ and α-GTP subunits. The lifetime of the α-GTP subunit is determined by the GTPase activity of the α-subunit. After hydrolysis of bound GTP, the α- and $\beta\gamma$ subunits recombine, completing the cycle. A receptor/ligand complex is only shown in the G-protein activation cycle steps in which it participates.

binding characteristics, the rates of encounter and reaction of receptor/ligand complexes and G-protein, and the GTPase activity of the G-protein α-subunit. Bacterial toxins, such as pertussis and cholera toxin, interfere with the G-protein cycle and alter the cell response, suggesting that proper regulation of the G-protein cycle and G-protein activity are important in maintaining cell function.

Development of a mathematical model that relates receptor/ligand binding to G-protein activation while incorporating these effects would be valuable. As more information becomes available, the model could be linked to models for the generation of specific cellular responses and could be used in predicting the effect of manipulating specific parameters on the subsequent responses of cells in culture or in vivo. Such a model could also be useful in uncovering the underlying basis of varying agonist efficacies. The purpose of this paper is to explore such a model formulation for G-protein activation.

A simple model relating receptor/ligand binding to G-protein activation can be written as follows. For the binding of monovalent ligand to a homogeneous set of monovalent receptors, receptor/ligand complex (C) formation is expressed as

$$\frac{d[C]}{dt} = k_f[L][R] - k_r[C] \quad (1)$$

with

$$[R_t] = [R] + [C] \quad (2)$$

where k_f is the receptor/ligand association rate constant (M^{-1} s^{-1}) and k_r is the receptor/ligand dissociation rate constant (s^{-1}). Species in brackets indicate quantity, #/cell for membrane-associated species and mol/l for ligand in solution. L is ligand, R is free receptors on the cell surface, and R_t is total receptors on the cell surface, usually assumed constant on the time-scale of signal transduction (s-min) (Lauffenburger and Linderman, 1993). This formulation assumes that the concentrations of all species are spatially homogeneous.

To compare the time of a C/G-protein encounter (several milliseconds; Taylor, 1990) with the likely time *between* encounters (determined by the diffusivity of receptor/ligand complexes and G-proteins) the time between encounters can be estimated from (Einstein, 1905),

$$t = \frac{d^2}{4D}, \quad (3)$$

where t is the time between encounters of a receptor and any G-protein, D is the sum of the receptor and G-protein diffusion coefficients, and d is one-half the mean distance between G-proteins. If G-proteins are assumed to be uniformly distributed, d can be estimated by

$$d = \sqrt{\frac{A}{\pi N}}, \quad (4)$$

where A is the cell surface area and N is the number of inactive G-proteins in the cell membrane (Lauffenburger and Linderman, 1993). Estimates of these parameters are $D = 1 \times 10^{-10}$ cm^2/s (Lauffenburger and Linderman, 1993), $A = 2200$ μm^2 (Alberts et al., 1989), and $N = 100000$/cell, although G-proteins may number between hundreds of thousands and millions per cell (Bokoch et al., 1988; Neubig et al., 1985).

Given these estimates, the time between collisions of a single receptor with any G-protein is approximately 170 ms, an order of magnitude greater than the time required for activation of a G-protein in contact with a receptor/ligand complex. Thus, the time required for a two-dimensional (2-D) diffusion encounter between receptors and G-proteins appears to be a limiting factor in the activation of G-proteins. The direct in vivo measurements of the effect of receptor diffusion on G-protein-coupled signal transduction have not been made; however, the role of receptor lateral diffusion in signal transduction is supported by experiments in which the membrane fluidity of the cells is altered and G-protein-stimulated enzyme activity is affected (Hanski et al., 1979; Bakardjieva et al., 1979; Moscona-Amir et al., 1989; Gorospe and Conn, 1987). Reactions between receptor/ligand complexes and G-protein are thus assumed to be diffusion-limited in the formulation of the model equations.

If G-protein molecules are uniformly distributed within the plasma membrane, the diffusion-limited encounter rate constant between C and G, k_c, can be estimated using

$$k_c = \frac{2\pi D}{A \ln(d/s)}, \quad (5)$$

where s is the encounter radius (≈1–10 nm) (Lauffenburger and Linderman, 1993).

Taylor (1990) states that the transient encounter between an agonist-occupied receptor and a G-protein lasts for only a few milliseconds. Thus, the intermediates C-βγ-GDP, C-βγ-, and C-βγ-GTP are neglected, and formation of α-GTP is treated as a direct result of collision between C and G-protein. With inactivation of α-GTP dependent on the intrinsic GTPase activity of the α-subunit, a simple expression for α-GTP formation is written as

$$\frac{d[\alpha\text{-GTP}]}{dt} = k_c[C][G] - k_i[\alpha\text{-GTP}], \tag{6}$$

where k_c is the overall reaction rate constant for collision of C and G-protein and release of α-GTP ((#/cell)$^{-1}$ s^{-1}), and k_i is the GTPase activity (s^{-1}). If each α-GDP formed by inactivation of α-GTP quickly reassociates with a βγ, the number of α-GDP molecules in the membrane can be assumed to be small. For a constant total concentration of G-protein, $[G_t]$, α-GDP can be neglected in the overall G-protein balance, or

$$\begin{aligned}[G_t] &= [\beta\gamma\alpha\text{-GDP}] + [\alpha\text{-GTP}] + [\alpha\text{-GDP}] \\ &\approx [\beta\gamma\alpha\text{-GDP}] + [\alpha\text{-GTP}],\end{aligned} \tag{7}$$

and the kinetics of α-GDP/βγ recombination can be neglected.

This model, although appealing in its simplicity, may be severely limited in its ability to represent accurately the dynamics of G-protein activation. Its shortcomings are inherent not only in some of its key assumptions, but also in the basic formulation using ordinary differential equations (ODEs).

For receptor/ligand complex formation described by Eq. 1, the equilibrium number of receptor/ligand complexes, $[C_{eq}]$, is given by

$$[C_{eq}] = \frac{[R_t][L]}{[L] + K_D}, \tag{8}$$

where K_D, the equilibrium receptor/ligand dissociation constant, is equal to the ratio k_r/k_f. Even at equilibrium, receptor/ligand binding remains dynamic as ligand continues to bind and unbind to receptors with rates proportional to k_f and k_r, respectively. Stickle and Barber (1989) have termed the movement of ligand among free receptors by binding and unbinding "switching." Experimental evidence suggests that "switching" of ligand among receptors may significantly contribute to enzyme activation (Stickle and Barber, 1989) and calcium mobilization (Mahama and Linderman, 1994b).

When receptor/ligand binding and dissociation kinetics are rapid, the lifetime of a receptor/ligand complex is short, and ligand "switches" quickly among the free receptors on the cell surface. The fraction of total receptors bound at any time can be thought of more accurately as the fraction of time each receptor is occupied by ligand. Conversely, when receptor/ligand binding and dissociation kinetics are slow, the lifetime of each complex is long, and ligand movement among free receptors by "switching" is minimized. In this case, the fraction of total receptors bound can be reasonably thought of as representing receptors nearly continuously bound by ligand.

For cases of equal equilibrium numbers of receptor/ligand complexes, a significant physical difference exists between low and high levels of ligand "switching." Within the simple model formulation (Eqs. 1–2 and 5–7), however, the situation at steady state is dependent only on the ratio of the dissociation and association rate constants (K_D) and not their absolute values. Thus, the steady-state number of α-GTP molecules predicted from this model will be independent of ligand "switching."

