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Monte Carlo Simulations of Membrane Signal Transduction Events: Effect of Receptor Blockers on G-Protein Activation

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Abstract—Cells have evolved elaborate strategies for sensing, responding to, and interacting with their environment. In many systems, interaction of cell surface receptors with extracellular ligand can activate cellular signal transduction pathways leading to G-protein activation and calcium mobilization. In BC_3H1 smooth muscle-like cells, we find that the speed of calcium mobilization as well as the fraction of cells which mobilize calcium following phenylephrine stimulation is dependent upon receptor occupation. To determine whether receptor inactivation affects calcium mobilization, we use the receptor antagonist prazosin to block a fraction of cell surface receptors prior to phenylephrine stimulation. For cases of equal receptor occupation by agonist, cells with inactivated or blocked receptors show diminished calcium mobilization following phenylephrine stimulation as compared to cells without inactivated receptors. Ligand/receptor binding and two-dimensional diffusion of receptors and G-proteins in the cell membrane are studied using a Monte Carlo model. The model is used to determine if receptor inactivation affects G-protein activation and thus the following signaling events for cases of equal equilibrium receptor occupation by agonist. The model predicts that receptor inactivation by antagonist binding results in lower G-protein activation not only by reducing the number of receptors able to bind agonist but also by restricting the movement of agonist among free receptors. The latter process is important to increasing the access of bound receptors to G-proteins.

Keywords—Signal transduction, Antagonist, Mathematical model, Diffusion, Computer simulation, Smooth muscle cells, Phenylephrine, Prazosin.

INTRODUCTION

A central issue to all of cellular and tissue engineering is the role that receptors play in eliciting cell responses. Ligand/receptor binding on a cell surface can lead to responses as diverse as contraction, secretion, proliferation, migration, and differentiation. A quantitative understanding of the relationship between cell receptor binding and such responses will aid in the "engineering" or manipulation of cells and tissues for biotechnological and medical benefit.

The events following receptor/ligand binding and leading to cellular responses are generally termed signal transduction. In this paper we focus on our efforts to link receptor/ligand binding with early events in the signal transduction cascade. In many systems ligand occupation of cell surface receptors may lead to activation of GTP-binding proteins, or G-proteins, in the cell membrane and release of calcium from intracellular stores via the signal transduction cascade shown in Fig. 1. The basic features of this signal transduction cascade have been characterized through experimental work in the past several years and are detailed in recent reviews (5,27).

To quantitatively examine the link between receptor occupation and calcium mobilization, we use the α_1-adrenergic receptor agonist phenylephrine (PhE). PhE stimulation of α_1-adrenergic receptors on single smooth muscle-like BC_3H1 cells results in intracellular calcium mobilization (7,19). For this experimental system, agonist/receptor binding is rapid. Even for PhE concentrations as low as 0.1 μM, 95% of equilibrium receptor occupation is reached in less than 0.1 sec. In comparison with agonist/receptor binding, calcium mobilization in these cells is slow, occurring 3–25 sec after PhE stimulation. Therefore, we measure both the fraction of cells which mobilize calcium and the speed of calcium mobilization as a function of equilibrium receptor occupation. We find that the fraction of cells that mobilize calcium as well as the speed of the mobilization is dependent on the equilibrium receptor occupation. To determine whether receptor inactivation affects calcium mobilization, we use the receptor antagonist prazosin (Pz) to block receptors from participation in the signaling pathway prior to PhE stimulation. We find that for cases of equal equilibrium receptor occupation by the agonist PhE, increasing receptor inactivation via antagonist binding diminishes the fraction of cells that mobilize calcium and slows the calcium mobilization. From this point on we will use the term "receptor occupation" to mean the occupation of receptors by agonist

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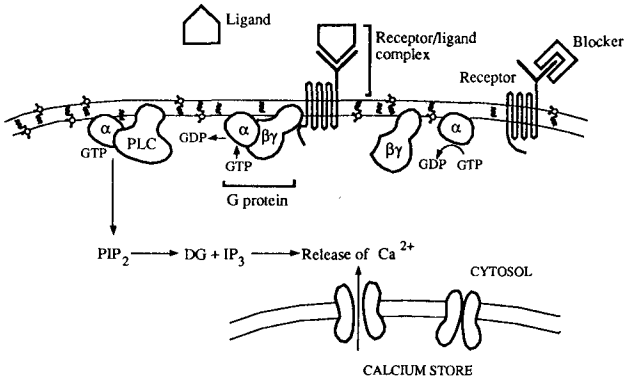

FIGURE 1. Signal transduction pathway. Ligand (agonist) binds to a receptor on the cell membrane forming a receptor/ligand complex. The receptor/ligand complex associates with a G-protein (βγα-*GDP*) in the cell membrane, decreasing the G-protein's affinity for GDP and increasing its affinity for GTP. Following exchange of GTP for GDP on the G-protein, the receptor and G-protein dissociate, and the trimeric G-protein separates into two parts: the βγ subunit and the active α-*GTP* subunit. α-*GTP* stimulates the activity of a phospholipase C (PLC), which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DG) and inositol 1,4,5,-trisphosphate (IP_3). IP_3 is soluble in the cytosol and binds to receptors on an internal calcium store, causing the release of calcium into the cytosol. The α-*GTP* subunit is able to inactivate itself by hydrolyzing bound GTP to GDP. The inactive α-*GDP* subunit can recombine with the βγ subunit to reform the G-protein trimer. Also shown is a receptor antagonist or blocker which is able to bind to a receptor without activating the signaling pathway.

(PhE) and "receptor inactivation" or "blocked receptors" to mean the occupation of receptors by antagonist (Pz).

