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## Diffusion-limited reactions in G-protein activation: Unexpected Consequences of Antagonist and Agonist Competition

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### Abstract

In this work, we ask whether the simultaneous movement of agonist and antagonist among surface receptors (i.e. continually associating and dissociating from individual receptors according to specified kinetics) has any unexpected consequences for G-protein activation and receptor desensitization. A Monte Carlo model framework is used to track the diffusion and reaction of individual receptors, allowing the requirement for receptors and G-proteins or receptors and kinases to find each other by diffusion (collision coupling) to be implemented explicitly. We find that at constant agonist occupancy the effect of an antagonist on both G-protein activation and the ratio of G-protein activation to receptor desensitization can be modulated by varying the antagonist dissociation kinetics. The explanation for this effect is that antagonist dissociation kinetics influence the ability of agonists to access particular receptors and thus reach G-proteins and kinases near those receptors. Relevant parameter ranges for observation of these effects are identified. These results are useful for understanding experimental and therapeutic situations when both agonist and antagonist are present, and in addition may offer new insights into insurmountable antagonism.

### Keywords

G-protein coupled receptor; desensitization; simulation; model

### Introduction

Agonist binding to G-protein coupled receptors (GPCRs) allows receptors to activate G-proteins, initiating a signal transduction cascade and ultimately cellular responses that are important in processes including regulation of heart contractility, pain and the immune response (reviewed in (George *et al.*, 2002)). Agonist-occupied GPCR are also targeted for phosphorylation, a key first step in the desensitization pathway. It is estimated that GPCRs represent 1% of the human genome (Breitwieser, 2004) and that 50% of modern therapeutics act by modulating GPCRs (George *et al.*, 2002). One type of therapeutic is an antagonist, a molecule that binds to the target receptor but does not activate it. The antagonist functions by blocking agonist binding and reducing signaling.

The interaction between receptors and G-proteins has been described by the collision coupling model (Tolkovsky and Levitzki, 1978), reviewed in Lauffenburger and Linderman (1993),

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which allows for receptors and G-proteins to diffuse in the membrane and interact when they collide. The diffusion of receptors and G-proteins in the membrane is sufficiently slow as to limit the number of G-proteins that can be activated by a single receptor (Mahama and Linderman, 1995). Similarly, the phosphorylation of agonist-bound receptors is believed to first require the diffusion of receptors and membrane-bound kinases (Krupnick and Benovic, 1998).

Agonist or antagonist ligands continually associate and dissociate from individual receptors according to (ligand-specific) kinetics. This allows, even when binding has equilibrated, for the continual movement of ligands among surface receptors. In previous theoretical and experimental studies, when comparing cases of *equal occupancy of receptors by agonist*, increased movement of agonist (shorter half-life of the receptor-agonist complex) resulted in increased G-protein activation, as receptors newly occupied by agonist have access to G-proteins close by (Mahama and Linderman, 1994; Shea *et al.*, 1997; Stickle and Barber, 1993). Such movement has also been predicted to partially decouple G-protein activation from receptor phosphorylation (Woolf and Linderman, 2003).

In this work, we ask whether *antagonist* dissociation kinetics can also play a role in modulating G-protein activation and receptor desensitization. In other words, we determine the consequences of the simultaneous movement of agonist and antagonist among surface receptors for G-protein activation and receptor desensitization. A Monte Carlo model framework is used to track the diffusion and reaction of individual receptors, allowing the requirement for receptors and G-proteins or receptors and kinases to find each other by diffusion (collision coupling) to be implemented explicitly. Simulations are used to scan a broad range of conditions and to identify regimes that may be of experimental interest.

## Methods

### Estimating the role of diffusion

The reactions producing GPCR activation and phosphorylation are shown schematically in Fig. 1. In order to accurately simulate these reactions, we first determine which bimolecular reactions are likely to be diffusion-limited. The reactions we evaluate are ligand binding, G-protein activation,  $G_{\beta\gamma}$  recruitment of receptor kinase, receptor phosphorylation, and G-protein recombination. We compare the overall observed reaction rate constant ( $k_f$ ) with the transport rate constant ( $k_+$ ). We estimate  $k_+$  in the appropriate dimensionality with equations given in Lauffenburger and Linderman (1993):

$$2 \text{ dimensions: } k_+ = \frac{2\pi D}{\ln(b/s)} \quad (1)$$

$$3 \text{ dimensions: } k_+ = 4\pi Da \quad (2)$$

$$b = \sqrt{\frac{SA}{\pi[G]}} \quad (3)$$

where  $D$  is the diffusion coefficient,  $b$  is half the mean separation distance between reactants,  $s$  is the encounter radius,  $a$  is the cell radius,  $SA$  is the total surface area and  $[G]$  is the average G-protein concentration. This estimation assumes that the reactants are evenly distributed on the surface. If the reactants are locally enriched or depleted in one area the actual value of  $k_+$  could vary by as much as 10-fold and can be more accurately determined by our simulations (Shea and Linderman, 1998).

For the two dimensional reactions in the cell membrane, G-protein activation and receptor phosphorylation, we used values of  $D = 10^{-11} - 10^{-9} \text{ cm}^2/\text{sec}$  (Lee *et al.*, 1993; Sako and Kusumi),  $SA = 1000 \mu\text{m}^2$ , and  $[G] = 10 - 100/\mu\text{m}^2$  (Pardo *et al.*, 1997). For G-protein activation we then calculate range for  $k_+$  of  $10^{-6}$  to  $10^{-3} (\#/cell)^{-1} \text{ sec}^{-1}$ . This range overlaps with observed values of  $k_f$  ( $10^{-7} (\#/cell)^{-1} \text{ sec}^{-1}$  to  $10^{-4} (\#/cell)^{-1} \text{ sec}^{-1}$  (Bornheimer *et al.*, 2004; Simons *et al.*, 2004; Yi *et al.*, 2003)). This broad range of values for  $k_f$  is likely due to the differences in the cell types used and the concentrations of the receptors and G-proteins in these experiments. For receptor phosphorylation a similar value of  $k_+$  is calculated because the diffusion coefficient and the mean separation between reactants is similar. Values of  $k_f$  for GPCR phosphorylation have not been widely reported, but the rate constant for receptor phosphorylation estimated for T cell receptors is of the same range as for G-protein activation (Schade and Levine, 2002). Thus for these two reactions, diffusion likely limits the rate of reaction and the locations of the relevant molecules will be explicitly tracked in our simulations.