The assumption of a well mixed, homogeneous system may not hold. Dimensionality is important in diffusion/reaction problems; the kinetics of three-dimensional bulk phase reactions are significantly different than those of two-dimensional surface reactions (Torney and H. M. McConnell, 1983). Bimolecular reactions in this latter system are restricted to two dimensions, and the rates of these reactions may be limited by diffusivity. The formulation of the model equations and the estimate of the diffusion-limited encounter rate constant k_c (Eq. 5) are based on the assumption of a well mixed system. As receptor/ligand complexes and G-protein diffuse, their numbers and distributions within the membrane may change. Development of spatial heterogeneity in the species concentrations would undermine the model formulation as well as the estimation of the encounter rate constant.

Depending on the diffusivity and the distributions of molecules in the membrane, the recombination of α-GDP and βγ may be slow. As signaling progresses, the assumption that the α-GDP concentration is small may be poor, and a significant fraction of the total G-protein pool may exist as α-GDP. A differential equation for α-GDP with formation by inactivation of α-GTP and removal by collision of α-GDP and βγ would also have to be included. However, the recombination rate constant for α-GDP and βγ is unknown and likely to be diffusion-limited. As with receptor/ligand complexes and G-protein, the numbers and distributions of α-GDP and βγ in the cell membrane are time-dependent. As a result, the encounter rate constant for the bimolecular recombination of α-GDP and βγ may not be a true constant. Even if a time- and concentration-dependent recombination rate constant is included in the model, its form would be difficult to determine.

If this reaction system is not well mixed, then ODEs cannot accurately represent the events occurring in the system. A further complication is that the membrane concentrations of species in this signaling pathway, although numbering in the tens or hundreds of thousands, are very dilute and, thus, the validity of the continuum approximation is questionable. Finally, stochastic effects may be significant in the interaction of membrane species and G-protein activation. Again, with our simple model of G-protein activation, these effects cannot be considered.

To summarize, in the simple model described above, the time-evolution of ligand-bound receptors and active G-proteins is assumed to be adequately described by Eqs.1–2 and 5–7. To examine this model formulation, to allow for stochastic effects and "switching" to play a role in single cell behavior, to predict more quantitatively the diffusion-limited rate constant for the activation of G-protein by bound receptors (k_c), to include the diffusion-limited recombination of α and $\beta\gamma$ subunits, and to allow for nonhomogeneous distribution of molecules on the membrane surface, a Monte Carlo framework for examining the dynamics of active G-protein formation is formulated.

MONTE CARLO MODEL DESCRIPTION

The Monte Carlo model includes receptor/ligand binding, collision of C and G-protein to form α-GTP, inactivation of α-GTP to produce α-GDP, and recombination of α-GDP and $\beta\gamma$. All bimolecular membrane reactions in the Monte Carlo simulations are considered to be diffusion-limited.

Binding and unbinding of ligand to receptors occurs with probabilities proportional to $k_f[L]$ and k_r, respectively.

$$R \underset{k_r}{\overset{k_f[L]}{\rightleftharpoons}} C \tag{9}$$

The ligand concentration, $[L]$, is assumed to be uniform and constant. Collision of a receptor/ligand complex and an inactive G-protein results in the activation of G-protein.

$$C + \beta\gamma\alpha\text{-GDP} \xrightarrow{k_c} C + \alpha\text{-GTP} + \beta\gamma, \tag{10}$$

where k_c is the diffusion-limited encounter rate constant between C and G-protein. Inactivation of α-GTP by hydrolysis of GTP to GDP occurs with a probability proportional to the GTPase activity of the α-subunit, k_i.

$$\alpha\text{-GTP} \xrightarrow{k_i} \alpha\text{-GDP} \tag{11}$$

Diffusion-limited recombination of α-GDP and $\beta\gamma$ occurs upon collision. The encounter rate constant, k_α, for recombination of $\beta\gamma$ and α-GDP can be calculated from the model results.

$$\alpha\text{-GDP} + \beta\gamma \xrightarrow{k_\alpha} \alpha\beta\gamma\text{-GDP} \tag{12}$$

The total number of receptors (R_t) and G-proteins (G_t) in the cell membrane is constant during the course of a simulation. Constraining relationships for receptor and G-protein species are given by the following equations:

$$[R_t] = [R] + [C] \tag{13}$$

$$[G_t] = [\beta\gamma\alpha\text{-GDP}] + [\alpha\text{-GTP}] + [\alpha\text{-GDP}] \tag{14}$$

$$[\beta\gamma] = [\alpha\text{-GTP}] + [\alpha\text{-GDP}]. \tag{15}$$

Parameter values used in the simulations are given in Table 1.

The characteristic time of each simulation, t_s, is calculated from the fastest event in the system, either a reaction event or a move event governed by the diffusivity. The characteristic time associated with each reaction event, t_r, is

$$t_r = k^{-1}, \tag{16}$$

where k is the rate constant of a reaction with units of inverse time ($k_f[L]$, k_r, or k_i). The characteristic time per move, t_m, of a molecule in 2-D is given by the relation (Einstein, 1905)

$$t_m = \frac{d_n^2}{4D}, \tag{17}$$

where d_n is the lattice spacing, and D is the diffusion coefficient. Calculations of t_r and t_m are made for every reaction rate constant and diffusion coefficient used in the simulation. The characteristic time of the simulation, t_s, is set equal to the characteristic time of the fastest event (the smallest value of t_r or t_m). The Monte Carlo time-step is thus t_s/N, where N, the number of particles in the simulation, may change over the course of the simulation. The probability of a reaction or move event, P_r or P_m, is set equal to the ratio of the characteristic time of the simulation, t_s, to the characteristic time of an event, t_r or t_m.

$$P_r = \frac{t_s}{t_r} = k\,t_s \tag{18}$$

$$P_m = \frac{t_s}{t_m} = \frac{4Dt_s}{d^2}. \tag{19}$$

The fastest event in the simulation, which has a characteristic time equal to t_s, thus occurs with a probability of 1, and all other events occur with probabilities between 0 and 1.

To ensure that the model results are independent of the Monte Carlo time-step, control simulations are run with time-steps smaller than those calculated above, with the reaction and move probabilities scaled accordingly. Simulation results with the calculated Monte Carlo time-step and with smaller time-steps are indistinguishable.

Results shown are from runs using a square grid with periodic boundary conditions and a lattice spacing of 7 nm, approximately a protein diameter. A square mesh is chosen because of the low density of molecules in the simulations. For $[L] < 10$ μM, simulations are run on an 1800×1800-site lattice, and simulations for all other ligand concentrations are

TABLE 1 Parameter definitions and values used in the Monte Carlo model

Parameter	Definition	Value
$[R_t]$	Total cell receptors	20000/cell
$[R_b]$	Blocked or inactivated receptors	$(0.048–0.98)R_t$
$[L]$	Ligand concentration	1–1000 μM
k_f	Ligand/receptor association rate constant	4×10^4, 1×10^6 $M^{-1}s^{-1}$
k_r	Ligand/receptor dissociation rate constant	2, 50 s^{-1}
k_r/k_f	Equilibrium receptor/ligand dissociation constant	50 μM
$[G_t]$	Total G-protein	100,000/cell
k_i	G-Protein inactivation rate constant	0.02–2 s^{-1}
D	Diffusion coefficient	10^{-11}–10^{-9} cm^2/s
A	Surface area of a cell	2200 μm^2
d_n	Monte Carlo lattice spacing	7 nm

run on a 1000 × 1000-site lattice. Each simulation thus represents approximately 2–7% of the total cell surface area. Simulations are also run on simulation grids with lattice spacings larger than 7 nm to determine whether the results are independent of the lattice spacing. For lattice spacings between 7 and 28 nm, the results are independent of the lattice spacing for simulations in which receptor/ligand binding kinetics are rapid ($k_f = 1 \times 10^6\ M^{-1}\ s^{-1}$, $k_r = 50\ s^{-1}$). For simulations in which receptor antagonists are added and the effects of receptor/ligand binding kinetics are eliminated, the results do show a slight effect of lattice spacing on α-GTP production. For lattice spacings from 7 to 28 nm, α-GTP production increases with lattice spacing at a rate of approximately a 1% per nm. Lattice spacings lower than 7 nm, which are less than one protein diameter, were not tested because of the physical constraints of the system.