We did not expect receptor inactivation to have a significant effect on calcium mobilization for cases of equal equilibrium receptor occupation. To explain this result, we look closely at the initial membrane signaling events following agonist/receptor binding to see if G-protein activation, and thus the following signaling events, is affected by receptor inactivation. Using Monte Carlo simulations of two-dimensional (2-D) diffusion of receptors and G-proteins in the cell membrane, we study the effects of receptor occupation, receptor inactivation, and 2-D diffusion on the activation of G-proteins in the cell membrane. We find that for our experimental system, the frequency of agonist/receptor binding, or movement of agonist among available receptors, significantly affects the extent of G-protein activation and thus calcium mobilization.

EXPERIMENTAL PROCEDURES

Details on cell culture and the measurement of intracellular free calcium concentration in single cells can be found elsewhere (19). Briefly, BC_3H1 cells are grown on coverslips at least 24 hr prior to experiments. Cells adhered to a coverslip are incubated with 5 μM fura-2 AM (Molecular Probes, Eugene, OR) at 37°C for 20 min. The coverslip is then washed with pH 7.4 physiological buffer solution which contains (in mM): NaCl, 140; KCl, 10; $CaCl_2$, 1.8; $MgCl_2$, 1.0; Na_2HPO_4, 1.0; HEPES, 25; and glucose, 5. The coverslip is then transferred to a thermoregulated flow chamber that fits on a microscope stage (Nikon Diaphot, Nikon, Inc., Garden City, NY). For stimulation, 7 ml of PhE solution is rinsed through the flow chamber with a syringe in less than 5 sec. The PhE concentration in the flow chamber consistently reaches 95% of PhE stimulant concentration within the first 0.8 sec of addition. For experiments with receptor inactivation, Pz in buffer solution is initially added to the flow chamber to block receptors and then unbound Pz is removed by rinsing. PhE stimulation then proceeds as above. Fura-2 is excited at 334 and 365 nm, and fluorescence emissions are collected with a charge-coupled device (CCD) camera (Photometrics Ltd., Tucson, AZ). Images are collected for at least 20 sec prior to PhE stimulation to establish a calcium baseline for each cell. Background and calibration images are taken for each experiment as described in (19).

Three concentrations of Pz are used in the pretreatment experiments to produce different levels of receptor blockage. The fraction of receptors blocked (F_b) by Pz pretreatment is calculated from

$$F_b = \frac{B}{R_t} = \frac{[A]}{[A] + K_{D,A}} (1 - e^{(-k_{f,A}[A] - k_{r,A})t}) \quad (1)$$

where B is the number of blocked receptors, R_t is the total number of receptors, $[A]$ is the antagonist concentration, $k_{f,A}$ is the receptor/antagonist association rate constant, $k_{r,A}$ is the receptor/antagonist dissociation rate constant, and $K_{D,A}$ is the equilibrium dissociation constant for the antagonist ($K_{D,A} = k_{r,A}/k_{f,A}$). The values used in our calculations were $k_{f,A} = 8.55 \times 10^6\ M^{-1}\ sec^{-1}$, $k_{r,A} = 0.00077\ sec^{-1}$ (21). Using these literature values for Pz binding rate constants and the time of Pz pretreatment, the fraction of blocked receptors on the cell surface is calculated. For the experiments shown here, the calculated values of F_b were 0.13, 0.31, and 0.61.

The number of Pz-blocked receptors is nearly constant over the time scale of PhE stimulation. For $k_{r,A} = 0.00077\ sec^{-1}$, 95% of the receptors blocked by Pz remain blocked after 1 min. Thus, the number of blocked receptors is assumed constant during PhE stimulation. The equilibrium number of receptor/agonist complexes, C_{eq}, on the cell surface following PhE stimulation is given by

$$C_{eq} = \frac{R_t(1 - F_b)[L]}{[L] + K_D} \quad (2)$$

where $[L]$ is the agonist concentration, K_D is the equilibrium dissociation constant for the agonist, and $R_t(1 - F_b)$

is equal to the total number of *unblocked* receptors. Experiments were done for $[L] = 10$ μM, and calculations used the values $K_D = 5.8$ μM and $R_t = 19{,}000$/cell (see Table 1).

Image data are collected and analyzed using the ISee graphical programming system (Inovision Corp., Durham, NC) as described elsewhere (19). The intracellular free calcium concentration is calculated from the raw image data as described by Grynkiewicz (13). Responding cells are defined as cells that have at least a 30% increase in intracellular free calcium concentration within 2 min following PhE stimulation. Calcium response latency is defined as the time between PhE addition and the maximum rate of increase in intracellular free calcium concentration.

Mathematical Model

Monte Carlo simulations of G-protein activation include both 2-D diffusion and the reaction of species in the cell membrane. The membrane reactions included in the simulations are discussed below. Agonist/receptor binding on the cell surface is described by

$$R + L \underset{k_r}{\overset{k_f}{\rightleftharpoons}} C. \tag{3}$$

where R is a free receptor, L is a ligand (agonist) molecule, and C is the agonist/receptor complex. The ligand concentration is assumed uniform and constant.

The equilibrium agonist/receptor dissociation constant, K_D, is equal to the ratio of the dissociation rate constant and the association rate constant, k_r/k_f. In the Monte Carlo simulations, conversion of receptors between agonist-occupied and unoccupied states is allowed to occur with a probability proportional to the reaction rate constants, k_f and k_r.