For the remaining three reactions, ligand binding, recruitment of receptor kinase and G-protein recombination, one of the reactants diffuses in three dimensions (Eqn. 2). For these cases  $k_+ > k_f$  and the overall reaction rate is limited by intrinsic reaction kinetics and not the diffusion of reactants (Lauffenburger and Linderman 1993). Thus the locations of individual ligand,  $G_\alpha$  and (non membrane-bound) receptor kinase molecules are not necessary to track in simulations.

### Simulation procedure

To accurately simulate the reactions in GPCR signaling and phosphorylation we track the spatial location of the receptors and G-proteins (to incorporate diffusion) and their discrete states (e.g. bound to agonist, phosphorylated) using a kinetic Monte Carlo model (Brinkerhoff and Linderman, 2005). A two-dimensional triangular lattice was used to represent the cell membrane. The lattice was 3000 by 3000 with a spacing of 2 nm. This simulates a total area of  $36 \mu\text{m}^2$ ; periodic boundary conditions are used to approximate an entire cell. The lattice contains receptors and G-proteins with a diameter of 4 lattice spacings or 8 nm.

The simulation algorithm is similar to previous models (Woolf and Linderman, 2004). Briefly, a receptor or G-protein is chosen at random and attempts to move, react, bind, or dissociate as appropriate. This procedure is repeated until on average each protein has gotten one opportunity to act, which completes one time step (approximately 0.3 msec). For a diffusion event the molecule moves a single lattice space in a random direction, unless the move would cause two molecules to overlap, in which case the move is rejected.

An agonist-bound receptor will exist in one of many different rapidly interconverting conformations or energy states that dictate the possible interactions for the receptor (Kenakin, 2002). This is generally simplified to allow two possible states, active and inactive (e.g. Kinzer-Ursem *et al.*, 2006). Each time the algorithm chooses an agonist-receptor complex it is randomly assigned to be either active or inactive, independent of its previous state or the status of other receptors. The percentage of agonist-bound receptors that are active on average is given by  $\alpha K_{ACT}/(\alpha K_{ACT} + 1)$ . We set  $K_{ACT} = 10^{-6}$  and  $\alpha = 10^5$  or  $10^8$ , corresponding to 9% or 99% of agonist-bound receptors in the active conformation. Antagonist-bound receptors can only exist in the inactive state, and thus are incapable of activating G-proteins and of being phosphorylated. In other words, the value of  $\alpha$  for an antagonist is 0. Antagonist dissociation from these receptors allows the receptors to be available for agonist or antagonist binding.

Agonist-bound active receptor can activate a G-protein if one is within the interaction radius of one lattice space, or 2 nm (i.e. receptor and G-protein must be neighbors to interact). Activation is recorded as the entry of a  $G_{\alpha-GTP}$  subunit into the cytosol while the  $G_{\beta\gamma}$  subunit remains at the membrane.  $G_{\beta\gamma}$  subunits can bind to receptor kinases.  $G_{\beta\gamma}$ -kinases can diffuse

to and phosphorylate active agonist-bound receptors. Phosphorylated receptors, when agonist-bound and active, can also activate G-proteins in the same way as unphosphorylated agonist-bound active receptors (Jin *et al.*, 2000). It is believed that steps subsequent to phosphorylation, i.e. arrestin binding, desensitize the receptor and prevent it from activating G-proteins, although we focus on shorter time-scale events and thus do not include these in our simulations. GTP is hydrolyzed with rate constant  $k_{GTP}$  to give  $G_{\alpha-GDP}$ ; recombination of  $G_{\alpha-GDP}$  and  $G_{\beta\gamma}$  reforms the inactive G protein.

Model output is the number of active G proteins ( $G_{\alpha-GTP}$ ) at steady state or the initial rates of G-protein activation and receptor phosphorylation as calculated by a least squares fit of the simulation output over the first 30 seconds. In addition, the ratio of the rate of G-protein activation to the rate of receptor phosphorylation (GARP) is calculated (Woolf and Linderman, 2003). The ratio is interesting as an indicator of the relative initial amounts of each process, and is similar to the relative agonist signaling vs. endocytosis measurements of Whistler *et al.* (1999) except that our measure follows events at earlier times.

### Parameter values and probabilities

For discrete time models, reactions can be modeled as Poisson processes and the probabilities of reaction can be derived from the bulk reaction rates using a Poisson distribution. With sufficiently small time steps ( $\Delta t$ ), the probabilities of reaction are nearly proportional to the intrinsic reaction rate constants, according to (Rowley, 1994)

$$P = 1 - \exp(-k\Delta t) \approx k\Delta t \quad (4)$$

The probability of a diffusion event,  $P_{\text{move}}$ , was calculated using the translational diffusion coefficient  $D$  of proteins in a lipid bilayer. For a single particle exhibiting Brownian diffusion on a triangular lattice, the probability of a particle moving at least one lattice spacing,  $l$ , in one iteration time step,  $\Delta t$ , can be approximated with

$$P_{\text{move}} = 1 - \exp\left(\frac{-6\Delta t D}{l^2}\right) \approx \frac{6\Delta t D}{l^2} \quad (5)$$

### Parameter values used in the simulations are listed in Table 1

*To compare equivalent conditions while varying the antagonist dissociation rate constant, we simultaneously vary the concentrations of agonist and antagonist to maintain a constant occupancy of the receptors by antagonist for any given agonist occupancy.* For example, if the antagonist dissociation rate constant  $k_{\text{off-antag}}$  is increased by 10 fold the concentration of antagonist is also increased by 10 fold as indicated by the familiar Gaddum equation (Colquhoun 2006):

$$\% \text{antagonist occupancy} = \frac{[Antag]/K_{D-antag}}{1 + [Ag]/K_{D-ag} + [Antag]/K_{D-antag}} * 100\% \quad (6)$$

where  $K_{D-antag} = k_{\text{off-antag}}/k_{\text{on-antag}}$  and  $K_{D-ag} = k_{\text{off-ag}}/k_{\text{on-ag}}$ .