The simulations are initialized by placing particles randomly on the simulation grid. No two molecules are allowed to occupy the same lattice site (because of the physical constraint of the spacing), so if the selected site is occupied, a new random lattice site is selected. After a particle is placed on a lattice site, it is randomly identified as a receptor or a G-protein based on their relative proportions in the membrane. This results in slight variations in the total number of receptors and G-proteins among simulations, while the total number of molecules remains constant. For simulations of antagonist-pretreated cells, a particle is identified as a receptor, G-protein, or blocked receptor (R_b) during grid initialization, again based on their relative proportions in the membrane. Antagonist-receptor binding is treated as irreversible over the time scale of the simulation, so all receptors designated as R_b during grid initialization remain inactive throughout the simulation.

Each particle is chosen randomly and, based on its identity, reaction and move probabilities, P_r and P_m, are calculated. For reactions that occur independently of movement, such as binding and unbinding of ligand to a receptor and inactivation of α-GTP, a successful reaction results in the reidentification of the particle. If movement is accepted based on the move probability, and the selected lattice site is unoccupied, the particle is moved. If movement is accepted based on the move probability and the selected lattice site is occupied by another particle, collision reactions are considered. If the collision occurs between two nonreacting particles, e.g., a receptor colliding with a receptor, the move is rejected. Although some restriction in mobility occurs in not allowing two particles to occupy the same lattice site, the concentrations of species in these simulations are dilute (on the order of 0.3% of the lattice sites filled), so species concentration does not significantly affect the diffusivity. This is in contrast to simulations by Saxton and Owicki (1989) of the effect of diffusivity of rhodopsin and transducin on activation of transducin in which the concentrations of both species are relatively high.

Collision between an α-GDP and a $\beta\gamma$ results in reformation of inactive G-protein for every collision and produces a net loss of one particle. For collision between a C and a G-protein, activation results in the net formation of one particle. Because no two particles are allowed to occupy the same lattice site, the move is accepted and activation occurs as long as at least one of the four nearest-neighbor sites around the nonmoving particle is unoccupied. If all four nearest-neighbor sites around the nonmoving particle are occupied, the move is rejected. Again, because species concentrations in these simulations are dilute, this restriction on diffusion-limited activation occurs very infrequently.

The degree of spatial heterogeneity in the distribution of a particular molecule is determined from the model simulations by calculating its probability distribution function, $P(x)$, where x is distance (McQuarrie, 1976). For a random distribution of molecules, $P(x)$ is independent of distance, time, and particle identity. If molecules A and B are not randomly distributed, then $P(x)_{A\rightarrow B}$, which is $P(x)$ of particle A around particle B, may be a function of time and distance. From the Monte Carlo simulations, $P(x)_{A\rightarrow B}$ at a given time is calculated by counting As in the horizontal and vertical directions from each B for all distances. This value is then normalized by the total number of sites around all Bs at that distance, which for our square simulation grid is 4 times the number of Bs. The choice of counting only particles at a particular horizontal and vertical distance, as opposed to counting particles at a particular radial distance, is suggested by the grid configuration.

Results of the model, in the form of $P(x)$ and species numbers, are the average of 10–20 simulations converted to a per cell basis. Because the numbers of species continue to fluctuate because of random variation, the numbers of species at steady state are calculated by averaging over at least 15 s.

The encounter rate constants k_c and k_α are calculated from the Monte Carlo simulation results. All species concentrations are known as a function of time; therefore, k_c and k_α can be approximated from Eqs. 20–21 where $\Delta[\alpha\text{-GTP}]$ and $\Delta[\alpha\text{-GDP}]$ are the changes in [α-GTP] and [α-GDP] occurring over the time interval Δt, respectively.

$$k_c = \left(\frac{\Delta[\alpha\text{-GTP}]}{\Delta t} + k_i[\alpha\text{-GTP}]\right) \Big/ ([C][\beta\gamma\alpha\text{-GDP}]) \quad (20)$$

$$k_\alpha = \left(k_i[\alpha\text{-GTP}] - \frac{\Delta[\alpha\text{-GDP}]}{\Delta t}\right) \Big/ ([\beta\gamma][\alpha\text{-GDP}]) \quad (21)$$

RESULTS

Typical simulation results from the Monte Carlo model for one set of parameter values are shown in Fig. 2. The model predicts that the number of receptor/ligand complexes increases to a steady-state level. Fluctuations in the number of complexes about the steady-state level occur as receptors continue to bind and unbind ligand. Formation of α-GTP by collision of C and G-protein lags behind complex formation. In this particular simulation, the number of α-GTP molecules peaks near 15 s, and then tails off to a steady-state level after 60 s.

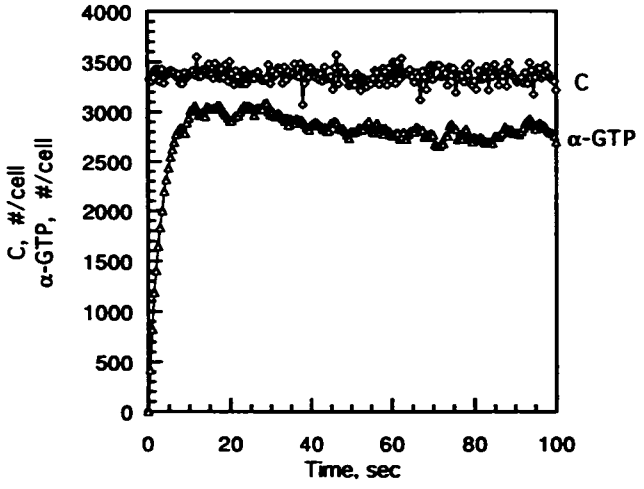

FIGURE 2 Monte Carlo model predictions of the number of receptor/ligand complexes (C) and α-GTP are shown. All species numbers reach a steady state after an initial transient, the length of which is dependent on receptor/ligand binding kinetics, ligand concentration, diffusivity, and GTPase activity. Parameters used in this simulation are $[L] = 10$ μM, $k_f = 1 \times 10^6$ M^{-1} s^{-1}, $k_r = 50$ s^{-1}, $D = 1 \times 10^{-11}$ cm^2/s, $k_i = 0.2$ s^{-1}, and $[R_t] = 0$. All other parameters are given in Table 1.