G-protein is a trimer consisting of α, β, and γ subunits, with GDP bound to the α subunit. Inactive G-protein, *βγα-GDP*, is represented in the model equations as G. Collision between an agonist/receptor complex and a G-protein results in the formation of a ternary agonist/receptor/G-protein complex. The α subunit of the ternary complex has an enhanced affinity for GTP and a diminished affinity for GDP (27). GTP binds to the α subunit within milliseconds, resulting in the dissociation of the ternary complex. Because the ternary complex is short-lived, G-protein activation is approximated by

$$C + G \rightarrow C + \alpha\text{-}GTP + \beta\gamma. \tag{4}$$

The activation of G-protein is assumed to be diffusion-limited and thus occurs with every collision of an agonist/receptor complex with an inactive G-protein. Direct *in vivo* measurements of the effect of receptor or G-protein diffusion on G-protein–coupled signal transduction have not been made. The role of lateral diffusion in signal transduction is supported by experiments in which the membrane fluidity of cells is altered and G-protein stimulated enzyme activity is affected (4,12,15,23). Further, it is believed that values of the rate constants for other membrane reaction events, such a the crosslinking of receptors by a multivalent ligand, the trapping of receptors in coated pits, and the coupling of receptors to membrane-associated components, are close to the diffusion limit (9,11,18,22).

The active *α-GTP* subunit has a limited lifetime since its intrinsic GTPase activity results in hydrolysis of GTP to GDP:

TABLE 1. Parameters used in Monte Carlo simulations.

Parameter	Meaning	Value	Reference
K_D	Equil. agonist dissociation constant	5.8×10^{-6} M	1,3,14,21
k_f	Agonist association rate constant	1×10^7 M^{-1} sec^{-1}	[a]
k_r	Agonist dissociation rate constant	58 sec^{-1}	
k_i	G-protein inactivation rate constant	2 sec^{-1}	28
R_t	Total number of α_1-adrenergic receptors	19,000 cell^{-1}	3,6,21
G_t	Total number of G-proteins	100,000 cell^{-1}	6
A_c	Cell surface area	2,200 μm^2	2
D	Diffusion coefficient	1×10^{-10} cm^2 sec	10
d_n	Distance between MC sites	7 nm	
t_s	Time step, $d_n^2/4D$	0.001225 sec	
N	Number of sites/edge (square grid)	1000	
P_1	Probability of $R \rightarrow C$, $k_f[L]t_s$	0.0245,0.1225,1.0[b]	
P_{-1}	Probability of $C \rightarrow R$, $k_r t_s$	0.07105,0.0[b]	
P_i	Probability of $\alpha\text{-}GTP \rightarrow \alpha\text{-}GDP$, $k_i t_s$	0.00245	

[a]Measurement of k_f and k_r have not been reported for the PhE/α_1-adrenergic receptor system. Values used here are similar to those for epinephrine binding to β-adrenergic receptors (Richard Neubig, personal communication).

[b]For test case 3, reaction probabilities used for agonist/receptor binding were $P_1 = 1.0$, and $P_{-1} = 0.0$.

$$\alpha\text{-}GTP \xrightarrow{k_i} \alpha\text{-}GDP. \quad (5)$$

Conversion of α-*GTP* to α-*GDP* in the simulation occurs with a probability proportional to the α-*GTP* inactivation rate constant, k_i. The inactive α-*GDP* subunit may combine with a βγ subunit to reform the complete G-protein:

$$\alpha\text{-}GDP + \beta\gamma \rightarrow G. \quad (6)$$

This reaction is also assumed to be diffusion-limited and occurs upon collision of the two subunits.

All reaction rate constants mentioned above as well as other parameters used in the simulations are summarized in Table 1. The value of the receptor diffusion coefficient in these cells has not been measured. Typical receptor diffusion coefficients are in the range of 10^{-9} to 10^{-11} cm^2/sec (10). The value of the G-protein diffusion coefficient has not been measured in any cell type, although Kwon *et al.* (17) have measured the diffusion coefficient for βγ subunits in NG-108-15 cells. We choose a reasonable value of 10^{-10} cm^2/sec for the receptor, G-protein, and G-protein subunit diffusion coefficients.

All Monte Carlo simulations are run on a 1,000 × 1,000-site square grid with lattice spacing of 7 nm, which is approximately a protein radius. Periodic boundary conditions are used. For simulations with inactive receptors, a fraction of the receptors placed on the grid are randomly selected and reidentified as antagonist-blocked receptors. The simulations are initialized by placing receptors and G-proteins randomly on the simulation grid. All membrane species are allowed to move randomly and independently with the same mobility or diffusion coefficient. If a random move results in the collision of two nonreacting species, *e.g.*, a receptor colliding with a receptor, the move is rejected. Results shown are the average of 20 simulations converted to a single cell basis according to the cell surface area.

RESULTS

Figure 2 shows a typical single cell response to PhE stimulation. We observe a brief time lag between agonist addition and calcium mobilization. The time lag, or latency, between the addition of PhE and the calcium response is dependent on the agonist concentration. Agonist/receptor binding is rapid compared with the calcium response in this experimental system (for [L] = 0.1 μM, binding reaches 95% of equilibrium binding in less than 0.1 sec), so the response can be related to the equilibrium receptor occupation. The calcium response latency and fraction of cells responding as a function of equilibrium receptor occupation are shown in Fig. 3. Solid circles represent data for cells stimulated with PhE only. Equilibrium receptor occupation ranges from 2–90% of the cell receptors. At high fractional receptor occupation, the speed of calcium mobilization and the fraction of cells responding reach a maximum.