## Results

With our model of agonist and antagonist binding to receptors we explore a range of parameters to determine their impact on G-protein activation and receptor phosphorylation.

## Antagonist dissociation rate constant can modulate G-protein activation

Steady state G-protein activation was simulated for constant agonist binding and dissociation kinetics ( $k_{\text{on-ag}}$ ,  $k_{\text{off-ag}}$ ), antagonist association kinetics ( $k_{\text{on-antag}}$ ) and agonist concentration. When we varied the antagonist dissociation rate constant ( $k_{\text{off-antag}}$ ) we made an equivalent adjustment in antagonist concentration so that at a given agonist concentration the antagonist occupancy was constant (Eqn. 6). We plot our results for G protein activation at steady state against the level of agonist occupancy in order to compare cases with equal numbers of receptors bound by agonist. Previous models have shown that at constant occupancy of receptors by agonist (with no antagonist present) the agonist dissociation rate constant  $k_{\text{off-ag}}$  can modulate the number of active G-proteins (Shea *et al.*, 1997); we use similar conditions in this model of competition between agonist and antagonist.

When comparing cases of equal agonist occupancy, simulations show that the presence of the antagonist reduces G-protein activation and, in addition, that the antagonist dissociation kinetics can modulate the amount of this reduction (Fig 2a). For example, at 10% agonist occupancy and an antagonist with  $k_{\text{off-antag}} = 100/\text{sec}$ , the agonist is able to activate ~3 times as many G-proteins as compared to an antagonist with  $k_{\text{off-antag}} = 0.1/\text{sec}$ . The highest levels of activation, comparable to those achieved in the absence of antagonist, are found when antagonist dissociates quickly. In this case, the occupancy of any individual receptor by antagonist is fleeting, freeing the receptor to be next occupied by agonist and then able to activate nearby G-proteins. At a given agonist occupancy, the amount of G-protein activation decreases with decreasing antagonist dissociation kinetics.

Interestingly, when antagonist dissociation is very slow ( $k_{\text{off-antag}}$  less than about 0.001/sec), the tightly binding antagonist effectively removes some G-proteins from the total by not allowing access of agonist to the receptors near these G-proteins. Thus the maximum extent of activation is reduced as compared to less tightly binding antagonists.

The steady state results of Fig. 2a are replotted in the more traditional format of a dose-response curve in Fig. 2b. We note that our Hill coefficients are larger than typically observed. There are two possible explanations for this. First, our simulations here do not incorporate cell-to-cell variation in parameter values. We have previously shown that such variation, particularly when threshold-like behavior is present in later steps in the activation pathway, leads to a flattening (smaller Hill coefficient) of the dose-response curve (Mahama and Linderman, 1994). In addition, large Hill coefficients in our simulations may also be due to particular assumptions about molecular movement and reaction (e.g., movement is constrained to a lattice and the entire circumference of a molecule is considered to be equally reactive). However, the qualitative trends observed here with regard to the role of antagonist dissociation kinetics are expected to hold over a range of assumptions about how to code molecular movement and collision events (data not shown).

We next tested a range of parameter values (Fig. 3a,b) to determine when the extent of G-protein activation is most influenced by antagonist dissociation kinetics, as determined by comparing activation by agonist in the presence of an antagonist that moves quickly among the receptors ( $k_{\text{off-antag}} = 100/\text{sec}$ ) with an antagonist with a longer half-life ( $k_{\text{off-antag}} = 0.1/\text{sec}$ ). Simulations with changed parameter values are compared to the base case shown in Fig. 2 and reproduced in Fig. 3 as the black curves.

We begin with two agonist parameters, the agonist dissociation rate constant  $k_{\text{off-ag}}$  and the agonist efficiency ( $\alpha$ ). When  $k_{\text{off-ag}}$  is reduced (to 0.1/sec; Fig. 3, green curves) agonist movement among receptors is reduced, a smaller number of G-proteins are activated per agonist-bound receptor, and the modulation of activation by antagonist dissociation kinetics is reduced. In other words, because agonist movement among receptors plays a smaller role in

activation, interference of that movement by a slowly dissociating antagonist has little effect. When  $\alpha$  is reduced (to  $10^5$ ; Fig. 3, purple curves) receptors will be in an active conformation only a small fraction of the time that they are bound by agonist. As compared to a higher value of  $\alpha$ , a smaller number of G-proteins are activated per agonist-bound receptor and the modulation of activation by antagonist dissociation kinetics is reduced. A low efficiency agonist binds many receptors and only activates some of them. The specific receptors that are bound by agonist and in an active conformation changes rapidly and this changing of receptors between active and inactive conformations takes the place of the direct movement of agonist among receptors. Thus the effect of antagonist dissociation kinetics ( $k_{\text{off-antag}}$ ) on G-protein activation is most likely to be observed with an agonist that has a high dissociation rate constant ( $k_{\text{off-ag}}$ ) and a high efficiency ( $\alpha$ ).