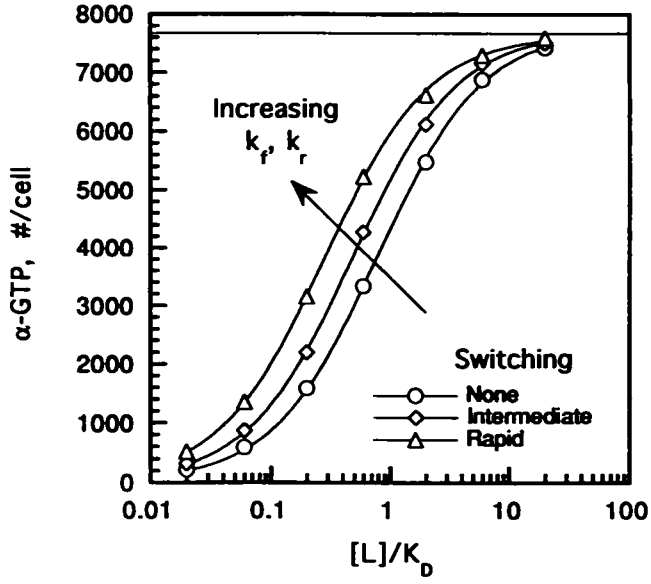

FIGURE 3 Effect of receptor/ligand binding kinetics on steady-state G-protein activation. The three data sets differ in values of ligand/receptor association and dissociation rate constants. For the "no switching" set, a fraction of receptors is initially blocked by a receptor antagonist and the remaining receptors bind ligand according to $k_f[L] = 50$ s^{-1} and $k_r = 0$ to ensure that all unblocked receptors are occupied by ligand. Results for the "no switching" case are plotted against the ligand concentration that produces an equal equilibrium number of complexes in the absence of blocker. Maximum G-protein activation occurs when all receptors are bound by ligand continuously, and is represented by the horizontal line at the top of the figure. For intermediate ligand switching, $k_f = 4 \times 10^4$ M^{-1} s^{-1} and $k_r = 2$ s^{-1}. For rapid ligand switching, $k_f = 1 \times 10^6$ M^{-1} s^{-1} and $k_r = 50$ s^{-1}. Other parameters used are $D = 1 \times 10^{-11}$ cm^2/s and $k_i = 0.2$ s^{-1}.

Effect of ligand "switching" on steady-state G-protein activation

To consider the effect of receptor/ligand binding kinetics on steady-state G-protein activation, systems with equal equilibrium levels of receptor/ligand complexes (calculated from Eq. 8) and varying levels of "switching" are compared over a range of ligand concentrations. A "no switching" case is included in which a particular fraction of receptors (F_b) are always blocked by a receptor antagonist and receptor/ligand binding is allowed to occur irreversibly. In this situation, the effects of "switching" are eliminated because the unblocked receptors are bound by ligand at all times. The number of equilibrium receptor/ligand complexes for this case is then simply $[R_t](1 - F_b)$. Results from these receptor blocker/no switching simulations are plotted against the ligand concentration calculated using Eq. 8 that would produce the same equilibrium number of receptor/ligand complexes in the absence of receptor blocker.

The steady-state number of α-GTP molecules produced by the 2-D diffusion and collision of C and G-protein with varying ligand "switching" contributions is shown in Fig. 3. Symbols show model predictions, and curves are drawn to illustrate the trends. The three data sets shown represent no switching, intermediate switching, and rapid switching cases. The model predicts that steady-state levels of α-GTP increase with increasing ligand "switching." As the ligand concentration increases, the number of receptor/ligand complexes approaches the total number of receptors, and the steady-state number of α-GTP approaches a maximum level of activation.

The contribution of ligand "switching" to the steady-state level of α-GTP can be more readily assessed by ratioing the steady-state levels of α-GTP produced by 2-D diffusion and collision with ligand "switching" (α-GTP$_S$) to the level of α-GTP produced by 2-D diffusion and collision without "switching" (α-GTP$_{NS}$). The ratio (α-GTP$_S$/α-GTP$_{NS}$) is a measure of the enhancement of α-GTP production by ligand movement among receptors. The values of α-GTP$_S$/α-GTP$_{NS}$ for the simulations of Fig. 3 are shown in Fig. 4. G-protein activation enhancement by ligand "switching" is most pronounced at low ligand concentrations when the pool of free receptors is large. For rapid "switching," nearly a 2.5-fold increase in α-GTP is seen in this region. As ligand concentration increases and the number of free receptors decreases, contributions of ligand "switching" to G-protein activation diminish, and α-GTP$_S$/α-GTP$_{NS}$ reaches unity at high ligand concentrations.

Effect of diffusivity on steady-state G-protein activation

The Monte Carlo model of G-protein activation is used to determine whether the diffusivity of membrane species significantly affects α-GTP production. For a given simulation, the diffusivities of all species are equal. Results of altering the diffusion coefficient for the case of rapid "switching" are shown in Fig. 5. Diffusion coefficients are varied from 1×10^{-11} to 1×10^{-9} cm^2/s, within ranges measured for receptors (Gennis, 1989). With the value of GTPase activity used

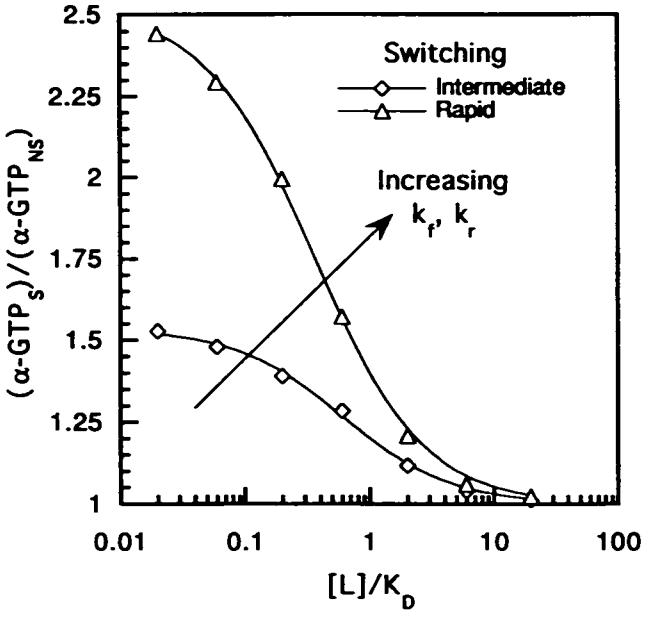

FIGURE 4 Enhancement of steady-state G-protein activation by ligand "switching." Ratios of steady-state numbers of α-GTP produced by 2-D diffusion and collision and ligand "switching" to steady-state numbers of α-GTP produced by 2-D diffusion and collision alone (α-GTP_S/α-GTP_{NS}) are shown for the simulations presented in Fig. 3.

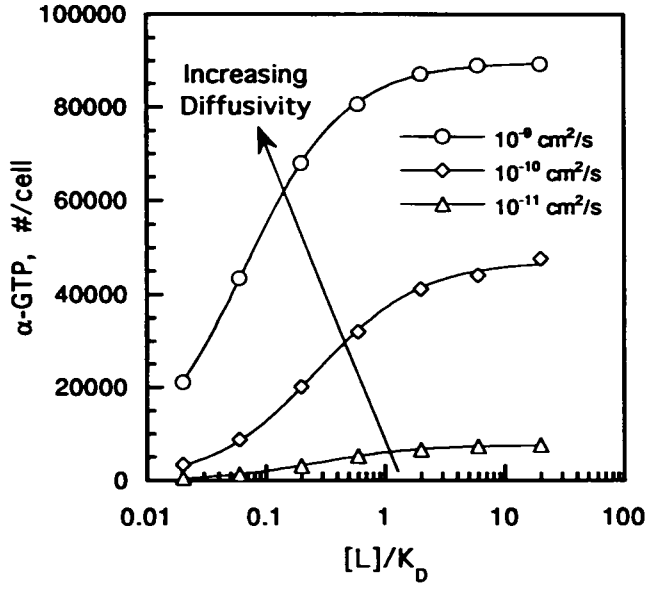

FIGURE 5 Effect of diffusivity on steady-state G-protein activation. Steady-state levels of α-GTP produced with rapid ligand "switching" are shown. As the diffusivity increases, the steady-state level of α-GTP increases. For $D = 1 \times 10^{-11}$ cm²/s, α-GTP production is less than 10% of the total G-protein pool over the entire range of ligand concentration. For $D = 1 \times 10^{-9}$ cm²/s, significant G-protein activation occurs at very low ligand concentrations, and α-GTP production saturates at high ligand concentrations. G-protein activation for the intermediate diffusivity, $D = 1 \times 10^{-10}$ cm²/s, reaches a maximum of approximately 50% of all G-protein in the α-GTP form. Other parameters used are $k_f = 1 \times 10^6$ M^{-1} s^{-1}, $k_r = 50$ s^{-1}, and $k_i = 0.2$ s^{-1}.

in these simulations ($k_i = 0.2\ s^{-1}$), changes in the diffusivity significantly affect α-GTP production. For the lowest diffusivity, α-GTP production is low, even at high ligand concentrations. For the highest diffusivity, α-GTP production saturates for $[L]/K_D \geq 5$, and α-GTP production is significant at very low ligand concentrations. For the intermediate diffusivity, α-GTP production increases steadily over the entire range of ligand concentrations shown. In addition, low ligand stimulation results in limited G-protein activation. Trends for the effect of diffusivity on the steady-state level of α-GTP are independent of the receptor/ligand association and dissociation kinetics, although absolute numbers of α-GTP produced vary.

