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Modeling Activation and Desensitization of G-Protein Coupled Receptors Provides Insight into Ligand Efficacy

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Signaling through G-protein coupled receptors is one of the most prevalent and important methods of transmitting information to the inside of cells. Many mathematical models have been proposed to describe this type of signal transduction, and the ternary complex (ligand/receptor/G-protein) model and its derivatives are among the most widely accepted. Current versions of these equilibrium models include both active (i.e. signaling) and inactive conformations of the receptor, but do not include the dynamics of G-protein activation or receptor desensitization. Yet understanding how these dynamic events effect response behavior is crucial to determining ligand efficacy. We developed a mathematical model for G-protein coupled receptor signaling that includes G-protein activation and receptor desensitization, and used it to predict how activation and desensitization would change if either the conformational selectivity (the effect of ligand binding on the distribution of active and inactive receptor states) or the desensitization rate constant was ligand-specific. In addition, the model was used to explore the implications of measuring responses far downstream from G-protein activation. By comparing the experimental data from the β_2-adrenergic, μ-opioid, D_1 dopamine, and neutrophil *N*-formyl peptide receptors with the predictions of our model, we found that the conformational selectivity is the predominant factor in determining the amounts of activation and desensitization caused by a particular ligand.

Introduction

The most common pathways for transmitting information to the inside of cells involve signaling through G-protein coupled receptors. It has been estimated that as much as 5% of the human genome codes for receptors of this type (Kenakin, 1996), and 60% of all pharmaceuticals used today are believed to act through these pathways (Roush, 1996). Because of the predominance of G-protein coupled receptors in cellular signaling and their involvement in many disease states, understanding what determines the efficacy of ligands that bind to these receptors (i.e. the ability of bound ligands to elicit responses) is of great interest. Successfully achieving this understanding could have broad implications for medicine and biology and greatly aid in the rational design of drugs. Mathematical modeling of G-protein coupled receptor signaling provides a useful tool for reaching this goal. By combining mathematical

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modeling with experimental data, one can bring a greater quantitative understanding to the data and maximize the potential of the information available.

Building on Clark's (1937) classic model of receptor/ligand binding, the mobile receptor hypothesis of Cuatrecasas (1974) first posed the idea that receptors can move in the membrane and interact with accessory proteins. This in turn gave rise to the ternary complex (ligand/receptor/G-protein) model (TCM) of De Lean *et al.* (1980) which explicitly allowed receptors to interact with G-proteins as well as ligands. This simple model has been expanded upon as more information about G-protein coupled receptors has been uncovered.

One variation of the TCM, known as the extended ternary complex model (eTCM), was developed by Samama *et al.* (1993) to account for constitutive activity in G-protein coupled receptor mutants and the observance of two receptor affinity states in the absence of G-protein. The eTCM includes both an active, signaling receptor state (R*) and an inactive, non-signaling receptor state (R) (see Fig. 1). These two states are thought to represent two distinct conformations of the receptor. The ability of a ligand to stabilize or bind to the R* state is related to the ligand's efficacy. Full agonists strongly favor the R* state, partial agonists favor the R* state less strongly, neutral antagonists favor both states equally, and inverse agonists favor the R state (Lefkowitz *et al.*, 1993).

The TCM and eTCM [and the related but more thermodynamically complete cubic ternary complex model (Weiss *et al.*, 1996)] are equilibrium models and therefore do not include the dynamics of the critical processes of receptor desensitization or G-protein activation. Shea (1997) has performed theoretical studies in which the dynamics of G-protein activation have been added to ternary complex models and has shown that including this non-equilibrium process can dramatically effect the model predictions for cellular responses. That is, the levels of activated G-protein do not necessarily parallel the concentrations of various receptor species predicted from equilibrium models.

Desensitization of receptors allows cells to adapt to repeated or persistent stimulation by providing a mechanism of turning down cellular responses, while G-protein activation provides a timed molecular switch for signaling responses. The efficacy of ligands that bind to G-protein

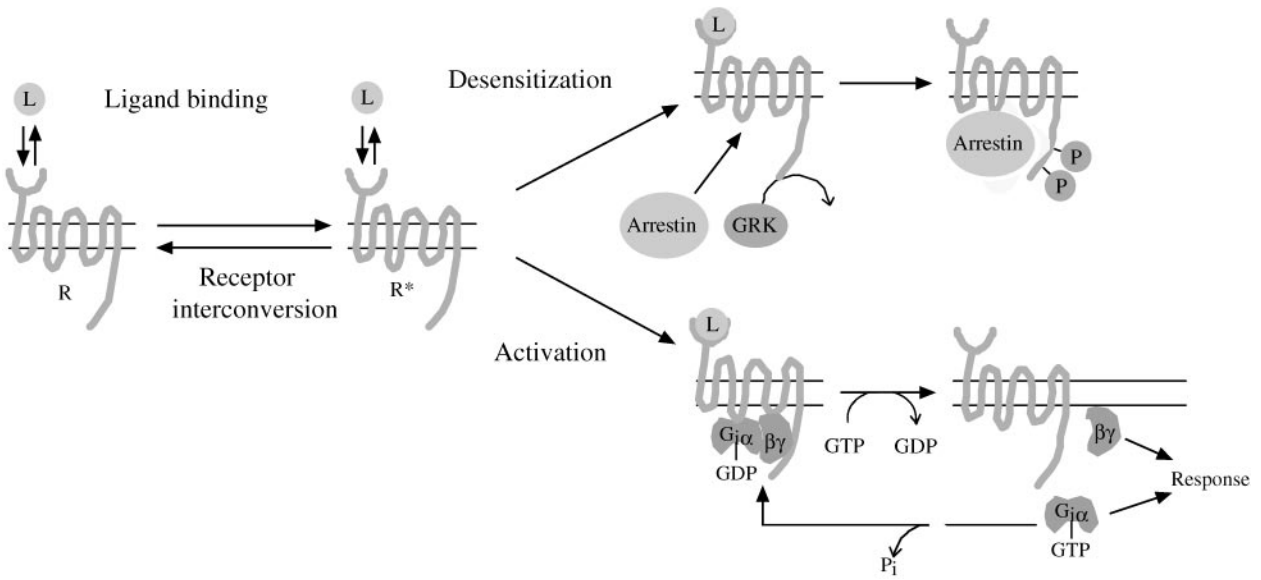

FIG. 1. Pathways of ligand binding, receptor interconversion, G-protein activation and receptor desensitization. Receptor interconversion from R (inactive) to R* (active) is thought to involve a conformational change. G-protein activation causes the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP). The G-protein then dissociates into α and $\beta\gamma$ subunits which can activate effectors and are deactivated by rebinding to each other following GTP hydrolysis by the α subunit. In many systems desensitization occurs as a result of receptor phosphorylation by G-protein coupled receptor kinases (GRKs) followed by the binding of arrestins to prevent further signaling.

coupled receptors may be determined in a large part by the dynamics of G-protein activation and receptor desensitization that they elicit. Receptor desensitization and G-protein ctivation act together to control response behavior.