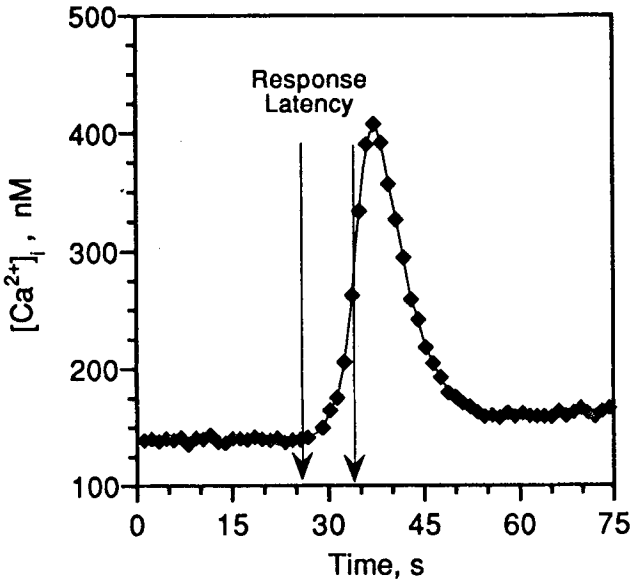

FIGURE 2. Single cell calcium response. Shown is the concentration of intracellular free calcium in a single cell as a function of time. The cell is stimulated with 10 μM PhE at a time indicated by the first arrow. The second arrow represents the time of maximum rate of calcium increase as determined by a nonlinear fit of the data. The time lag between the addition of PhE and the time of maximum rate of calcium increase is defined as the calcium response latency.

By using Pz to inactivate, or block, cell receptors prior to PhE stimulation, we compare the latency and fraction of cells responding for cases of equal equilibrium receptor occupation and varying degrees of receptor inactivation. These data are also shown in Fig. 3. Open circles represent data for cells treated with Pz prior to PhE stimulation. The fraction of cell receptors blocked by Pz ranges from 13–61%. For cases of equal equilibrium receptor occupation, the difference between calcium responses with and without receptor inactivation increases with the fraction of receptor inactivation.

The cause of the decreased cell responsiveness to PhE stimulation resulting from receptor inactivation is unknown and suggests that more than the equilibrium receptor occupation may play a role in G-protein activation and mobilization of intracellular calcium. For example, Pz may have other physiological effects on the cells than receptor inactivation; however, we and others (16,21) find no evidence for such effects. Alternatively, the dynamics of agonist/receptor binding, rather than simply the equilibrium receptor occupation, may affect calcium mobilization in our system. However, for cases of equal equilibrium receptor occupation, the time course of binding is faster in our experiments with inactivated receptors be-

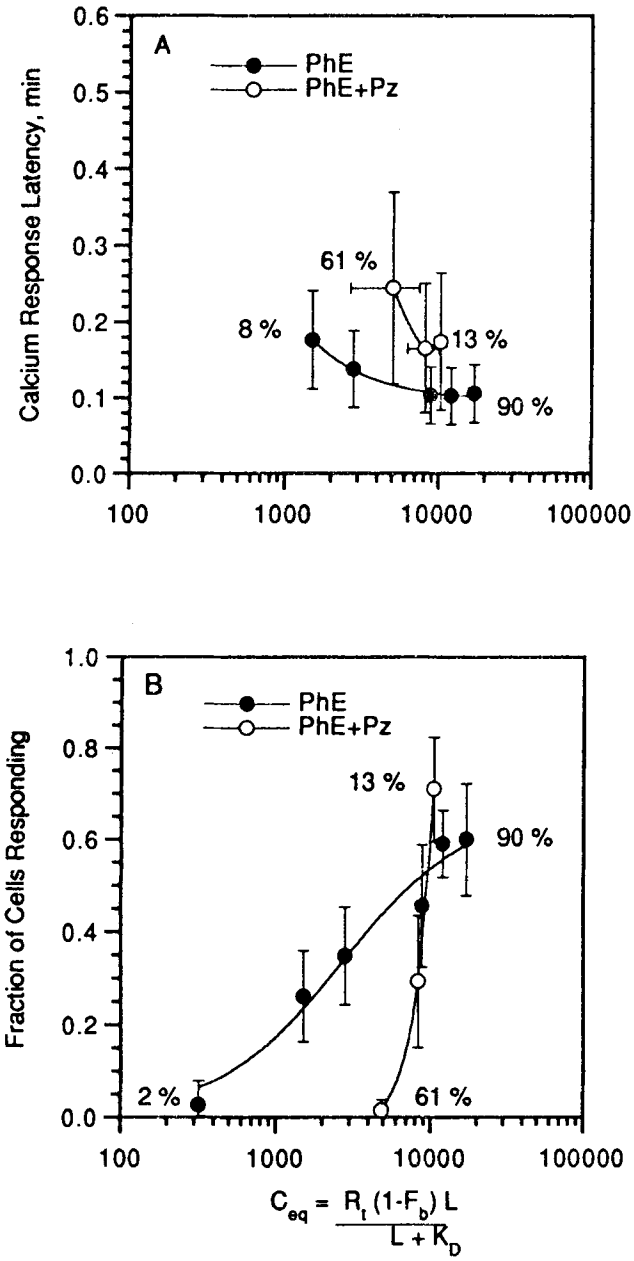

FIGURE 3. Calcium response latency and fraction of cells responding. Both the calcium response latency and the fraction of cells responding depend on the equilibrium receptor occupation, C_{eq}. Data without Pz pretreatment are shown as solid circles. Equilibrium receptor occupation for these data ranges from 2 to 90%. Open circles represent data where cells were pretreated with Pz to block cell receptors. For the three cases shown, Pz treatment inactivates between 13% and 61% of the receptors. (A) The calcium response latency reaches a minimum at high equilibrium receptor occupation. For equal equilibrium receptor occupation, pretreatment with receptor blocker slows the calcium response. (B) The fraction of cells responding increases to a maximum at high equilibrium receptor occupation. As the fraction of receptors blocked by Pz increases, the fraction of cells responding drops sharply.

cause a higher agonist dose is used to achieve the same equilibrium receptor occupation. Therefore, agonist/receptor binding dynamics cannot account for the difference in calcium mobilization seen with receptor inactivation.