The relative roles of ligand "switching" and diffusivity in α-GTP production can be compared by examining the ratio α-GTP_S/α-GTP_{NS} as a function of diffusivity. Enhancement in α-GTP production for the simulations of Fig. 5 is shown in Fig. 6. Maximum enhancement in α-GTP production occurs for the lowest diffusivity, when the time between collisions of receptor/ligand complexes and G-protein is the longest. Ligand "switching" aids in mixing the system by allowing receptor/ligand complexes access to a larger fraction of the membrane surface, increasing chances of collision between C and G-protein. Even at the highest diffusivity, a 60% enhancement in α-GTP production is seen for low $[L]/K_D$.

Effects of ligand "switching" and diffusivity on spatial distribution of molecules

Movement of ligand among free receptors, or "switching," and 2-D diffusion play a major role in determining the magnitude of the steady-state number of α-GTP molecules as well as in determining the homogeneity or heterogeneity of molecule distributions in the membrane. To measure quantitatively the degree of heterogeneity in molecule distributions, probability distribution functions, $P(x)$, are calculated.

As C and G-protein diffuse and collide, α-GTP is produced. α-GTP diffuses as well, but has a limited lifetime determined by its GTPase activity. The degree of clustering of α-GTP near receptor/ligand complexes can be assessed by computing $P(x)_{\alpha GTP \to C}$. The steady-state values of $P(x)_{\alpha GTP \to C}$ (shown for $t = 15$ s) for high diffusivity/no switching, low diffusivity/rapid switching, and low diffusivity/no switching are shown in Fig. 7. As expected, for high diffusivity (10^{-9} cm²/s) the distribution of α-GTP near C is nearly independent of distance. The system appears to be well mixed with little clustering of α-GTP near receptor/ligand complexes.

For low diffusivity (10^{-11} cm²/s)/no ligand switching, the $P(x)_{\alpha GTP \to C}$ shows that α-GTP is likely to be found near a C. For distances from a complex greater that 25 lattice spaces (175 nm), the probability of finding an α-GTP is approximately zero. This distance is on the order of the distance an α-GTP may diffuse before being inactivated by hydrolysis of its bound GTP, d_i:

$$d_i = \sqrt{4Dt_i} = \sqrt{\frac{4D}{k_i}}, \qquad (22)$$

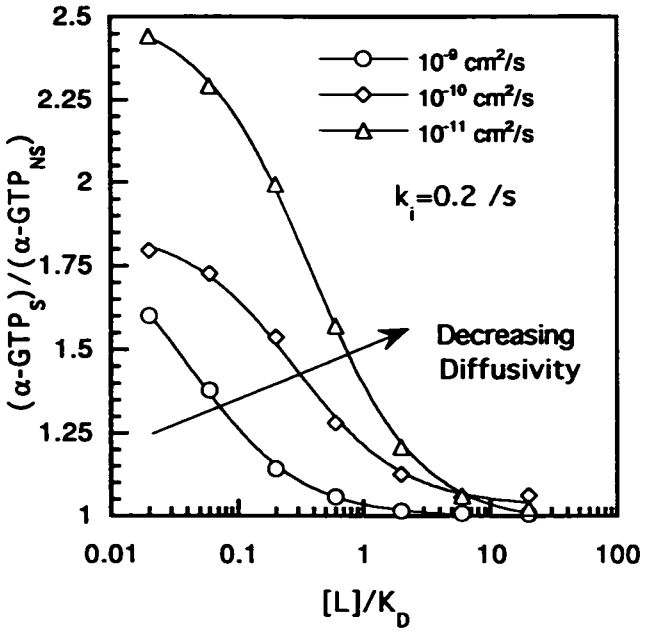

FIGURE 6 Effect of diffusivity on enhancement of steady-state α-GTP production. The ratio (α-GTP$_S$/α-GTP$_{NS}$) is shown for the simulations presented in Fig. 5.

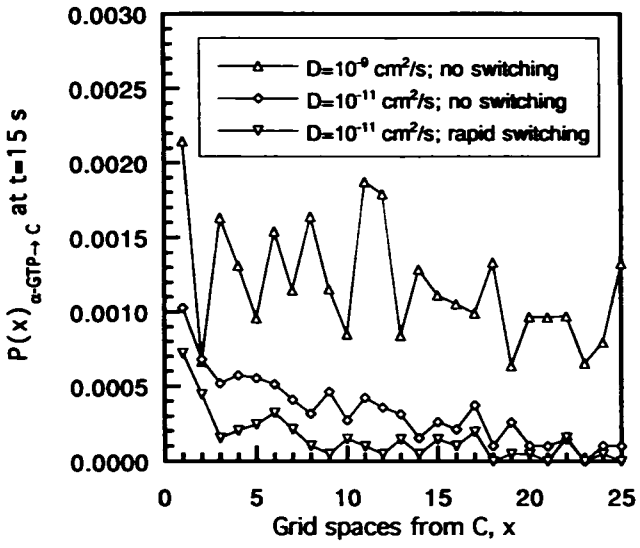

FIGURE 7 Probability distribution function of α-GTP near receptor/ligand complexes, $P(x)_{\alpha\text{-GTP}\to C}$, at t = 15 s (steady state). The effect of diffusivity and ligand "switching" on the clustering of α-GTP near receptor/ligand complexes is calculated. For high diffusivity, the concentration of α-GTP near C is nearly independent of distance, indicating a homogeneous distribution of α-GTP. For low diffusivity, significant clustering of α-GTP near receptors is observed, although rapid ligand "switching" reduces the clustering effect. Parameters used in the simulations are: $[L]$ = 10 μM; high diffusivity, $D = 1 \times 10^{-9}$ cm²/s; low diffusivity, $D = 1 \times 10^{-11}$ cm²/s; rapid ligand switching, $k_f = 1 \times 10^6$ M^{-1} s^{-1} and k_r = 50 s^{-1}; k_i = 0.2 s^{-1}.

where the time before inactivation, t_i, can be estimated as $1/k_i$. For the parameters used in Fig. 7, d_i is approximately 140 nm.

For low diffusivity/rapid switching, the $P(x)_{\alpha GTP\to C}$ is smaller and less steep than for the low diffusivity/no switching case, whereas the actual number of α-GTP molecules is greater. During rapid "switching," each receptor is transiently occupied by ligand. In calculating $P(x)_{\alpha GTP\to C}$, α-GTP is only counted near those receptors currently bound to ligand, and at any time the distribution of α-GTP near a C varies depending upon the total fraction of time the receptor has been bound by ligand. This is consistent with the presumption that ligand "switching" distributes active G-protein over the cell surface by allowing all receptors to activate G-protein for some fraction of time.