Figure 1 shows the steps involved in receptor desensitization and G-protein activation as well as the interconversion of the receptor between active and inactive states. G-protein activation results in the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) on the α subunit of the G-protein. The G-protein then dissociates into the α and $\beta\gamma$ subunits which can both go on to activate effectors. The α subunit possesses intrinsic GTPase activity that hydrolyzes the bound GTP back to GDP, and allows the α and $\beta\gamma$ subunits to recombine to turn off the signal (Coleman & Strand, 1996). Because the ternary complex models do not include the steps of G-protein activation, they are best used to model systems in the absence of guanine nucleotides.

Desensitization of G-protein coupled receptors is thought to involve phosphorylation of the ligand-occupied receptor by a G protein coupled receptor kinase (GRK) followed by the binding of arrestin to the phosphorylated receptor to inhibit further signaling (Freedman & Lefkowitz, 1996). This then marks the receptor for sequestration, internalization, and eventually recycling to the cell surface. This pathway of desensitization has been well characterized for the β-adrenergic receptor and rhodopsin (Freedman & Lefkowitz, 1996). In addition, receptor phosphorylation by GRKs is known to be important in the desensitization of many other G-protein coupled receptors including the dopamine (Lewis *et al.*, 1998), muscarinic cholinergic (Hosey, 1994), and *N*-formyl peptide receptors (Prossnitz, 1997). It has been shown in some systems that GRKs are recruited to the cell membrane by the $\beta\gamma$ subunits of the G-protein, possibly making desensitization a function of G-protein activation (Pitcher *et al.*, 1992). However, an affinity conversion of the *N*-formyl peptide receptor believed to be related to desensitization was not abolished in cells treated with pertussis toxin to uncouple G-proteins from receptors (Sklar *et al.*, 1987; Hoffman *et al.*, 1996a).

We have developed a model for G-protein coupled receptor signaling based on the eTCM that includes G-protein activation and receptor desensitization. The model was used to address the following questions: How does including G-protein activation and receptor desensitization in a model for G-protein coupled receptor signaling affect the predicted response behavior, and how does changing the parameters involved in these processes shift the dose–response curves? Can this model be used to make predictions about ligand-specific parameters, which are critical in evaluating ligand efficacy, by comparing results from the model with previously reported data for activation and desensitization of G-protein coupled receptors? Can new data for the activation and desensitization of the neutrophil *N*-formyl peptide receptor be explained using this model, and what insight does this provide about the properties of these ligands?

Model Description

Our model for G-protein coupled receptor signaling is shown in Fig. 2. The model equations, procedures for solving the equations and a table of parameter values are given in Appendix A. This model is partly based on the eTCM and a previously reported model for neutrophil *N*-formyl peptide receptor binding (Hoffman *et al.*,

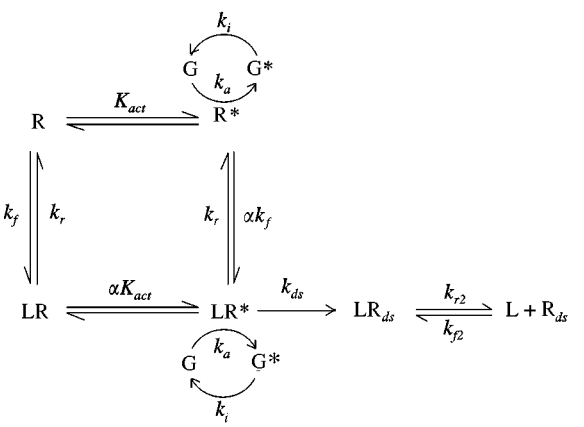

FIG. 2. Our model of G-protein coupled receptor signaling including G-protein activation and receptor desensitization. The response is controlled largely by the parameters α and k_{ds}. R is the inactive form of the receptor, R* is the active form of the receptor, LR is the inactive ligand/receptor complex, LR* is the active ligand/receptor complex, LR_{ds} is the desensitized ligand/receptor complex, G is inactive G-protein, G* is activated G-protein, L is free ligand, and R_{ds} is the desensitized receptor. Parameter values and definitions are given in Table A1 in Appendix A.

1996a), but also includes the steps of G-protein activation and receptor desensitization. As in the eTCM, our model includes two receptor states, an inactive, non-signaling state (R), and an active, signaling state (R*). Because conformational changes can occur extremely rapidly (Bray, 1998), the R to R* and LR to LR* conversions are assumed to be at equilibrium and are characterized by the equilibrium constants K_{act} and αK_{act}, respectively. The effect of ligand binding on the distribution between the active and inactive receptor states is thus controlled by the parameter α, also known as the conformational selectivity. The signaling receptor state can activate a G-protein equally well in both the ligand bound (LR*) and unbound (R*) forms. The ligand-bound activated receptor is allowed to desensitize with a rate constant k_{ds}. Only LR* is allowed to desensitize because only the ligand-bound receptor appears to be phosphorylated by GRKs (Freedman & Lefkowitz, 1996; Krupnick & Benovic, 1998), and R* would be present in insignificant quantities in systems with little or no constitutive activity (the focus of this paper). Activation of G-protein is represented by a single reaction between the G-protein (G) and LR* or R* since this is believed to be rate limiting in the production of activated G-protein (G*) (Neubig & Sklar, 1993; Posner *et al*., 1994). Furthermore, the recombination of the α and $\beta\gamma$ subunits of the G-protein is considered to be fast, so the deactivation of G-protein is modeled as a single reaction representing the hydrolysis of GTP to GDP (Adams *et al*., 1998).

Some parameters in the model should be dependent on the identity of the ligand used, while others will be tissue dependent and should not vary between ligands acting on the same receptor. Determining which parameters differ among ligands is essential for gaining a better understanding of ligand efficacy. Changing parameter values in the model and comparing the results with the experimental data for activation and desensitization of G-protein coupled receptors should provide insight into which parameters are ligand dependent.

By definition, the conformational selectivity (α) represents the effect of ligand binding on receptor activation (or vice versa), and is an intrinsic property of the ligand (Weiss *et al*., 1996). Similarly, it has been shown with a simpler model for the neutrophil *N*-formyl peptide receptor that the values of a desensitization rate constant fit to high-time-resolution binding data can vary for different *N*-formyl peptides (Hoffman *et al*., 1996b). In addition, different ligands for the β-adrenergic receptor are known to stimulate different amounts of receptor phosphorylation and desensitization, suggesting that they may have different values of α or k_{ds} (Benovic *et al*., 1988; January *et al*., 1997). Therefore, we expect the parameters α and k_{ds} to depend on the ligand's identity, and our model can be used to predict how changing these parameters will effect the resulting activation and desensitization.

The ligand binding rate constants (k_f, k_{f2} and k_{r2}) are also ligand dependent, but differences in these parameters are unlikely to be a major determinant of a ligand's efficacy. The ligand dissociation rate constant k_r may play a role in efficacy (Shea & Linderman, 1997) but probably not sufficient to account for the variations in efficacy discussed here (simulations not shown). Because any receptor in the R* form (whether ligand-bound or not) is assumed to be equally capable of activating G-protein, we take the G-protein activation rate constant k_a to be ligand independent and assume that different ligands cause different levels of activation primarily through different values of α and k_{ds}. While RGS (regulator of G-protein signaling) proteins may affect the G-protein inactivation rate constant k_i, these proteins act at the level of the dissociated α subunit and should not depend on the identity of the ligand that activated the receptor (Berman & Gilman, 1998). It has also been demonstrated that the Michaelis constant for GTP hydrolysis is not dependent on the nature of the ligand (Colquhoun, 1998). Therefore, k_i should not vary to a large extent between ligands. However, as a test of this our model can be used to predict how much k_i and k_a would have to change to produce the observed differences in activation and desensitization stimulated by different ligands.