Two-dimensional diffusion and collision of agonist/receptor complexes and G-proteins in the cell membrane result in activation of G-protein and calcium mobilization. In our experimental system, equal receptor occupation does not produce equal calcium mobilization when a fraction of the receptors are blocked by an antagonist. Stickle and Barber (24) study a similar experimental system, the binding of epinephrine to β-adrenergic receptors and the resulting activation of adenylate cyclase, and suggest that the agonist/receptor binding frequency may be important for enzyme activation at low agonist concentrations. The rapid movement of agonist among receptors, which may mean the dissociation and then rebinding of the same agonist molecule or the dissociation of one agonist molecule and the binding of another agonist molecule, could enhance the access of bound receptors to G-proteins. This may partly explain the effect of receptor inactivation on calcium mobilization.

An alternative model incorporating agonist/receptor binding frequency is offered by Stickle and Barber (25,26) who use a deterministic formalism to calculate adenylate cyclase activation. They assume that the encounter between receptors and adenylate cyclase is of finite duration and that receptors may change state (bound/unbound) during the encounter with a resulting effect on the ability of the receptor to activate adenylate cyclase. The results of their model hinge on a relatively long encounter time between receptors and adenylate cyclase and apply only at steady state.

We use Monte Carlo simulations to follow the dynamics of binding of agonist to receptors and the diffusion of receptors and G-proteins. We make the assumption that the activation of G-protein is diffusion-limited and that the G-protein/receptor encounter duration is short (on the order of 10^{-7} sec). Our Monte Carlo simulations of receptors and G-proteins in the cell membrane allow us to determine whether or not receptor inactivation significantly affects G-protein activation and thus calcium mobilization. Comparisons between the experimental data and the simulations are limited to the test cases given in Table 2. All test cases have equal values of G-protein number and equilibrium receptor occupation. In test case 1, no receptors are blocked. In test cases 2 and 3, 59.5% and 74.4% of the receptors are blocked prior to agonist stimulation.

Monte Carlo predictions for the time-course of G-protein activation for the three test cases are shown in Fig. 4. Test case 1, with no receptor inactivation, leads to activation of approximately 20% more α-*GTP* than test case 2 and 40% more than test case 3 in the first 3 sec.

TABLE 2. Test cases used in Monte Carlo simulations. Cases compared have equal equilibrium receptor occupation. Case 1 has no blocked or inactivated receptors. Cases 2 and 3 have 59.5% and 74.4% of receptors blocked prior to agonist stimulation, respectively.

Parameters	Case 1 $F_b = 0$	Case 2 $F_b = 0.595$	Case 3 $F_b = 0.744$
Agonist concentration, [L]	2 μM	10 μM	10 mM
Total receptors, R_t	19,000	19,000	19,000
Total G-protein, G_t	100,000	100,000	100,000
Equil. agonist/receptor complexes, C_{eq}	~4,900	~4,900	~4,900
Blocked receptors, R_b	0	~11,300	~14,100
Free receptors at equil., R_f	~14,100	~2,800	~0

The encounter rate constant between agonist/receptor complexes and G-protein is shown for the three test cases in Fig. 5. The encounter rate constant for test case 1 remains approximately constant over the course of agonist stimulation. For cases 2 and 3, the encounter rate constants are initially lower than for test case 1. In addition the encounter rate constants for cases 2 and 3 drop off quickly within the first 2 sec of agonist stimulation to lower, approximately constant values.

The differences in agonist/receptor complex and G-protein encounter rate constants among cases with varying degrees of receptor inactivation can be attributed to differences in the distribution of agonist/receptor complexes on the cell surface. To illustrate this difference, snapshots of agonist/receptor complexes and α-*GTP* from two Monte Carlo simulations are shown in Fig. 6. The snapshots are from simulations of test cases 1 and 3. More α-*GTP* is produced in case 1 (Fig. 6a) than in case 3 (Fig. 6b), and the α-*GTP* is more evenly distributed over the cell membrane in case 1. In case 3, α-*GTP* is segregated in patches in the membrane. For test case 3, all α-*GTP* is located very near an agonist/receptor complex. In case 1, some α-*GTP* subunits are found more distant from agonist/receptor complexes than is possible by diffusion alone.

For test case 1, no receptors are blocked, and the cells are stimulated with a low agonist concentration. Agonist binds to receptors on the cell surface, and by 2-D diffusion, agonist/receptor complexes collide with several G-proteins in their vicinity to produce α-*GTP*. Agonist may dissociate and bind to other receptors in the cell membrane producing complexes over the entire cell surface.

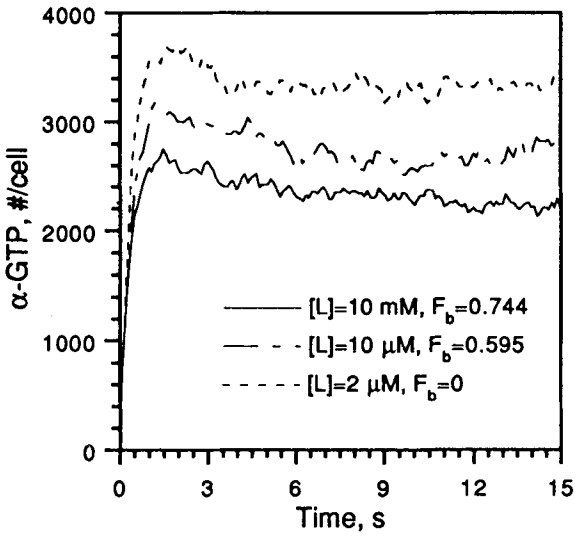

FIGURE 4. Predictions of α-*GTP* production. For the three test cases with equal equilibrium receptor occupation, the simulations predict that inactivation of receptors inhibits G-protein activation. In the first 3 sec following agonist stimulation, tests case 1 with $F_b = 0.0$ produces approximately 20% more α-*GTP* than test case 2 with $F_b = 0.595$% and 40% more α-*GTP* than test case 3 with $F_b = 0.744$%.