We next examined the role of diffusivity and receptor number. In general, the movement of agonist among receptors contributes significantly to G-protein activation when receptors are fairly isolated and each receptor essentially has its own “pool” of G-proteins to activate; it is under this circumstance that changes in antagonist dissociation kinetics should significantly modulate activation. As expected, when diffusion in the membrane is faster ( $D = 10^{-10}$  cm<sup>2</sup>/sec vs.  $10^{-11}$  cm<sup>2</sup>/sec) each active agonist-receptor complex has access to a large section of the membrane and activates many more G-proteins. However, receptors are now less isolated and can activate G-proteins near other receptors, reducing the effect of antagonist dissociation kinetics (Fig 3, orange curves). Similarly, increasing the receptor concentration results in higher G-protein activation but, because receptors can more easily reach G-proteins near another receptor, less of an effect of antagonist dissociation kinetics (Fig. 3, red curves). Thus the effect of antagonist dissociation kinetics ( $k_{\text{off-antag}}$ ) on G-protein activation is most likely to be observed when diffusion is slow and receptor number is low.

### Antagonist dissociation rate constant can modulate the relative rates of G-protein activation and receptor phosphorylation

We now investigate signaling and desensitization at an early physiological time point. We calculate the initial rates of G-protein activation and receptor phosphorylation as well as their ratio, GARP (G-protein activation/receptor phosphorylation), a measure we previously introduced and explored as it relates to *agonist* dissociation kinetics (Woolf and Linderman, 2003). A change in GARP indicates that activation and phosphorylation can be partially decoupled. For the parameter values of Fig. 2, antagonist dissociation kinetics have little effect on these rates or their ratio (Fig. 4a,b). However, conditions exist for which GARP is significantly influenced by antagonist dissociation kinetics (Fig. 4c,d). This new set of parameters has two key differences from previous conditions. First, the agonist dissociation rate constant  $k_{\text{off-ag}}$  is increased to 1000/sec to allow improved access of antagonist to receptors previously occupied by agonist. Second, the antagonist occupancy is high (85%) and agonist occupancy is low (2.5%) to increase the chances that a receptor previously occupied by an agonist will next be occupied by an antagonist. In this new parameter regime, antagonist dissociation kinetics have no noticeable effect on G-protein activation over the range  $k_{\text{off-antag}} = 1-300/\text{sec}$  (Fig. 4c); agonist-bound receptors have sufficient access to G proteins throughout the range. Receptor phosphorylation, however, is a minimum at an intermediate value of  $k_{\text{off-antag}} \sim 100/\text{sec}$ . The explanation of this effect involves the timing of several events (and thus depends on several rates) and is as follows. A receptor occupied by agonist will activate a nearby G protein that in turn will recruit a receptor kinase. If agonist dissociates from the receptor and then antagonist binds before the receptor kinase phosphorylates the receptor, then the antagonist-bound receptor cannot be phosphorylated; phosphorylation is reduced. This effect is most pronounced at  $k_{\text{off-antag}} \sim 100/\text{sec}$ . This reduction in receptor phosphorylation produces a maximum in the GARP ratio (Fig. 4d), demonstrating a partial uncoupling of



activation and desensitization as the antagonist dissociation rate constant increases from  $\sim 1$  to  $\sim 100$ .

We further explore the sensitivity of the GARP ratio to the antagonist dissociation rate constant for a range of conditions. In Fig. 5 we demonstrate the effects of four parameters that modulate the GARP ratio over the range of  $k_{\text{off-antag}} = 1\text{--}1000/\text{sec}$  and compare them to the result in Fig. 4d (reproduced as the top curve in Figs. 5a and b). When  $k_{\text{off-ag}}$  is reduced, agonist moves less quickly from one receptor to another, reducing the access of antagonist to receptors once bound by agonist and thus limiting the effect that antagonist dissociation kinetics can have on phosphorylation (Fig. 5a). Similarly, when the agonist efficiency  $\alpha$  is reduced, receptors are in an active conformation only a small fraction of the time that they are bound by agonist and this toggling between active and inactive conformations plays a greater role than movement of agonist and antagonist among receptors in determining the GARP ratio (Fig. 5a). Thus the effect of  $k_{\text{off-antag}}$  on the GARP ratio is most likely to be observed with an agonist that has a high  $k_{\text{off-ag}}$  and efficacy.

Receptor, G-protein and receptor kinase diffusion in the membrane also play a role in determining the GARP ratio, much as seen in Fig. 3 for activation alone. When diffusion is slow each molecule is effectively more isolated and  $k_{\text{off-antag}}$  has a larger effect on the GARP ratio than when diffusion is fast (Fig. 5b). Similarly, decreasing the receptor concentration increases the distance between receptors and increases the effect of  $k_{\text{off-antag}}$  on the GARP ratio. In other words, the effect of  $k_{\text{off-antag}}$  on the GARP ratio is most likely to be observed when diffusivity and receptor number are small.

## Discussion

In this work, we examine the effect of competition between agonist and antagonist, and in particular antagonist dissociation kinetics, on G-protein activation and receptor phosphorylation over a range of parameters. We find two effects of antagonist. The first and of course well-known effect is that antagonist reduces agonist occupancy by blocking agonist binding. Normalizing the results for agonist occupancy removes this effect from the analysis. The second, unexpected effect is that the antagonist dissociation rate constant  $k_{\text{off-antag}}$  can modulate the amounts of G-protein activation and receptor phosphorylation by blocking access of the agonist to particular receptors. As shown in Fig. 2a, *for the same agonist and antagonist occupancy levels*, antagonists that dissociate quickly allow more G-protein activation than antagonists that dissociate more slowly. The effect of antagonist dissociation kinetics on the ratio of the initial rates of G-protein activation to receptor phosphorylation is more nuanced: both increases and decreases in the ratio are seen as  $k_{\text{off-antag}}$  is increased (Figs. 4,5).

Our simulations further explore the range of conditions that would be necessary to observe an effect of  $k_{\text{off-antag}}$  in GPCR systems. For example, the antagonist dissociation rate constant must be small, as an antagonist with a larger dissociation rate constant (e.g.  $k_{\text{off-antag}} = 100/\text{sec}$ ) could allow the agonist to activate the same number of G-proteins at constant agonist occupancy as if the antagonist was not present at all (Fig 2). In addition, the agonist must have a fast dissociation rate constant and be a highly efficient agonist (Figs. 3 & 5). The diffusion of receptors and G-proteins in the membrane must be slow and the numbers of receptors and G-proteins must be relatively small (Figs. 3 & 5).