Experimental Methods

Although some literature data are available for comparison with model results, we also

performed experiments to generate activation/desensitization data for the *N*-formyl peptide receptor on neutrophils. Activation and desensitization were measured in human neutrophils isolated from the blood of healthy donors on the day of the experiment. The neutrophils were partially separated from the blood by gelatin sedimentation, and further isolated to >98% purity using the counterflow elutriation protocol of Tolley *et al.* (1987). Following isolation, the cells were resuspended in a physiological buffer (5 mM KCl, 147 mM NaCl, 1.9 mM KH_2PO_4, 0.22 mM Na_2HPO_4, 5.5 mM glucose, 0.3 mM $MgSO_4$, 1 mM $MgCl_2$, 10 mM HEPES, pH 7.4) at 10^8 ml^{-1}.

Activation of the neutrophils was measured as *N*-formyl peptide induced oxidant production at 37°C using the spectrofluorometric assay of Hyslop & Sklar (1984). Neutrophils at 2×10^6 ml^{-1} were stimulated in the presence of 167 μg ml^{-1} parahydroxyphenylacetic acid (PHPA), 53 μg ml^{-1} superoxide dismutase, and 53 μg/ml horse radish peroxidase. Superoxide dismutase rapidly converts O_2^- to H_2O_2, which in the presence of peroxidase converts PHPA to a fluorescent diadduct. Fluorescence was monitored on an SLM 8100 spectrofluorometer with an excitation wavelength of 323 nm, and an emission wavelength of 400 nm.

Desensitization of the oxidant response was evoked by dual stimulation of the neutrophils with *N*-formyl peptides (Model & Omann, 1998). Cells were first stimulated with a submaximal concentration (180 nM) of *N*-formyl-norleucyl-leucyl-phenylalanine (CHO-NLF), and 3 min later a saturating amount (3 nM) of *N*-formyl-norleucyl-leucyl-phenylalaninyl-nor-leucyl-tyrosyl-lysine-fluorescein (CHO-NLFNTK-fl) was added. Oxidant production was monitored continuously at 37°C, and desensitization was reported as the percent reduction in maximal oxidant production caused by the initial submaximal stimulus. Figure 3 shows typical data from one of these experiments and defines how desensitization was quantified. Note that the response to 3 nM CHO-NLFNTK-fl alone represents a maximum response, and is equivalent to the response generated when 180 nM CHO-NLF and 3 nM CHO-NLFNTK-fl are added simultaneously rather than in succession (data not shown). We therefore compare the response generated by 3 nM CHO-NLFNTK-fl added 5 min after 180 nM CHO-NLF with the response generated by 3 nM CHO-NLFNTK-fl alone to determine the percent desensitization.

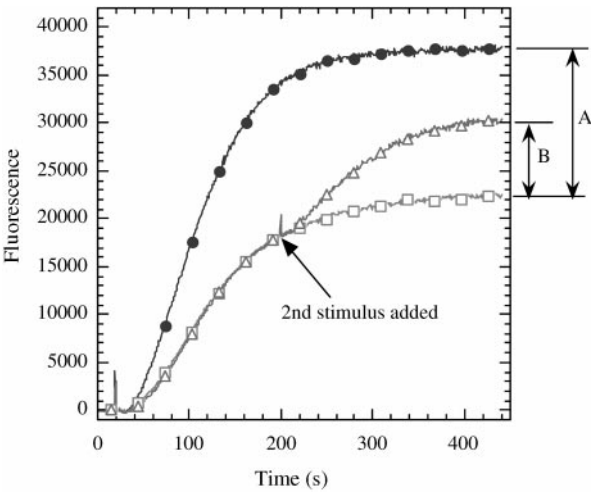

FIG. 3. Measurement of desensitization of the neutrophil *N*-formyl peptide receptor from repeated stimulation as determined by oxidant production at 37°C. 3 nM CHO-NLFTNK-fl added alone at 20 s (●); 180 nM CHO-NLF added at 20 s followed by 3 nM CHO-NLFTNK-fl 180 s later as indicated (△); 180 nM CHO-NLF added alone at 20 s. Percent desensitization was calculated as $(1 - B/A) \times 100$ (□).

Results

Model predictions for the activation of G-protein as a function of dimensionless ligand concentration ($[L]/K_d$ where $K_d = k_r/k_f$) at several different values of the conformational selectivity (α) and the desensitization rate constant (k_{ds}) are shown in the dose–response curves of Fig. 4 (see Appendix A for details of the calculations). This figure demonstrates that as α is increased, the maximum of the dose–response curve increases and the midpoint (EC_{50}) shifts to the left. However, when the rate constant for desensitization (k_{ds}) increases, the maximum of the dose–response curve decreases while the midpoint is relatively unchanged. Thus, our model predicts that different ligands have different values of k_{ds} if only the maximum response stimulated by these ligands is different, and different values of α if both the maximum response and the midpoint of the dose–response curves are different. Changes

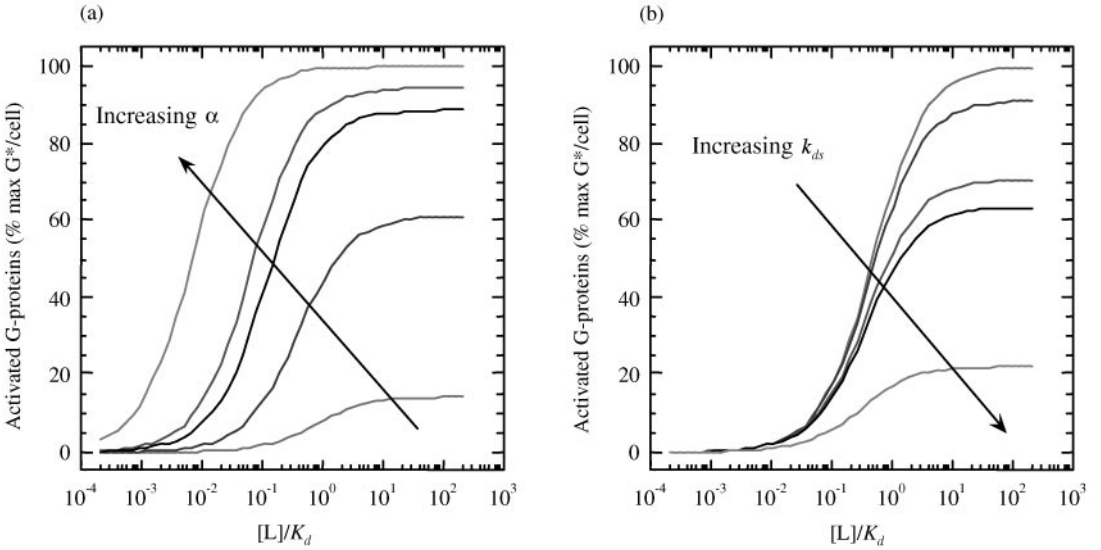

FIG. 4. Model predictions of activation as a function of dimensionless ligand concentration for changing values of α (a) and k_{ds} (b). Activation here is reported as the amount of G* (percent maximum). Ranges for α and k_{ds} are given in Table A1 in Appendix A. The value of k_{ds} was held constant at 0.065 s^{-1} in panel (a), and the value of α was held constant at 10^4 in panel (b). The general trends for the movement of the dose–response curves seen in this figure do not depend on the starting value of the midpoint.

in the maximum and midpoint of the dose–response curves could also result if the ligands had different values of the G-protein activation and inactivation rate constants k_a and k_i (data not shown). However, to see a significant effect these parameters would have to vary over orders of magnitude, which is not expected for different ligands as discussed above.