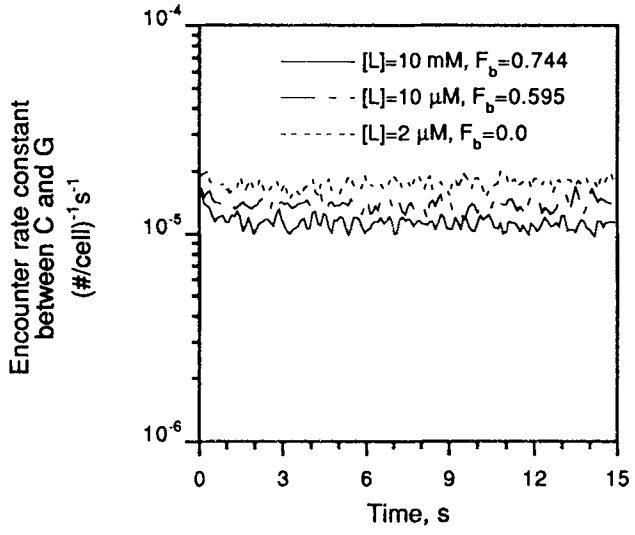

FIGURE 5. Agonist/receptor complex and G-protein encounter rate constant. The encounter rate constant for agonist/receptor complexes and G-protein is calculated using k_i and the numbers of agonist/receptor complexes, G-protein, and α-*GTP* predicted from the Monte Carlo model. For the three test cases with equal equilibrium receptor occupation, the simulations predict that inactivation of receptors reduces the encounter rate constant between G-protein and agonist/receptor complexes.

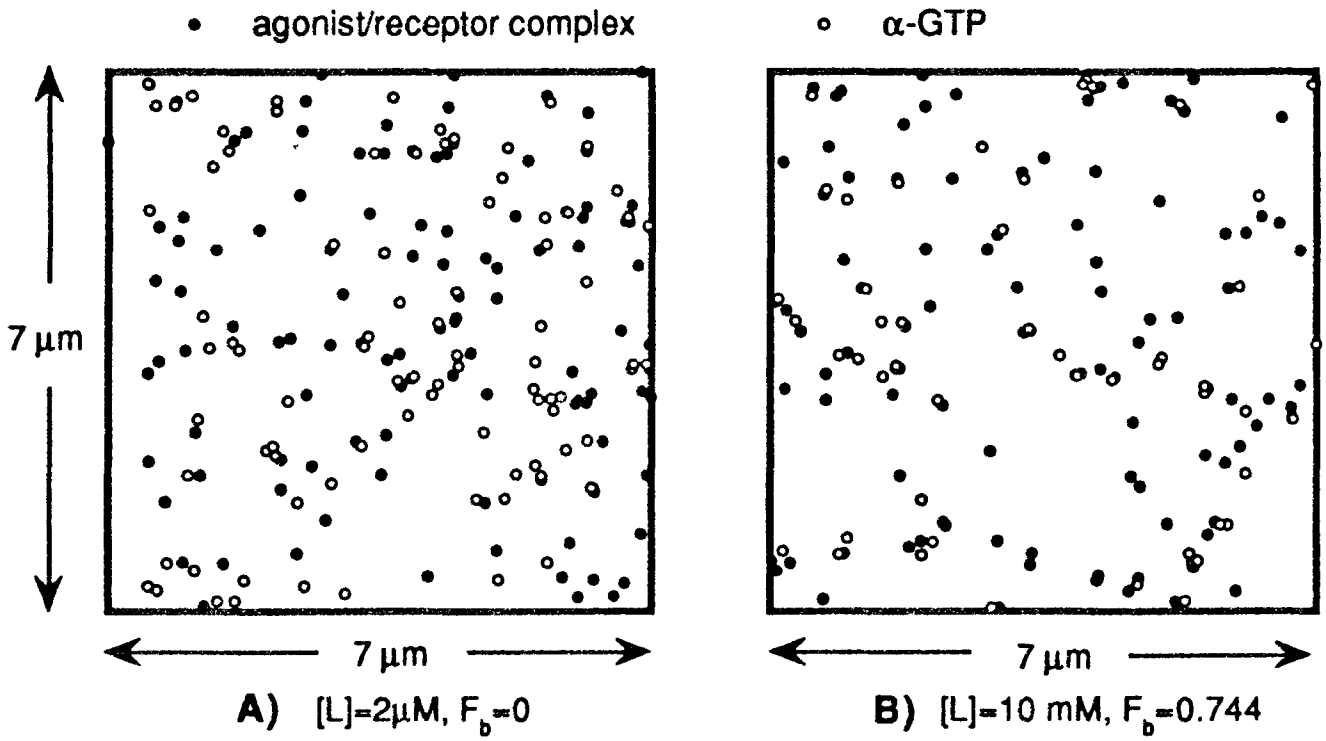

FIGURE 6. Snapshots of agonist/receptor complexes and α-*GTP*. The simulation snapshots are for test cases 1 and 3 with equal equilibrium receptor occupation, and are taken at t = 3 sec. For (A), movement of agonist among free receptors and diffusion are important in activation of G-protein. For (B), agonist movement among receptor is insignificant in G-protein activation because the number of free receptors is nearly zero; G-protein activation occurs almost exclusively by 2-D diffusion. Because of the movement of agonist among receptors, α-*GTP* formation is greater and more evenly distributed in (A) than (B). Also, α-*GTP* is seen at greater distances from agonist/receptor complexes in (A) because of the effect of agonist movement. Symbols are not drawn to scale.