To determine whether local depletion of G-protein occurs around receptor/ligand complexes, the $P(x)$ of inactive G-protein around C, $P(x)_{G\to C}$ is examined (data not shown). For the three situations discussed in Fig. 7, the density of G-protein molecules near complexes is lower than the density further away, with the difference being most pronounced for the low diffusivity/no ligand switching simulation. This suggests that the diffusion-limited encounter rate constant between C and G-protein, k_c, decreases with time because of the local depletion of G-protein near receptor/ligand complexes.

Calculation of diffusion-limited encounter rate constants

Turning from steady-state to transient results of the model, the cell-averaged encounter rate constant between C and G-protein, k_c, is examined. Values of k_c calculated for the three simulations of Fig. 7 are shown in Fig. 8. In addition, the theoretical predictions of k_c calculated from Eqs. 4 and 5 with $N = [G_t] = 100{,}000$ and s = 3.5 nm are shown for both diffusivities. After a slight initial drop in k_c seen in all simulations because of local depletion of G-protein near receptor/ligand complexes, the encounter rate constant k_c is approximately constant. For high diffusivity/no switching,

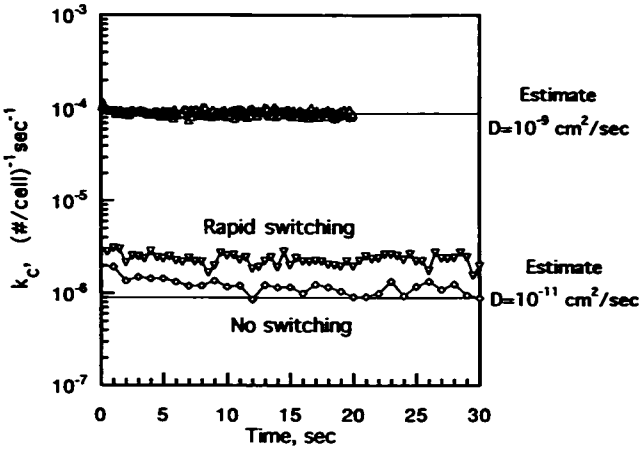

FIGURE 8 Effect of ligand "switching" and diffusivity on encounter rate constant between C and G-protein, k_c. Estimates of k_c are made using Eq. 5. The Monte Carlo model predictions for k_c are calculated from transient species numbers as given by Eq. 20. Parameters used in these simulations are the same as those in Fig. 7.

the encounter rate constant predicted by the Monte Carlo simulation is almost identical to the theoretical value predicted by Eq. 5. For low diffusivity/no switching, the theoretical value calculated for k_c is slightly lower than the Monte Carlo simulation prediction. For low diffusivity/rapid switching, k_c is 2–3 fold higher that the theoretical value because ligand movement among free receptors increases the probability of collision between receptor/ligand complexes and G-protein.

The cell-averaged recombination rate constant between $\beta\gamma$ and α-GDP, k_α, again calculated for the three cases discussed in Fig. 7, is shown in Fig. 9. A sharp drop in the recombination rate is seen for all simulations as G-protein activation progresses. For the high diffusivity/no switching simulation, k_α is high because the concentration of $\beta\gamma$ is high, and α-GDP and $\beta\gamma$ encounter one another quickly by diffusion. For the low diffusivity/no switching simulation, several G-proteins may be activated in the same region by a receptor/ligand complex, resulting in a slight clustering of $\beta\gamma$ and α-GDP. Thus, for low diffusivity, k_α is lower for rapid "switching" than for no "switching" because the distributions of both $\beta\gamma$ and α-GDP are more homogeneous in the former situation.

Steady-state numbers of α-GTP predicted from both the Monte Carlo model and the simple model of Eqs. 1–2 and 5–7 are given in Table 2 for the three situations shown in Fig. 7. In the simple model, the steady-state level of α-GTP ($[\alpha\text{-GTP}_{ss}]$) is given by

$$[\alpha\text{-GTP}_{ss}] = \frac{k_c[C_{eq}][G_t]}{k_c[C] + k_i}, \qquad (23)$$

where the equilibrium number of receptor/ligand complexes, $[C_{eq}]$, is calculated from Eq. 8, and k_c is estimated using Eq. 5. Because $[C_{eq}]$ is dependent only on the ratio of k_r to k_f (K_D) and not on the individual values, the simple model predictions are independent of receptor/ligand binding kinetics. It is unable to distinguish between a fraction of the receptors bound by ligand all of the time and all of the receptors bound by ligand a fraction of the time. Therefore, the simple model predicts the same steady-state level of α-GTP for both rapid "switching" and no "switching" situations. For low diffusivity, the simple model predictions for steady-state numbers of α-GTP are lower than those predicted by the Monte Carlo simulations. This difference is more pronounced for the low diffusivity/rapid switching case. The simple model cannot account for the increased encounter rate constant between C and G-protein that results from ligand "switching" (Fig. 8). In addition, neglecting accumulation of α-GDP in the simple model (Eq. 7) is a poor assumption, because the Monte Carlo model predicts that approximately 18% of the G-protein pool exists as α-GDP at steady state. For high diffusivity/no switching, the simple model estimate of steady-state α-GTP is in good agreement with the prediction of the Monte Carlo model. The estimate of k_c from Eq. 5 for this case is in good agreement with the Monte Carlo model prediction. In addition, the Monte Carlo simulations predict that little of the total G-protein pool exists in the form of α-GDP, so the simple model assumption of neglecting α-GDP is justified.

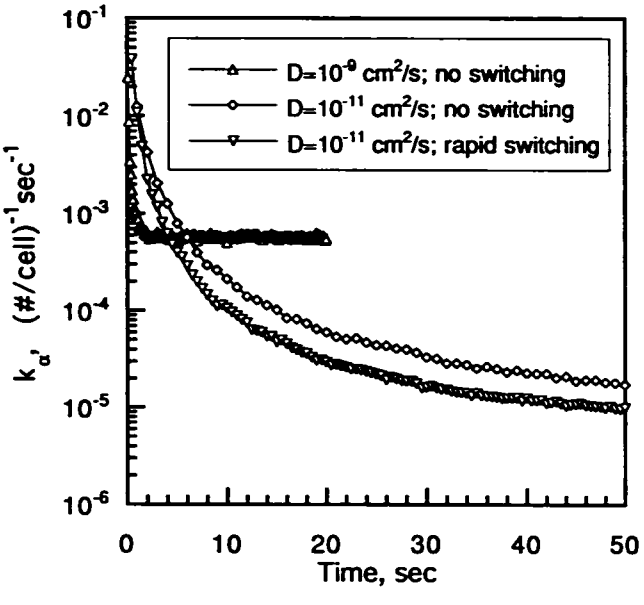

FIGURE 9 Effect of ligand "switching" and diffusivity on the recombination rate constant for α-GDP and $\beta\gamma$, k_α. Monte Carlo predictions for k_α are calculated from transient species numbers as given by Eq. 21. Parameters used in these simulations are the same as those in Fig. 7.

Effect of GTPase activity on steady-state G-protein activation

The GTPase activity of the α-subunit has been measured or estimated in several cell systems, and values of k_i range from 0.02 to 2 s^{-1} (Thomsen and Neubig, 1989; Im et al., 1990; Cassel et al., 1977; Brandt and Ross, 1985). As shown in Fig. 5 for rapid "switching," intermediate GTPase activity (k_i = 0.2 s^{-1}), and $D = 1 \times 10^{-10}$ cm^2/s, the steady-state level of α-GTP increases over the entire range of ligand concentration without saturating. Changes in GTPase activity dramatically affect the steady-state level of α-GTP. Steady-state levels of α-GTP with rapid switching and $D = 1 \times 10^{-10}$ cm^2/s for GTP hydrolysis rates of 0.02–2 s^{-1} are shown in Fig. 10. For low GTPase activity (k_i = 0.02 s^{-1}), the lifetime of an α-GTP is *tens* of seconds, and steady-state levels of α-GTP are high. This shift in the steady-state level of α-GTP resembles the shift occurring as the diffusivity increases (Fig. 5). Conversely, for high GTPase activity (k_i = 2 s^{-1}), the lifetime of α-GTP is *tenths* of seconds and conversion of α-GTP to α-GDP is rapid. Thus, steady-state levels of α-GTP are low, even for stimulation with high ligand concentrations. The shift in the steady-state number of α-GTP now resembles the shift occurring as the diffusivity decreases.