Experimental dose–response curves from January *et al.* (1997) for a double epitope modified human β_2-adrenergic receptor expressed in HEK-293 cells are shown in Fig. 5. The ligands used in these experiments have activity ranging from partial to full agonists. The dose–response curves for the different ligands have different values for both their maxima and midpoints. Comparing these data with Fig. 4 suggests that these ligands likely differ predominately in their values of α, with full agonists having large values of α relative to partial agonists.

In order to further resolve which parameters are actually different between ligands, we compared the amounts of desensitization and activation predicted by the model at various parameter values (see Appendix A for details). A plot of model predictions for activation and desensitization for varying values of α or k_{ds} is shown in Fig. 6. Our model predicts that as the value of α is increased for a given ligand, the amount of both activation and desensitization stimulated by that ligand increase. On the other hand, as k_{ds} is increased the amount of activation is predicted to decrease, while the amount of desensitization should increase.

We also examined model predictions of activation and desensitization at various values of the rate constants for activation and inactivation, k_a and k_i (data not shown). In this case, activation is changed as these parameters are varied, but desensitization remains constant. This is obvious from the model structure (Fig. 2) since changing k_a and k_i only changes the amount of G* produced and does not affect the amount of the desensitizing species (LR*). Therefore, it is not likely that ligands that cause different levels of both activation and desensitization differ only in these parameters.

Figure 7 shows activation vs. desensitization data elicited by several different ligands for three different G-protein coupled receptors (β_2-adrenergic, μ-opioid, and D_1 dopamine receptors) in four different experimental systems. In each case, the general trend observed is that ligands that cause more activation also cause more

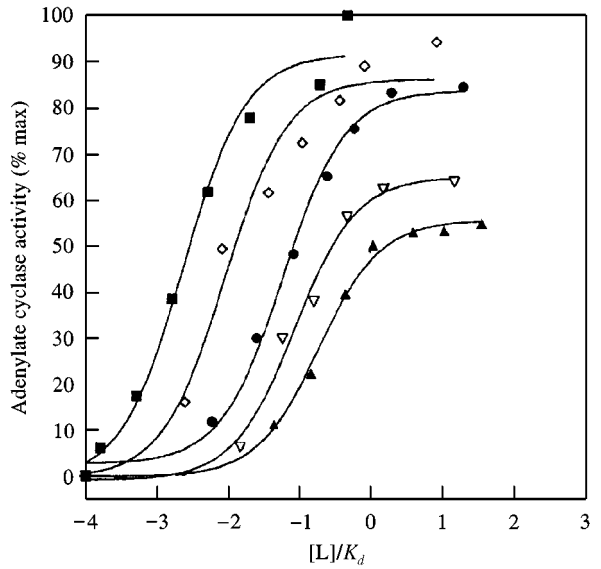

FIG. 5. Activation as a function of dimensionless ligand concentration for the β_2-adrenergic receptor. The ligands shown are epinephrine (■), fenoterol (◇), albuterol (●), dobutamine (▽), and ephedrine (▲). Redrawn from January *et al.* (1997). Ligands for the same receptor can vary widely in their ability to cause activation. Our model predicts that this may predominately be a result of a ligand's ability to hold the receptor in the active state (α).

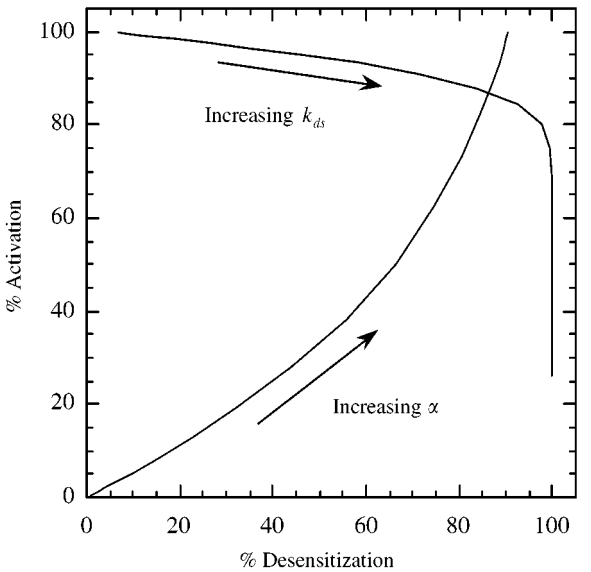

FIG. 6. Model predictions for activation vs. desensitization at various values of α and k_{ds}. Activation is reported as the amount of G* and desensitization as the sum of LR_{ds} and R_{ds} (both percent maximum). When α is varied, increasing activation correlates with increasing desensitization. When k_{ds} is varied, increasing activation correlates with decreasing desensitization. Ranges for α and k_{ds} are given in Table A1 in Appendix A. As in Fig. 4, the value of k_{ds} was held constant at 0.065 s^{-1} as α was varied, and the value of α was held constant at 10^4 as k_{ds} was varied.

desensitization. Based on our model predictions, this correlation between activation and desensitization can be explained if the different ligands differ only in their values of conformational selectivity. This is in agreement with our earlier prediction for the β_2-adrenergic receptor made by comparing experimental and theoretical dose response curves (see Figs 4 and 5).

Unfortunately, the situation is not always as unambiguous as in the case of the data shown in Fig. 7. Figure 8 shows activation vs. desensitization data from Lewis *et al.* (1998) for the rhesus macaque D_{1A} dopamine receptor expressed in C-6 glioma cells. There is no apparent correlation between the activation and desensitization elicited by the different ligands. If only the conformational selectivity were ligand dependent then one ligand could not cause more activation but less desensitization than another ligand, as is the case for several of the ligands shown in Fig. 8. This would suggest that the ligands differ in both their values of α and k_{ds} (Riccobene, 1999). Since increases in α result in an increase in both activation and desensitization and increases in k_{ds} cause an increase in desensitization and a decrease in activation, if both α and k_{ds} are varied at the same time a wide range of behavior can be elicited.

In order to further test the generality of our model for G-protein coupled receptor signaling, we took activation and desensitization data for the neutrophil *N*-formyl peptide receptor. The neutrophil *N*-formyl peptide receptor is a well-studied G-protein coupled receptor linked to several physiologically relevant responses. Activation was measured as the amount of oxidant production elicited. Desensitization was induced by stimulation of the receptor with a submaximal dose of ligand, and was measured as the decrease in oxidant production relative to controls caused by a subsequent maximal dose of ligand. Note that in contrast to the data shown in Fig. 7, the neutrophil oxidant response occurs far downstream of G-protein activation (Bokoch, 1995).