This movement of agonist among free receptors allows thorough access of agonist/receptor complexes to G-protein. Approximately 25% of the receptors are occupied at equilibrium, suggesting that a receptor is more likely to be unoccupied than occupied. Thus for this case, movement of agonist among receptors as well as receptor mobility is important for G-protein activation.

For test cases 2 and 3, a large fraction of the receptors are inactivated by the receptor blocker, so that much higher concentrations of agonist are needed to produce the same equilibrium receptor occupation as in test case 1. Agonist again binds to receptors on the cell surface, and agonist/receptor complexes activate G-proteins in their vicinity by 2-D diffusion and collision. However, because the agonist concentration is higher, each receptor has a higher probability of rebinding agonist than in test case 1. At equilibrium more than 63% and 99% of the receptors are occupied by agonist in test cases 2 and 3, respectively. Therefore, a receptor is more likely to be agonist-occupied than unoccupied. With fewer free receptors in these cases, movement of the agonist among receptors plays a less significant role in G-protein activation than in test case 1. For this reason the agonist/receptor complexes tend to activate most of the G-proteins in their vicinity by 2-D diffusion, causing a local depletion of inactive G-protein (not unexpected for 2-D reactions, see (11,29)). As this occurs, the encounter rate constant between G-proteins and complexes decreases to a steady-state value lower than that for test case 1.

DISCUSSION

In our experiments, we used the α_1-adrenergic receptor agonist PhE with and without the receptor blocker Pz to trigger calcium mobilization in individual BC_3H1 cells. Surprisingly, we find that in the presence of the antagonist the speed of calcium mobilization as well as the fraction of cells responding is diminished for similar rates of agonist/receptor binding and identical equilibrium receptor occupation by agonist. Such an effect will not be predicted from a model in which occupancy of a fraction of receptors 100% of the time is treated identically to occupancy of 100% of the receptors a fraction of time. For example one such model that we have developed to explain the effect of agonist concentration on the speed of calcium mobilization fails to account for the Pz data we report here (19).

Provided that activation of G-proteins by bound recep-

tors is diffusion-limited or at least partially diffusion-controlled, the results of our Monte Carlo simulations offer an explanation for the effect of receptor blockers. The Monte Carlo model predicts that for the same equilibrium dissociation constant, K_D, the association/dissociation rate constants of agonist/receptor binding may determine whether or not receptor blockers affect calcium mobilization. The behavior extremes occur when association/dissociation rates are fast and slow relative to the rate of 2-D diffusion.

For the case in which agonist/receptor binding is slow compared with 2-D diffusion, movement of agonist among free receptors will not play a significant role in G-protein activation at any agonist concentration. Activation of G-protein occurs mainly by 2-D diffusion, and activation by agonist movement is insignificant. In this situation then receptor inactivation will have little effect on G-protein activation. A simpler model of calcium mobilization (*e.g.*, Ref. 19) will make accurate predictions with and without receptor blockers in this case because G-protein activation occurs mainly by 2-D diffusion.

For the other extreme in which agonist/receptor binding kinetics are fast compared with 2-D diffusion, movement of agonist among free receptors may be a major contributor to G-protein activation. At low agonist concentrations, movement of agonist among receptors on the cell surface provides an increased encounter frequency between complexes and G-protein. The contribution of agonist movement to G-protein activation decreases as agonist concentration increases because the fraction of unoccupied receptors decreases. In this situation, then, receptor inactivation significantly reduces G-protein activation by reducing the component of G-protein activation produced by agonist movement among receptors. Models that do not account for this movement of agonist among receptors will fail to predict accurately G-protein activation and thus calcium mobilization in the presence of receptor blockers. The effects we show in this paper will be even more pronounced at lower, though still physiological, values of the diffusion coefficients (20).

Our experimental system falls closest to this second extreme, the case in which agonist/receptor binding kinetics are rapid compared with 2-D diffusion. This suggests that use of the receptor blocker Pz may significantly reduce G-protein activation by restricting agonist movement. Thus, the diminished cell responsiveness observed with Pz may be at least partially accounted for through reduced G-protein activation and thus lower calcium release. The movement of agonist among receptors is especially important for low levels of receptor occupation; this is where we see the greatest disparity in the calcium responses with and without receptor inactivation.

Receptor antagonists are commonly used pharmaceuticals, and more recently research has turned to the design of agents to inhibit receptor-catalyzed G-protein activation (*e.g.*, Ref. 8). Our findings suggest that a quantitative characterization of the effects of these agents may require analyses such as that presented here.