Because we found that the modulation of G-protein activation and receptor phosphorylation by  $k_{\text{off-antag}}$  is significant only within specific parameter ranges (Figs 3 & 5), there may be limited physiological systems in which our predictions can be thoroughly tested. The values of  $k_{\text{off-ag}}$  necessary are near the highest observed. Similarly, the value of the diffusivity necessary is at the lower end of the normal range, although some cell systems may have even

slower diffusion of receptors and G-proteins because of lipid rafts or the actin cytoskeleton constraining the diffusion of membrane proteins (Labrador *et al.* 2003;Ritchie *et al.*, 2005) and this would enhance the likelihood of observing an effect of  $k_{\text{off-antag}}$ . For most GPCRs it is likely an agonist with high efficiency ( $\alpha$ ) exists. In addition, the necessary receptor and G-protein numbers both are at the low end of the range of known concentrations. This means that activation in cells with overexpressed or upregulated receptors will likely be insensitive to varying  $k_{\text{off-antag}}$ .

Most interestingly, our simulations offer new hypotheses regarding the role of antagonist kinetics in influencing G protein activation and receptor phosphorylation. Recall that a slowly dissociating antagonist not only blocks receptors from being bound by agonist but also in effect (because diffusion is slow) blocks access to the G-proteins near those antagonist-bound receptors. The effect can limit the maximal amount of G-protein activation that an agonist can elicit (Fig. 2), suggesting a new mechanism to explain the phenomenon termed insurmountable antagonism. This mechanism could be experimentally tested and compared with other proposed mechanisms; for example, a two-state receptor model and a slowly binding antagonist model, both formulated as ordinary differential equation models, have been described (Lew and Ziozas 2004;Lew *et al.* 2001). As a second example, a correlation between antipsychotic side effects and antagonist dissociation kinetics has been observed and could be in part related to the role of antagonist kinetics in activation and desensitization demonstrated here (Kapur and Seeman, 2000,2001).

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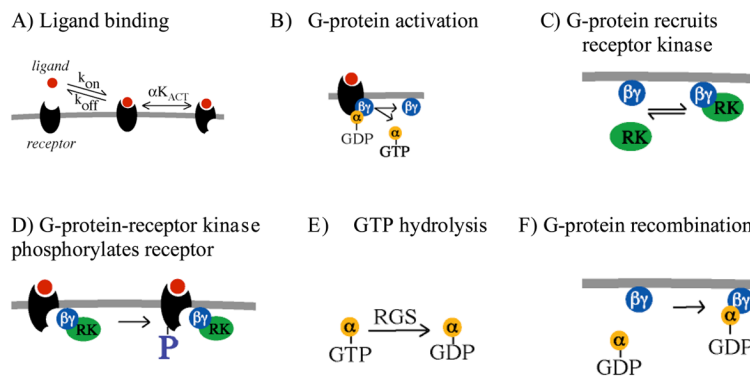
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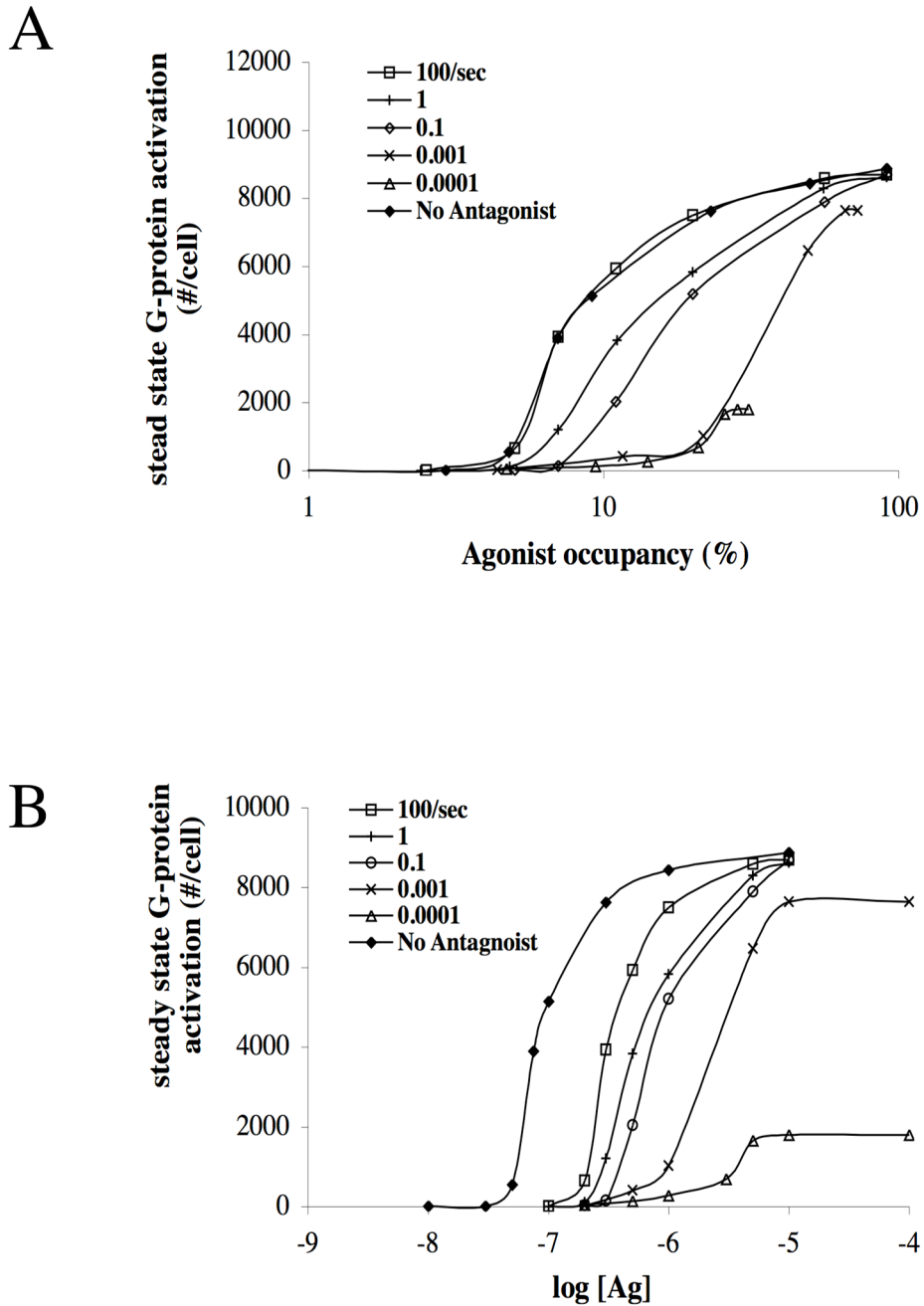
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**Figure 1. Six reactions in G-protein activation and receptor phosphorylation**