Although the steady-state behavior of α-GTP for high diffusivity and intermediate GTPase activity (k_i = 0.2 s^{-1}) with rapid "switching" is similar to that for intermediate diffusivity and low GTPase activity, there is an important dis-

TABLE 2 Steady-state predictions for the Monte Carlo model and the simple model

D	"Switching"	C_{eq} Simple	C_{eq} Monte Carlo	α-GTP$_{ss}$ Simple	α-GTP$_{ss}$ Monte Carlo	α-GDP$_{ss}$ Monte Carlo
10^{-9} cm^2/s	None	3333/cell	3400/cell	59,993/cell	59,500/cell	1,700/cell
10^{-11} cm^2/s	None	3333/cell	3300/cell	1,477/cell	1,600/cell	11,200/cell
10^{-11} cm^2/s	Rapid	3333/cell	3300/cell	1,477/cell	3,200/cell	18,700/cell

Parameters used are those from Fig. 7. For the simple model, k_c was calculated with s = 3.5 nm, A = 2200 μm^2, and N = 100,000.

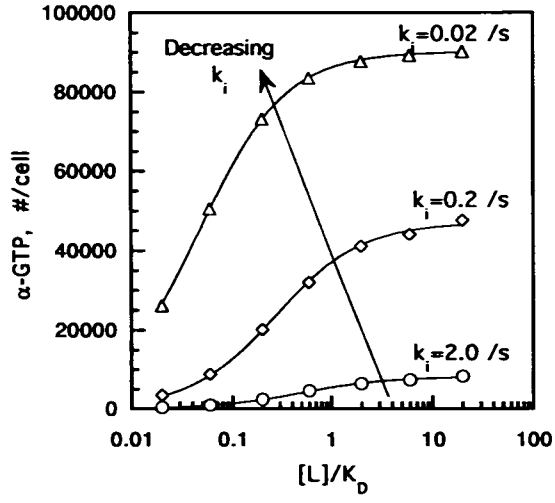

FIGURE 10 Effect of GTPase activity on steady-state G-protein activation. Steady-state levels of α-GTP produced with rapid ligand switching for three levels of GTPase activity are shown above. As the GTPase activity increases, the rate constant for α-GTP inactivation, k_i, increases, the lifetime of each α-GTP decreases, and the steady-state level of α-GTP decreases. Parameters used in the simulations are $D = 1 \times 10^{-10}$ cm^2/s and k_i = 0.02, 0.2, 2.0 s^{-1}.

tinction. In the former case, steady-state levels of α-GTP are reached quickly (≈5 s). In the latter case, steady state is reached much more slowly (≈80 s). Thus, although the same steady-state level of G-protein activation is achieved for both cases, temporal sensitivity is significantly compromised as the GTPase activity decreases.

Stochasticity and G-protein activation

The effects of stochasticity on α-GTP formation are examined by comparing the evolution of the species numbers in 30 simulations that have reached steady state. Each simulation is initialized identically, and then random move and reaction events proceed differently for each simulation. For $[L]/K_D = 0.2$, rapid ligand switching, $k_i = 0.2$ s^{-1}, and $D = 10^{-10}$ cm^2/s, stochastic fluctuations in the number of receptor/ligand complexes are approximately 11% of the equilibrium receptor/ligand complex number, whereas fluctuations in α-GTP are only 4% of the steady-state α-GTP number.

DISCUSSION

In this paper, results from a simple ODE model of G-protein activation are compared with results from a Monte Carlo model of G-protein activation. Both model formulations can be classified as collision-coupling models, in the sense that α-GTP formation occurs upon collision of a receptor/ligand complex with an inactive G-protein (Tolkovsky and Levitzki, 1981; Lauffenburger and Linderman, 1993). The Monte Carlo model is specifically developed to investigate the effects of receptor/ligand binding kinetics, 2-D diffusion, and GTPase activity on α-GTP production. In addition, the Monte Carlo model is used to predict quantitatively the effects of stochastic reaction and diffusion events, the development of heterogeneity in the distribution of molecules in the membrane, and the effective diffusion-limited encounter rate constants between receptor/ligand complexes and G-protein and between $\beta\gamma$ and α-GDP.

Predictions of the steady-state numbers of activated G-proteins from both models are in agreement when the diffusivity is high ($D = 10^{-9}$ cm^2/s). In this region, the Monte Carlo model predicts that less than 2% of the total G-protein pool exists as α-GDP, and the distributions of all molecules in the membrane are essentially homogeneous. Thus, the basic assumptions and formulation of the simple ODE model are valid. However, as the diffusivity decreases, the results of the simple ODE model deviate from those of the Monte Carlo model. The ODE model is unable to account for the movement of ligand among free receptors, treating the partial occupation of all receptors identically to continuous occupation of a fraction of receptors. As the diffusivity drops, the distribution of molecules in the membrane becomes more heterogeneous, and the validity of the ODE model formulation based on a well mixed system comes into question.

Monte Carlo simulations predict that the encounter rate constant for C and G-protein, k_c, is nearly constant and, in the absence of ligand "switching," is reasonably estimated using Eq. 5 (Fig. 8). However, in receptor/ligand systems where "switching" is significant, the effective rate constant between C and G-protein may increase by 2- to 3-fold over the Eq. 5 estimate. The recombination rate constant between α-GDP and $\beta\gamma$, k_a, predicted by the Monte Carlo model is strongly time-dependent and drops sharply as signaling progresses.

The results of the Monte Carlo simulations show that "switching" of ligand among receptors, which is affected by the receptor/ligand association and dissociation rate constants, k_f and k_r, produces a significant enhancement in G-protein activation. The greatest "switching" enhancement of G-protein activation occurs for rapid receptor/ligand binding kinetics at low ligand concentrations ($[L]/K_D < 1$), supporting experimental evidence that a low occupancy of the full receptor population by ligand promotes greater activa-

tion and signaling than for high occupancy of only a fraction of the receptor population (Stickle and Barber, 1992b; Mahama and Linderman, 1994b). This has direct implications for the use of receptor antagonists. For systems in which receptor/ligand binding kinetics are rapid and a large fraction of G-protein activation results from ligand movement by "switching," the action of the antagonist and the resulting reduction in G-protein activation will produce a reduction in cell response greater than that estimated based on the change in the number of receptor/ligand complexes. From a cellular perspective, reduction in the number of active receptor sites may be an effective method of desensitization. If following ligand stimulation receptors lose their signaling ability because of phosphorylation or other means, this may represent a cell's internal version of adding a receptor blocker to effectively reduce G-protein activation and prevent continued signaling.

From the Monte Carlo model, the steady-state number of α-GTP molecules is predicted to increase as the diffusivities of receptors and G-proteins in the membrane increase. Steady-state levels of α-GTP resulting from an increase in diffusivity (Fig. 5) resemble increases in steady-state α-GTP produced by decreasing the GTPase activity (Fig. 10). However, increases in diffusivity decrease the time needed to reach steady state (increase the temporal sensitivity) of the system, whereas increasing the lifetime of the α-GTP significantly decreases the temporal sensitivity of the system. Quite interestingly, experimental estimates of GTPase activity vary widely from 0.02 to 2 s^{-1} (Thomsen and Neubig, 1989; Im et al., 1990; Cassel et al., 1977; Brandt and Ross, 1985). An accurate measure of the GTPase activity as well as of the diffusivities of receptors, G-proteins, and G-protein subunits is needed to make an accurate estimate of the kinetics and extent of α-GTP production. Only one such attempt at measuring the diffusivity of G-protein subunits has been reported (Kwon et al., 1991).