Figure 9 shows activation vs. desensitization data for the two *N*-formyl peptides CHO-

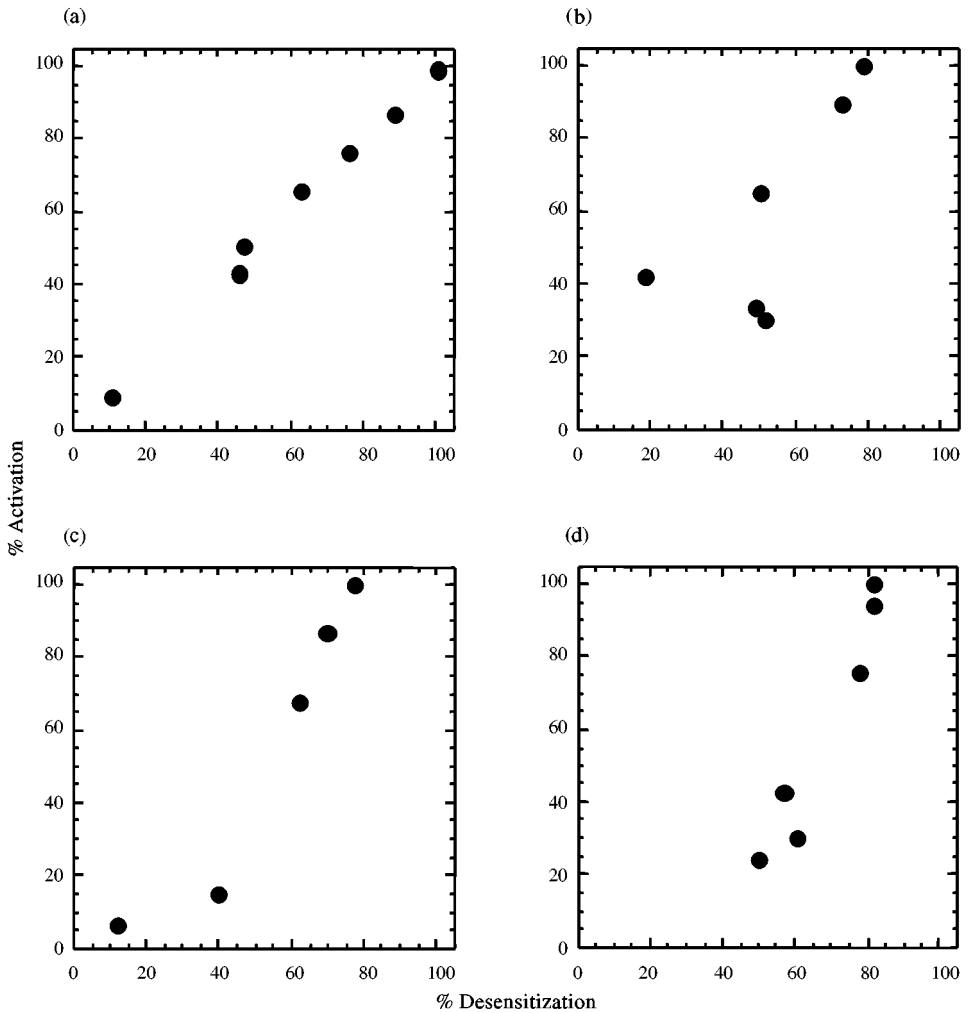

FIG. 7. Activation vs. desensitization plots for several different G-protein coupled receptor systems. (a) β_2-adrenergic receptor in a reconstituted membrane system. Redrawn from Benovic *et al.* (1988). Values represent % of activation and receptor phosphorylation produced by isoproterenol. (b) μ-opioid receptor in xenopus oocytes (from Yu *et al.*, 1997). Values represent % of activation caused by etorphine and % of desensitization relative to non-pretreated controls. (c) Dopamine D_1 receptor in D384 astrocytoma cells (from Balmforth *et al.*, 1990). Values represent % of activation caused by dopamine and % of desensitization relative to non-pretreated controls. (d) Dopamine D_1 receptor in NS20Y neuroblastoma cells (from Barton & Sibley, 1990). Values represent % of activation caused by dopamine and % of desensitization relative to non-pre-treated controls. In all plots, each point represents a different ligand. As a ligand's intrinsic activity increases, so does the amount of desensitization observed. Our model predicts that the different ligands have different values of α.

NLFNTK-fl and CHO-NLF. Unlike the activation vs. desensitization data presented earlier (Figs 7 and 8), a range of ligand concentrations were used to obtain data points for each of the ligands (i.e. each point on the plot represents a different initial ligand concentration). Both ligands cause similar amounts of desensitization at the same level of activation. Figure 10 shows model predictions for these two ligands using binding rate constants determined in our laboratory (Hoffman *et al.*, 1996a, b). These two ligands have equilibrium dissociation constant (K_d) values that differ by an order of magnitude (see Appendix A for values). The model predicts that the two *N*-formyl peptides should cause about the same amount of desensitization at a given level of activation if they have similar values of k_{ds} (differing by less than an order of magnitude) even though they have very different binding kinetics. These two ligands were reported to have different values of k_{ds} at 4°C when they were fit from binding data (Hoffman *et al.*, 1996b), but it

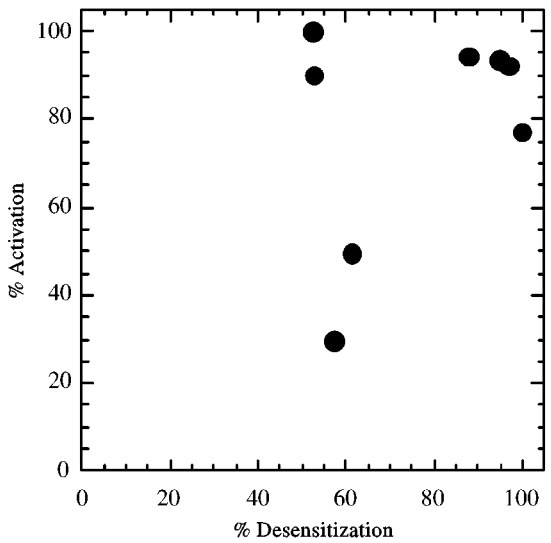

FIG. 8. Activation and desensitization for the D_{1A} dopamine receptor. Redrawn from Lewis *et al.* (1998). Values represent % of activation caused by dopamine and % of desensitization relative to non-pretreated controls. Each point represents a different ligand. There is no correlation between activation and desensitization for the ligands tested. Our model predicts that the different ligands have different values for both α and k_{ds}.

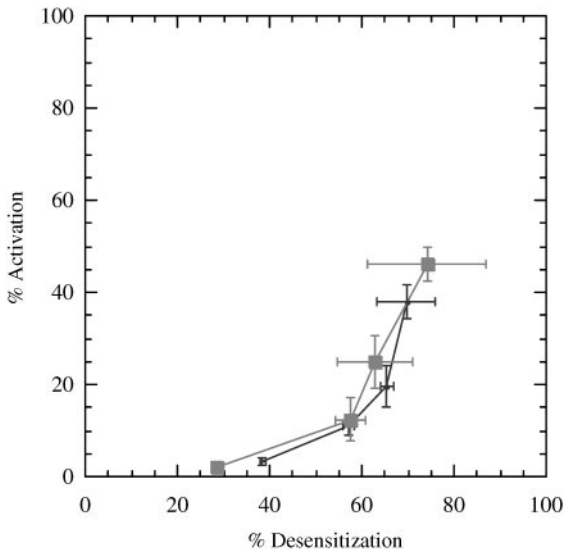

FIG. 9. Activation vs. desensitization for the neutrophil *N*-formyl peptide receptor for two different *N*-formyl peptides [CHO-NLFNTK-fl (●) and CHO-NLF (■)]. Activation was determined by measuring oxidant production using the parahydroxyphenylacetic acid assay of Hyslop & Sklar (1984), and desensitization was measured as described in Fig. 3 and Experimental Methods. Each point represents a different concentration of that ligand.

is not clear that the receptor species present at 4 and 37°C are the same. No specific predictions about α can be made by looking at the data in Figs 9 and 10 alone. However, in Appendix B we consider the implications of measuring a response such as neutrophil oxidant production, which occurs far downstream of G-protein activation, and show that CHO-NLFNTK-fl and CHO-NLF likely differ in their values of α.