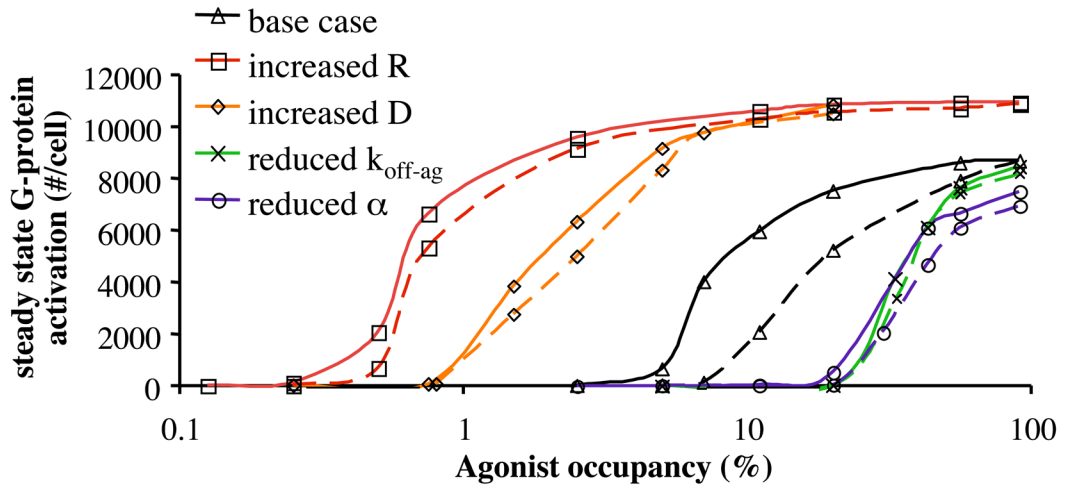
A) Signaling is initiated when ligand binds to receptor. The ligand-receptor complex establishes a rapid equilibrium between inactive and active states as determined by agonist efficiency  $\alpha$  (effectiveness of an agonist in causing the receptor to adopt an active conformation) and the receptor activation equilibrium constant  $K_{ACT}$  (discussed in (Kinzer-Ursem *et al.*, 2006; Woolf and Linderman, 2003)). B) Signaling occurs when an active ligand-receptor complex diffuses to and activates the G-protein heterotrimer, allowing the  $\alpha$  subunit to release GDP, bind GTP, and dissociate from the  $\beta\gamma$  subunit. C) The free G-protein  $\beta\gamma$  subunit recruits receptor kinase (RK) to the membrane. D) The  $\beta\gamma$ -kinase complex can phosphorylate a receptor, targeting the receptor for desensitization. E) The  $\alpha$  subunit is inactivated when GTP is hydrolyzed to GDP, a reaction that can be catalyzed by RGS proteins. F) The inactive  $\alpha$  subunit recombines with the  $\beta\gamma$  subunit to reform a heterotrimeric G-protein.



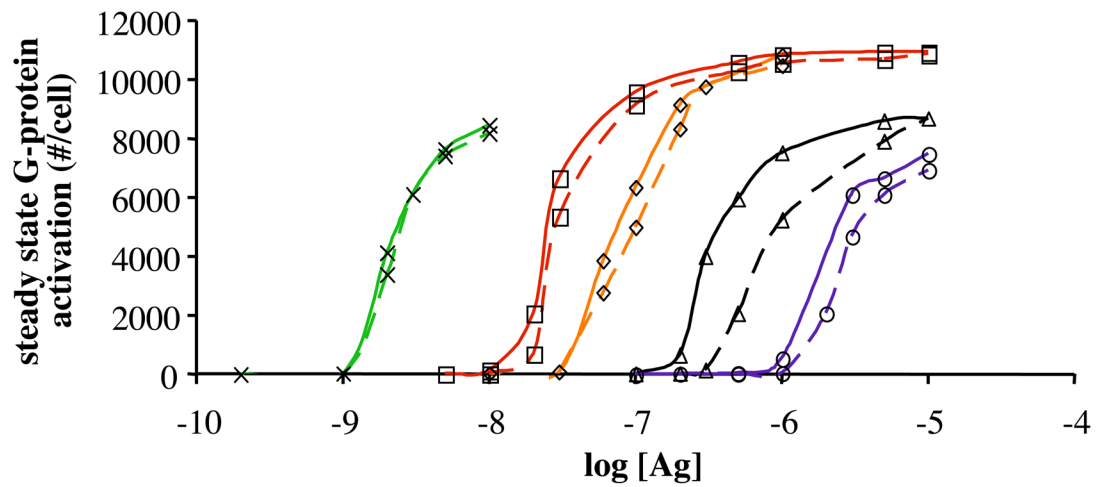
**Figure 2. Varying the antagonist dissociation rate constant  $k_{\text{off-antag}}$  can modulate the amount of G-protein activation**