REFERENCES

1. Abel, P. W., and K. P. Minneman. *Alpha*-1 adrenergic receptor binding and contraction of rat caudal artery. *J. Pharmacol. Exp. Ther.* 239(3):678–686, 1986.
2. Alberts, B., D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. Molecular Biology of the Cell. New York: Garland Publishing, Inc., 1989, 1219 pp.
3. Amitai, G., R. D. Brown, and P. Taylor. The relationship between α_1-adrenergic receptor occupation and the mobilization of intracellular calcium. *J. Biol. Chem.* 259(20): 12519–12527, 1984.
4. Bakardjieva, A., H. J. Galla, and E. J. M. Helmreich. Modulation of the β-receptor adenylate cyclase interactions in cultured chang liver cells by phospholipid enrichment. *Biochemistry* 18(14):3016–3023, 1979.
5. M. J. Berridge. Inositol trisphosphate and calcium signalling. *Nature* 361:315–325, 1993.
6. Bokoch, G. M., K. Bickford, and B. P. Bohl. Subcellular localization and quantitation of the major neutrophil pertussis toxin substrate, G_n. *J. Cell Biol.* 106:1927–1936, 1988.
7. Brown, R. D., K. D. Berger, and P. Taylor. α_1-Adrenergic receptor activation mobilizes cellular Ca^{2+} in a muscle cell line. *J. Biol. Chem.* 259(12):7554–7562, 1984.
8. Dalman, H. M., and R. R. Neubig. Two peptides from the $alpha_{2A}$-adrenergic receptor alter receptor G protein coupling by distinct mechanisms. *J. Biol. Chem.* 266:11025–11029, 1991.
9. Dembo, A., A. Kagey-Sobotka, L. M. Lichtenstein, and B. Goldstein. Kinetic analysis of histamine release due to covalently linked IgE dimers. *Mol. Immunol.* 19:421–434, 1982.
10. Gennis, R. R. *Biomembranes: Molecular Structure and Function.* Springer-Verlag, 1989, 533 pp.
11. Goldstein, B., C. Wofsy, and G. Bell. Interactions of low density lipoprotein receptors with coated pits on human fibroblasts: Estimate of the forward rate constant and comparison with the diffusion limit. *Cell Biol.* 78(9):5695–5698, 1981.
12. Gorospe, W. C., and P. M. Conn. Membrane fluidity regulates development of gonadotrope desensitization to GnRH. *Mol. Cell. Endocrinol.* 52:131–140, 1987.
13. Grynkiewicz, G., M. Poenie, and R. Y. Tsien. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260(6):3440–3450, 1985.
14. Guellaen, G., M. Yates-Aggerbeck, G. Vauquelin, D. Strosberg, and J. Hanoune. Characterization with [^{3}H]dihydroergocryptine of the α-adrenergic receptor of the hepatic plasma membrane. *J. Biol. Chem.* 253(4):1114–1120, 1978.
15. Hanski, E., G. Rimon, and A. Levitzki. Adenylate cyclase activation by the β-adrenergic receptors as a diffusion-controlled process. *Biochemistry* 18(5):846–853, 1979.
16. Hughes, R. J., M. R. Boyle, R. D. Brown, P. Taylor, and P. A. Insel. Characterization of coexisting $Alpha_1$- and $Beta_2$-adrenergic receptors on a cloned muscle cell line, BC_3H1. *Mol. Pharmacol.* 22(2):258–266, 1982.
17. Kwon, G., R. R. Neubig, and D. Axelrod. Lateral mobility

of tetramethylrhodamine labeled g protein βγ subunits in NG-108-15 cells. *FASEB J.* 5:A1595, 1991.

18. Lauffenburger, D. A., and J. J. Linderman. Receptors: Models for Binding, Trafficking, and Signaling. New York: Oxford University Press, 1993, 365 pp.
19. Mahama, P. A., and J. J. Linderman. Calcium signaling in individual BC_3H1 cells: Speed of calcium mobilization and heterogeneity. *Biotech. Prog.* 10:45–54, 1994.
20. Mahama, P. A., and J. J. Linderman. A Monte Carlo study of the dynamics of G-protein activation. *Biophys. J.* 67:1345–1357, 1994.
21. Mauger, J. P., F. Sladeczek, and J. Bockaert. Characteristics and metabolism of α_1 adrenergic receptors in a nonfusing muscle cell line. *J. Biol. Chem.* 257(2):875–879, 1982.
22. Mayo, K. H., M. Nunez, C. Burke, C. Starbuck, D. Lauffenburger, and C. R. Savage, Jr. Epidermal growth factor receptor binding is not a simple one-step process. *J. Biol. Chem.* 264:17838–17844, 1989.
23. Moscona-Amir, E., Y. I. Henis, and M. Sokolovsky. Aging of rat heart myocytes disrupts muscarinic receptor coupling that leads to inhibition of cAMP accumulation and alters the pathway of muscarinic-stimulated phosphoinositide hydrolysis. *Biochemistry* 28(17):7130–7137, 1989.
24. Stickle, D., and R. Barber. Evidence for the role of epinephrine binding frequency in activation of adenylate cyclase. *Mol. Pharmacol.* 36:437–445, 1989.
25. Stickle, D., and R. Barber. The encounter coupling model for beta-adrenergic receptor/GTP-binding protein interaction in the S49 cell. Calculation of the encounter frequency. *Biochem. Pharmacol.* 43(9):2015–2028, 1992.
26. Stickle, D., and R. Barber. Analysis of receptor-mediated activation of GTP-binding protein/adenylate cyclase using the encounter coupling model. *Mol. Pharmacol.* 43:397–411, 1993.
27. Taylor, C. W. The role of G proteins in transmembrane signalling. *Biochem. J.* 272;1–13, 1990.
28. Thomsen, W. J. and R. R. Neubig. Rapid kinetics of α_2-adrenergic inhibition of adenylate cyclase. Evidence for a distal rate-limiting step. *Biochemistry* 28(22):8778–8786, 1989.
29. Torney, D. C., and H. M. McConnell. Diffusion-limited reaction rate theory for two-dimensional systems. *Proc. R. Soc. Lond. A,* 387:147–170, 1983.