Discussion

It is well known that different ligands for the same receptor can elicit different levels of cellular response even when the same number of receptors are bound. These ligands are then said to have different values of efficacy, but what underlying ligand-specific parameters lead to this behavior? The ability of a receptor to transduce a response may depend on its "state" (e.g. active, desensitized), and this state may be influenced by the identity of the ligand that is bound. Unfortunately, isolating these individual receptor states experimentally has proved to be exceedingly difficult. However, combining available experimental data for response activation and receptor desensitization with a mathematical modeling approach can provide insight into the determinants of ligand efficacy. In order to accomplish this, we have taken equilibrium models for G-protein coupled receptor binding from the literature and modified them to include the dynamic processes of receptor desensitization and G-protein activation. Including these dynamic events in the model facilitates the making of predictions about ligand-specific parameters because the model results can then be more directly compared with the data on cellular responses, which are inherently non-equilibrium processes.

A wide range of response behavior as a function of ligand concentration can be achieved with our model by varying either the conformational selectivity (α) or the desensitization rate constant (k_{ds}). By looking at how the dose–response curves shift when these parameters are varied, one can make predictions about which ligand-specific parameters are different among a group of ligands. For example, if the maximum and midpoint of a dose–response curve are both shifted for one ligand relative to another, it

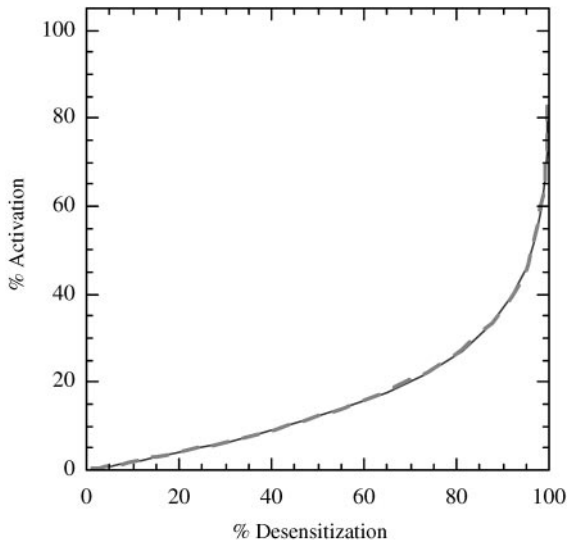

FIG. 10. Model predictions of activation vs. desensitization for various concentrations of CHO-NLFNTK-fl (—) and CHO-NLF (– – –). α is equal to 10 000 for CHO-NLFNTK-fl and 3000 for CHO-NLF and k_{ds} is 0.065 s^{-1} for both ligands. Binding rate constants for the two ligands are given in Table A2 of Appendix A and were from Hoffman *et al.* (1996a, b).

suggests that the two ligands have different values of the parameter α. However, if only the maximum is shifted, the ligands may differ only in their values of the parameter k_{ds}.

Plotting desensitization vs. activation data helps to further determine which ligand-specific parameters are different. If the amounts of both activation and desensitization stimulated by a particular ligand are greater (or less) than those stimulated by another ligand, it implies that α is larger (or smaller) for that ligand. Since both activation and desensitization are initiated by LR* in our model, they must both move in the same direction as α when α is changed. On the other hand, when k_{ds} is changed desensitization moves in the same direction as the change in k_{ds}, but activation moves in the opposite direction. By looking at the model in Fig. 2 the reason becomes obvious. As k_{ds} is decreased, LR* is less quickly converted to LR_{ds} leaving more of it available for activation of G-protein.

By comparing model results with data for partial and full agonists for several different G-protein coupled receptors, we found that these ligands likely differ predominantly in their conformational selectivity α. The conformational selectivity is a physical property of the ligand and determines how well a particular ligand stabilizes the active conformation of the receptor. The majority of the data found in the literature shows that ligands that induce more activation also cause more desensitization (Fig. 7), a result that can be explained by differences in α but not by differences in k_{ds} alone.

However, data from Lewis *et al.* (1998) for the D_{1A} dopamine receptor (Fig. 8) suggest that in some cases different ligands could have different values of both α and k_{ds}. If only α were ligand dependent than one ligand could not cause more activation but less desensitization than another ligand, as is the case for several of the ligands shown in Fig. 8. It should be noted that two of the plots in Fig. 7(c) and (d) show data from the D_1-dopamine receptor expressed in different cells than those used by Lewis *et al.* (1998). In both of these cases the data could be explained by differences in α alone, indicating that differences in response behavior could result from differences in the experimental system used independent of ligand-specific parameters [although most of the ligands shown in Fig. 8 are different than those shown in Fig. 7(c) and (d)]. This could be due to, for example, differences in the amount and/or type of G-protein coupled receptor kinase present in the cell system.

Our own data for activation and desensitization of the neutrophil *N*-formyl peptide receptor demonstrate a difference between two ligands for that receptor. The two *N*-formyl peptide ligands CHO-NLFNTK-fl and CHO-NLF appear to have different efficacies based on their dose–response curves for oxidant production (see Fig. 11). However, these ligands were shown to elicit similar amounts of desensitization at the same levels of activation (Fig. 9). Using our mathematical model to simulate these data leads to the prediction that the two ligands have similar values of the desensitization rate constant k_{ds}.

Should we then assume that the two *N*-formyl peptide ligands have different values of conformational selectivity to account for the observed differences in efficacy? Comparing Fig. 4 with Fig. 11 argues against this because changing α also changes the maximum of the dose–response curve. However, an additional point to consider when comparing experimental data with model

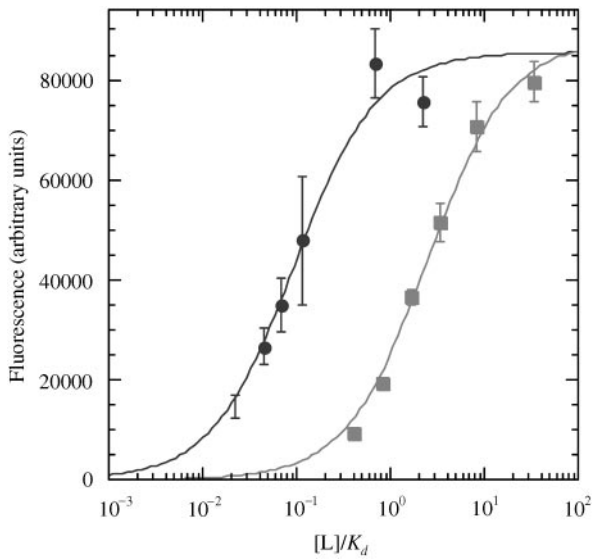

FIG. 11. Experimental dose–response curves for the two *N*-formyl peptides CHO-NLFNTK-fl (●) and CHO-NLF (■). The response measured was oxidant production which is far downstream of G-protein activation. Values for the K_{ds} of the two ligands are given in Table A2 of Appendix A.

results is the validity of assuming that activation is proportional to the amount of activated G-protein (G*) produced. If the response being measured is far down stream of the G-protein (e.g. neutrophil oxidant production), signal amplification at each step in the cascade may cause the final response to show a different type of behavior than G*.