(a) Activation as a function of agonist occupancy. Values of  $k_{\text{off-antag}}$  are listed. The extent of G protein activation is dependent on the value of  $k_{\text{off-antag}}$  when  $k_{\text{off-antag}}$  is less than 0.001/sec. Parameters:  $k_{\text{on-ag}} = 10^8/\text{M-sec}$ ,  $k_{\text{off-ag}} = 100/\text{sec}$ ,  $k_{\text{on-antag}} = 10^8/\text{M-sec}$ ,  $k_{\text{off-antag}} = 0.0001\text{--}100/\text{sec}$ .  $[\text{Antag}]/K_{\text{D-antag}} = 3$ ,  $D = 10\text{--}11 \text{ cm}^2/\text{sec}$ ,  $[\text{R}] = 2 \times 10^3/\text{cell}$ ,  $[\text{G}] = 10^4/\text{cell}$  and  $\alpha = 10^8$ . (b) Simulation results from (a) replotted to show activation as a function of agonist concentration.

A

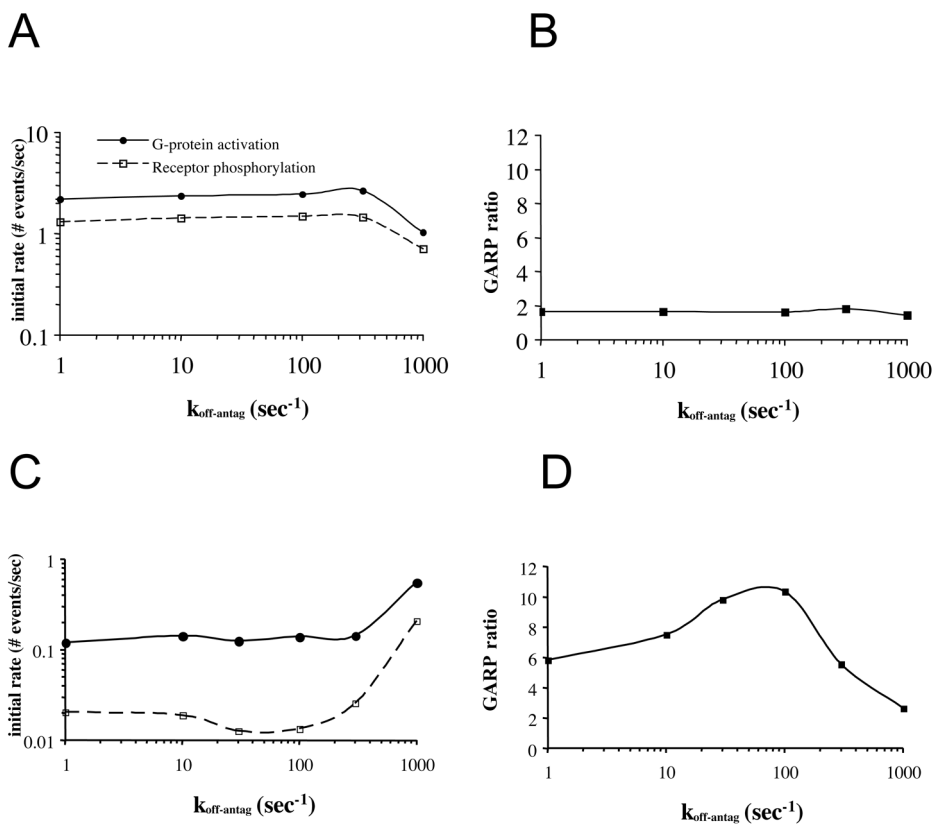


B



**Figure 3. Steady state G-protein activation as a function of agonist occupancy also depends on the receptor number (R), diffusivity (D), agonist dissociation rate constant ( $k_{\text{off-ag}}$ ) and agonist efficiency ( $\alpha$ )**

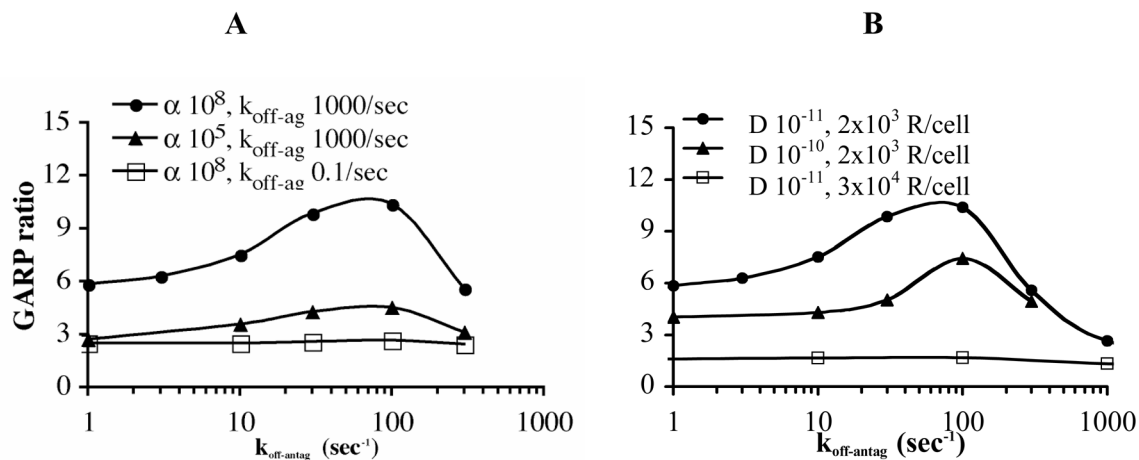
(a) By comparing cases when the antagonist dissociation rate constant  $k_{\text{off-antag}}$  is large (100/sec; solid lines) with  $k_{\text{off-antag}}$  small (0.1/sec; dotted lines) we show the effect of antagonist dissociation kinetics ( $k_{\text{off-antag}}$ ) on activation are less significant when receptor number (R) is large (red curve), diffusivity (D) is large (orange curve),  $k_{\text{off-ag}}$  is small (green curve) or agonist efficiency ( $\alpha$ ) is small (purple curve). Parameters for the base case are as in Fig. 2. The comparison cases have their respective parameters changed to  $[R] = 2 \times 10^4/\text{cell}$ ,  $D = 10^{-10} \text{ cm}^2/\text{sec}$ ,  $k_{\text{off-ag}} = 0.1/\text{sec}$  or  $\alpha = 10^5$ . (b) Simulation results from (a) replotted to show activation as a function of agonist concentration.