Alterations in the diffusivities of membrane species after ligand stimulation may represent another mechanism of cellular desensitization. Tethering of G-proteins and/or receptors to the cytoskeleton after prolonged exposure to ligand could result in decreased α-GTP formation because of decreased mobility. In neutrophils, recent evidence suggests that agonist binding to receptors results in clustering of receptor/ligand complexes into distinct regions of the cell membrane before internalization (Johansson et al., 1993). Alternatively, reduction in the mobility of the α-GDP and $\beta\gamma$ subunits would reduce the rate of recombination of α and $\beta\gamma$ subunits, resulting in depletion of inactive G-protein after extended ligand exposure and a corresponding reduction in α-GTP formation.

For low diffusivity/no switching (receptor antagonist present), heterogeneity develops in the distribution of molecules as signaling progresses. Accumulation of α-GTP near receptor/ligand complexes is significant (Fig. 7). Local depletion of G-protein near complexes is seen in varying degrees for all diffusion coefficients, with the most significant depletion occurring for the same combinations of diffusivity/switching where α-GTP clustering near receptors is most significant. Overall, the model simulations predict that ligand "switching" and high diffusivities of receptors and G-proteins increase the homogeneity in the distribution of the molecular species as well as produce increased G-protein activation.

Stickle and Barber (1989) have also suggested that the movement of ligand among free receptors, or "switching," significantly contributes to enzyme activation. In their models (Stickle and Barber, 1992a, b), they use an ordinary differential equation formalism to follow the activation of adenylate cyclase. The key aspect of their model is that the encounter time between a receptor and enzyme (theorized to represent a series of closely spaced collisions) is assumed to be long such that the receptor may bind or release ligand during the encounter with a resulting effect on the ability of that receptor to activate the enzyme. The encounter time is calculated to be on the order of several seconds to fit the model to their experimental data.

Our use of Monte Carlo simulations represents an alternate approach to understanding the dynamics of enzyme activation. Unlike Stickle and Barber (1992a, b), we assume that the duration of an encounter between a receptor/ligand complex and a G-protein is very small (equal to the Monte Carlo time step of the simulation, or on the order of 10^{-7} s). In the Stickle and Barber model, enhancement in enzyme activation with "switching" results from overcoming the "problem" of long encounter times. Once an enzyme is activated, the remaining encounter time between the receptor and enzyme is useless, so "switching," or the binding of ligand to another receptor, enhances activation. In contrast, in our Monte Carlo model, the enhancement in α-GTP production caused by "switching" is rooted in the fact that switching acts to "mix" the system, improving the access of receptor/ligand complexes to inactive G-proteins. A further difference between the two models is in the reforming of inactive G-protein or enzyme. In the Stickle and Barber model, G-protein activation is coupled to enzyme activation without explicit consideration of the G-protein activation cycle. Thus, enzyme inactivation resulting from the hydrolysis of GTP is assumed to be the rate-limiting step. In the Monte Carlo model, hydrolysis of GTP and recombination of α-GDP and $\beta\gamma$ subunits may both play significant roles. For some parameter ranges, a substantial fraction of the α-GDP may exist separate from $\beta\gamma$ subunits (see Table 2).

The Monte Carlo model described here can be used to make explicit predictions of the pharmacologic shift ratio K_D/EC_{50}, where K_D is the equilibrium dissociation constant (the ligand concentration at which half the receptors are bound) and EC_{50} is ligand concentration at which half the maximal response is achieved (Kenakin, 1993). In our case, we can define EC_{50} as the ligand concentration at which half the maximal amount of α-GTP is produced. For example, the top curve (rapid switching) in Fig. 3 gives a value of K_D/EC_{50} equal to about 3. The effect of "switching" is to increase the value of K_D/EC_{50}; thus, ligands with the same K_D values but different association and dissociation rate constants may

have different values of K_D/EC_{50}. Further, in the current Monte Carlo formulation, encounters between agonist-occupied receptors and inactive G-proteins, as well as encounters between α-GDP and $\beta\gamma$ subunits, are assumed to be completely diffusion-controlled. Some degree of reaction control could be easily incorporated by setting the probability of a reaction upon encounter to less than one. Thus, agonists with different abilities to activate G-proteins upon encounter, or different efficacies, could be simulated to determine their effect on G-protein activation and on the value of K_D/EC_{50}. The comparison of simulations with different receptor/ligand binding kinetics (different amounts of "switching") and varying degrees of reaction control (different efficacies) with experimental data may differentiate these two effects and allow testing of the model.

Such testing will require knowledge of the important inputs to the model, particularly the number of receptors and G-proteins on a cell. Such quantities are known to vary widely; for example, the number of G-proteins in different cell types has been reported to range from hundreds of thousands to millions (Bokoch et al., 1988; Neubig et al., 1985). A further complication is the fact that not all G-proteins may be accessible to receptors (Klotz and Jesaitis, 1994; Klotz et al., 1994). Finally, there may be significant differences between the activation of G-proteins in membrane preparations as compared with whole cells, perhaps because of differences in the relative ratios of receptors to G-proteins or in the accessibility of G-proteins to receptors.

Additional potential alterations to the Monte Carlo model are as follows. All species in our simulations are assigned the same diffusion coefficient, although the diffusivities of the membrane species may be different (Saxton and Owicki, 1989; Kwon et al., 1991). Further, in many cell systems, a significant fraction of receptors and G-proteins may be precoupled (Neubig et al., 1988; Fay et al., 1991). This would allow for a rapid increase in α-GTP formation after ligand addition. In addition, the precoupling of receptors and G-proteins may affect the ligand binding characteristics of the receptor, further influencing G-protein activation (Fay et al., 1991). Finally, Thomsen et al. (1988; Thomsen and Neubig, 1989), in both experimental and theoretical investigations of G-protein-coupled adenylate cyclase activation, suggest that in their system, the kinetics involved in the separation of the subunits after GTP binding are on the order of the kinetics of association for C and G-protein. Within the Monte Carlo framework, an alternative formulation of G-protein activation could be constructed to include a finite lifetime for the C-$\beta\gamma\alpha$-GTP complex. For rates of uncoupling lower than the GTPase activity, a significant fraction of G-protein is anticipated to accumulate in the C-$\beta\gamma\alpha$-GTP conformation with a resultant decrease in α-GTP formation.

Stochastic reaction and move events in G-protein activation result in small fluctuations in the steady-state level of α-GTP. For $[L]/K_D = 0.2$, $D = 10^{-10}$ cm^2/s, $k_i = 0.2$ s^{-1}, and rapid switching, the fluctuation in steady-state α-GTP is only 4%. When stochastic effects are included in the identification and placement of receptors and G-proteins as well, no increase in the stochastic fluctuations of steady-state α-GTP is seen. Unless the following steps in the signal transduction cascade are very sensitive to the steady-state level of α-GTP, this fluctuation in α-GTP production will not significantly affect the cell response. This supports experimental observations (Byron and Villereal, 1989; Prentki et al., 1988; Rooney et al., 1989) of calcium signaling in several cell types where the shape and timing of the intracellular calcium increase after repeated ligand stimulation have been shown to be reproducible. However, for variation in total receptors and G-protein that may represent cell-to-cell variability in protein concentration, the steady-state levels of α-GTP may vary significantly, suggesting that the unique calcium signature of each cell may be a function of its unique signal cascade composition (Mahama and Linderman, 1994a).

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