Trzeciakowski (1996) explored the implications of this type of signal amplification by using an iterative process with the ligand/receptor complex as the initial stimulus and assuming that each subsequent step in the cascade was represented by a hyperbolic function. He showed that as the number of steps increased, the maximum of the dose–response curve increased and the curve shifted to the left. Because of this, a ligand that appeared to be a partial agonist when looking at early steps in the signal transduction cascade could appear to be a full agonist when looking further downstream.

We used a similar approach to test the effects of stimulus amplification on our model, but in our case the initial stimulus was the amount of G* produced (see Appendix B). Our results match those of Trzeciakowski for the movement of the dose–response curves as the number of steps is increased. Adding these effects does not change our model predictions for activation and desensitization of the *N*-formyl peptide receptor. Two ligands with different binding kinetics are still predicted to cause the same amount of desensitization at a given level of activation if the values of k_{ds} are similar. However, one cannot assume that two ligands that appear to be full agonists have similar (large) values of α. Thus, in agreement with our earlier results, we conclude that the two *N*-formyl peptide ligands CHO-NLFNTK-fl and CHO-NLF have different values of α but similar values of k_{ds}.

Determining which parameters effect the ability of bound ligands to elicit cellular responses is a key problem in pharmacology and rational drug design. In this paper, we have demonstrated that both dose–response and activation vs. desensitization data for G-protein coupled receptors can be used in combination with a mathematical model to provide insight into the ligand-specific parameters underlying differences in efficacy. This interplay between mathematical modeling and experimental data is crucial to gaining a better understanding of ligand efficacy. Ultimately, it is an understanding of ligand efficacy that will allow quantitative manipulation of cellular responses and aid in the development of improved drug therapies for diseases involving G-protein coupled receptors.

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APPENDIX A

The differential equations that describe our model (shown in Fig. 2) are given below, and parameter definitions and values are listed in Tables A1 and A2.

$$d[R]/dt = k_r[LR] - k_f[L][R] - k_{fR}[R] + k_{fR}/K_{act}[R^*], \quad (A.1)$$

$$d[R^*]/dt = k_r[LR^*] - \alpha k_f[L][R^*] + k_{fR}[R] - k_{fR}/K_{act}[R^*], \quad (A.2)$$

$$d[LR]/dt = k_f[L][R] - k_r[LR] + k_{fR}/(\alpha K_{act})[LR^*] - k_{fR}[LR], \quad (A.3)$$

$$d[LR^*]/dt = k_{fR}[LR] - k_{fR}/(\alpha K_{act})[LR^*] - k_{ds}[LR^*] + \alpha k_f[L][R^*] - k_r[LR^*], \quad (A.4)$$

$$d[LR_{ds}]/dt = k_{ds}[LR^*] - k_{r2}[LR_{ds}] + k_{f2}[L][R_{ds}], \quad (A.5)$$

$$d[R_{ds}]/dt = k_{r2}[LR_{ds}] - k_{f2}[L][R_{ds}], \quad (A.6)$$

$$d[G^*]/dt = k_a[G]([LR^*] + [R^*]) - k_i[G^*], \quad (A.7)$$

$$d[G]/dt = k_i[G^*] - k_a[G]([LR^*] + [R^*]). \quad (A.8)$$

The ligand concentration [L] was assumed to remain constant (no depletion). No assumptions were made about the concentration of G-protein ([G]) in eqns (A.7) and (A.8).

In order to produce dose–response curves for different ligands, the above equations were solved using the ODE solver Berkeley Madonna (Kagi Shareware) with the Rosenbrock (stiff) integration method. This method is derived from the routine stiff (Press, 1992). Activation was reported as the maximum value of G* reached during the time course of the simulation. The model was run for 240 s based on the time course of neutrophil responses to *N*-formyl peptides, but this time is also appropriate for measurements of cAMP accumulation and desensitization measured by receptor phosphorylation. Desensitization was reported as the percent of total surface receptors

TABLE A1

Activation and desensitization parameters and total species concentrations used in our model of G-protein coupled receptor signaling

Parameter	Definition	Values
α	Effect of ligand binding on receptor activation	10^0–10^6
k_{ds}	Desensitization rate constant	10^{-4}–10^0 s^{-1}
k_{fR}	Forward rate constant for R to R* conversion	10 s^{-1}
K_{act}	Equilibrium constant for R to R* conversion	1.0×10^{-4}
k_a	Activation rate constant for G to G*	1.0×10^{-7} s^{-1}*
k_i	Inactivation rate constant for G*	2.0×10^{-1} s^{-1}*
L	Ligand concentration	10^{-12}–10^{-6} M
R_{tot}	Total number of surface receptors	5.5×10^4 $cell^{-1}$†
G_{tot}	Total number of G-proteins	1×10^5 $cell^{-1}$¶

* From Adams *et al.* (1998).

† From Sklar *et al.* (1984); Omann *et al.* (1987) for the neutrophil *N*-formyl peptide receptor.

¶ From Bokoch *et al.* (1988).

TABLE A2
Ligand binding rate constants used in our model for G-protein coupled receptor signaling

Parameter	Definition	CHO-NLFNTK-fl*	CHO-NLF†
k_f	Ligand association rate constant for R	$8.4 \times 10^7\ M^{-1}\,s^{-1}$	$1.2 \times 10^8\ M^{-1}\,s^{-1}$
k_r	Ligand dissociation rate constant for R	$0.37\ s^{-1}$	$7.3\ s^{-1}$
k_{f2}	Ligand association rate constant for R_{ds}	$8.4 \times 10^7\ M^{-1}\,s^{-1}$	$1.3 \times 10^2\ M^{-1}\,s^{-1}$
k_{r2}	Ligand dissociation rate constant for R_{ds}	$4.6 \times 10^{-3}\ s^{-1}$	$4.0 \times 10^{-5}\ s^{-1}$
K_d	Equilibrium dissociation constant (k_r/k_f)	4.4×10^{-9} M	6×10^{-8} M

* From Hoffman *et al.* (1996a). Note these values of the binding rate constants were used for all simulations shown in this paper except those in which CHO-NLF was being modeled. A wide range of binding rate constants were tested and results were qualitatively similar to the plots shown.
† Values from Hoffman *et al.* (1996b) for the CHO-NLF binding rate constants at 4°C were converted to 37°C values using the Arrenhius equation with an activation energy of 8000 cal/mol^{-1} (Sklar *et al.*, 1984).

that were in the R_{ds} and LR_{ds} forms at the end of the run (maximum value). Note that instead of reporting activation and desensitization as the maximum number of G* and R_{ds} plus LR_{ds}, respectively, the integrated values of these quantities over the time that the model was run could have been used. However, using the integrated values does not change our qualitative predictions for activation and desensitization as the values of parameters in the model are changed (data not shown).

APPENDIX B

Figure 11 shows dose–response curves for the two *N*-formyl peptides CHO-NLFNTK-fl and CHO-NLF. Our model predicts that these two *N*-formyl peptides have similar values of k_{ds} (see discussion of Figs 9 and 10). Even when the experimental dose–response curves are normalized to the K_ds of the ligands (as in Fig. 11) there is still a difference in the midpoints of the curves, but the curves have the same maximum value. Therefore, there must be some difference in ligand-specific parameters besides binding rate constants to account for the observed dose–response curves. What then is contributing to this shift in the midpoint? There is a possibility that the two ligands may differ in their values of α since this parameter is known to shift the midpoint and maximum of dose–response curves [see Fig. 4(a)]. However, if α is different but k_{ds} is the same, then the maxima should be different.

One possible reason that the dose–response curves for these ligands have the same maximum value is that the measured response (oxidant production) is far downstream of G-protein activation. The binding of a ligand to a receptor is just the first step in signal transduction by G-protein coupled receptors. Following this initial stimulus, a cascade of signaling events eventually gives rise to the final cellular response. For activation via the β-adrenergic receptor, the response is typically measured as adenyl cyclase activity which is only one step removed from G-protein activation. In this case, looking at predictions of the model for G* should be relevant. However, oxidant production in neutrophils follows activation of phospholipase C, protein kinase C, phosphatidylinositol-3 kinase, and several other steps (Bokoch, 1995). Because amplification and feedback can occur at each step, the final response may show a different type of behavior than G* does. The steps in this cascade involve enzymes such as kinases and phosphatases and may be represented by saturable functions.

Trzeciakowski (1996) used a nonlinear hyperbolic function of the following form to represent the steps in a signal transduction cascade:

$$E = \frac{S^\gamma}{S^\gamma + \beta^\gamma}, \tag{B.1}$$

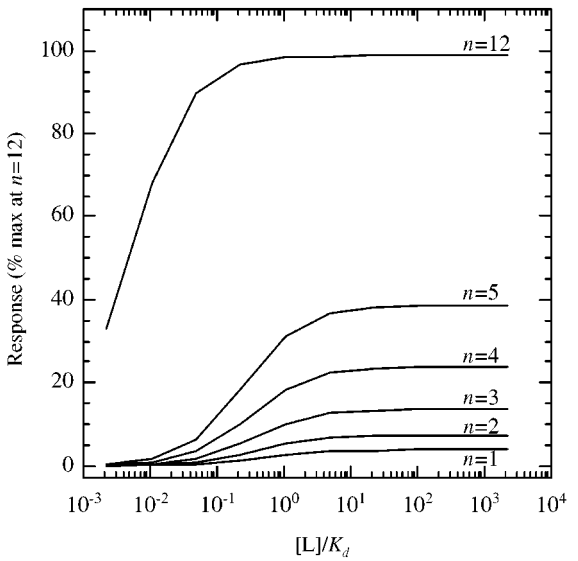

FIG. B1. Model dose–response curves as the number of steps in the signal transduction cascade (n) increases. A ligand may appear as a partial agonist if the response is measured early in the signal transduction cascade, but as a full agonist if it is measured further downstream. The responses in the figure are reported as a percentage of the maximum response generated when n equals 12 steps.

where E and S represent the response and stimulus, respectively, and β and γ are adjustable parameters. The initial stimulus (S_0) was taken as the number of ligand/receptor complexes multiplied by the intrinsic efficacy (ε) of the ligand, and the stimulus for each subsequent step was the response from the previous step in an iterative process:

$$S_0 = \varepsilon[\mathrm{LR}],$$

$$S_1 = f(S_0),$$

$$S_2 = f(S_1),$$

$$\ldots$$

$$S_n = f(S_n).$$

Trzeciakowski showed that as the number of steps increased, the maximum of the dose–response curve increased and the curve shifted left. Because of this, a ligand which appeared to be a partial agonist when looking at early steps in the signal transduction cascade could appear to be a full agonist when looking further downstream.

To test how adding this type of amplification effected our model, we used the same type of stimulus function and iterative process as Trzeciakowski (1996), but made the initial stimulus G* as generated by our model rather than LR. Figure B1 shows how the dose–response

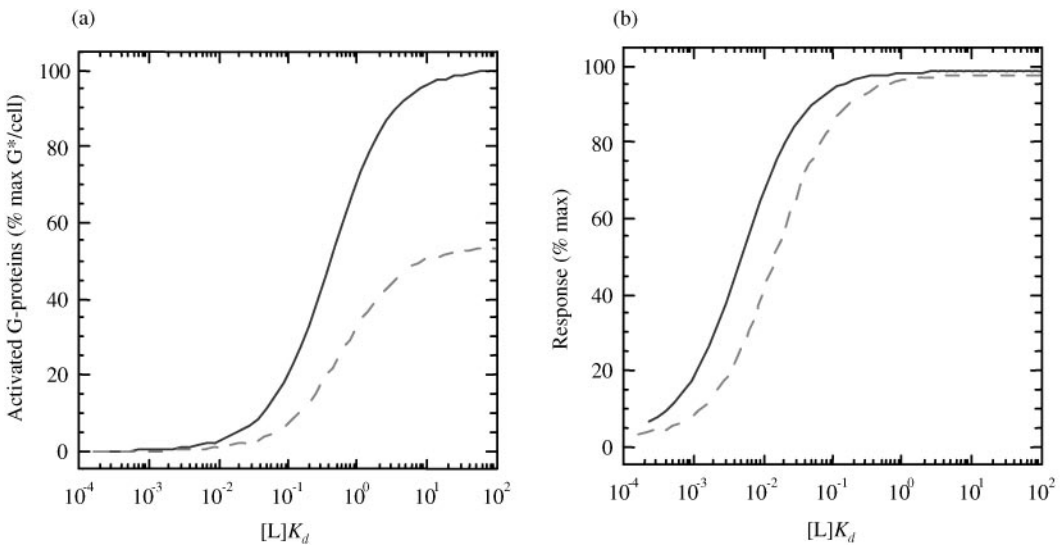

FIG. B2. Model dose–response curves for two ligands with different values of α (—CHO-NLFNTK-fl, $\alpha = 10\,000$; – – –CHO-NLF, $\alpha = 3000$). (a) The response is reported as activated G-protein, and one of the ligands appears to be a partial agonist. (b) The response is reported 12 steps downstream of G-protein activation with each step represented by a hyperbolic function, and both ligands appear to be full agonists. The dose–response curves have different midpoints due to the different values of α.

curves shift as the number of steps in the cascade increases. As in the case shown by Trzeciakowski, both the midpoint and the maximum of the dose–response curves are changed as the number of steps is increased. Figure B2 compares the dose–response curves for two ligands with different values of α when the response is taken as G* [Fig. B2(a)] with the dose–response curves for the same ligands when the response is 12 steps downstream of G-protein activation [Fig. B2(b)]. When the response is measured far downstream of G-protein activation, the two ligands appear to be full agonists even though one of the ligands would be a partial agonist if G* were taken as the response. This demonstrates that two ligands may have different values of conformational selectivity even if both appear to be full agonists depending on where the response is measured. This result does not change any of the previous predictions made using our model, but adds the extra caveat that when looking at a response far downstream of G-protein activation, ligands may differ in their values of α even if they appear to have the same maximum response.