**Figure 4. Varying the antagonist dissociation rate constant  $k_{\text{off-antag}}$  can modulate the initial rates of G-protein activation and receptor phosphorylation and their ratio**

a) The initial rates of G-protein activation and receptor phosphorylation are plotted as a function of the antagonist dissociation rate constant  $k_{\text{off-antag}}$ . Parameter values are as in Fig. 2 and 20% of receptors are occupied by agonist. b) The ratio of the initial rate of G-protein activation to the initial rate of receptor phosphorylation (GARP) is plotted from the simulation results of (a); there is no statistically significant variation in GARP with antagonist dissociation kinetics. c,d) A different parameter set than in a) gives a more pronounced effect of antagonist dissociation kinetics. Parameters:  $k_{\text{on-ag}} = 10^8/\text{Msec}$ ,  $k_{\text{off-ag}} = 1000/\text{sec}$ ,  $[\text{Ag}] = 2 \mu\text{M}$ ,  $[\text{Ag}]/K_{\text{D-ag}} = 0.2$ ,  $k_{\text{on-antag}} = 10^8$ ,  $k_{\text{off-antag}} = 1-1000/\text{sec}$ ,  $[\text{Antag}]/K_{\text{D-antag}} = 7$ ,  $D = 10^{-11} \text{ cm}^2/\text{sec}$ ,  $[\text{R}] = 2 \times 10^3/\text{cell}$ ,  $[\text{G}] = 10^4/\text{cell}$  and  $\alpha = 10^8$ .





**Figure 5. GARP ratio also depends on the parameters  $\alpha$ ,  $k_{\text{off-ag}}$ ,  $D$ , and receptor number**

a) Larger values of the agonist efficiency  $\alpha$  and the agonist dissociation rate constant  $k_{\text{off-ag}}$  are needed to see an effect of antagonist dissociation kinetics on the GARP ratio. For reference, the filled circles ( $\alpha = 10^8, k_{\text{off-ag}} = 1000/\text{sec}$ ) are the same simulations as in Fig. 4d. b) Smaller values of the diffusivity  $D$  and receptor number  $R$  are needed to see an effect of antagonist dissociation kinetics on the GARP ratio. For reference, the filled circles ( $D = 10^{-11}, 2 \times 10^3 \text{ R/cell}$ ) are the same as in Fig. 4d. Other parameters:  $k_{\text{on-ag}} = 10^8/\text{Msec}$ ,  $k_{\text{off-ag}} = 1000/\text{sec}$ ,  $[\text{Ag}] = 2 \mu\text{M}$ ,  $[\text{Ag}]/K_{\text{D-ag}} = 0.2$ ,  $k_{\text{on-antag}} = 10^8$ ,  $k_{\text{off-antag}} = 1-1000/\text{sec}$ ,  $[\text{Antag}]/K_{\text{D-antag}} = 7$ ,  $D = 10^{-11} \text{ cm}^2/\text{sec}$ ,  $[\text{R}] = 2 \times 10^3/\text{cell}$ ,  $[\text{G}] = 10^4/\text{cell}$  and  $\alpha = 10^8$ .

**Table 1**  
Parameter values for G-protein activation and receptor phosphorylation

Rate Constant	Definition	Value	References
$k_{\text{on-ag}} (M^{-1}s^{-1})$	agonist association rate constant	$10^{8a}$	<i>a</i>
$k_{\text{off-ag}} (s^{-1})$	agonist dissociation rate constant	$1 \checkmark 2500^{b,c}$	<i>c</i>
$k_{\text{on-antag}} (M^{-1}s^{-1})$	antagonist association rate constant	$10^{8a}$	<i>a</i>
$k_{\text{off-antag}} (s^{-1})$	antagonist dissociation rate constant	$0.0001 \checkmark 1000^{b,c}$	<i>c</i>
$k_{\text{hyd}} (s^{-1})$	GTP hydrolysis rate constant	$1^d$	<i>d</i>
$\alpha$	Agonist efficiency	$10^5, 10^8^d$	<i>d</i>
$k_{\text{on-RK}} (M^{-1}s^{-1})$	receptor kinase association rate constant	$1000^e$	<i>e</i>
$k_{\text{off-RK}} (s^{-1})$	receptor kinase dissociation rate constant	$1^d$	<i>d</i>
$k_{\text{G-recombine}} (s^{-1})$	G-protein recombination rate constant	$1000^f$	<i>f</i>
$D (cm^2/s)$	Diffusion coefficient	$10^{-11} \checkmark 10^{-9}^g$	<i>g</i>
<b>Concentrations</b>			
[L] (M)	ligand concentration	Varied	
[R] (#/cell)	Receptor concentration	$2 \times 10^3 - 2 \times 10^4^h$	<i>h</i>
[G] (#/cell)	G-protein concentration	$10^4^h$	<i>h</i>

<sup>a</sup>Varying the value of  $k_{\text{on-ag}}$  and  $k_{\text{on-antag}}$  between  $10^6$  and  $10^{10} M^{-1}s^{-1}$  has no noticeable effect on the results. The association rate constant in part determines the time course of receptor-ligand binding reaching equilibrium and under these conditions receptor-ligand equilibrium is reached sufficiently fast to have no noticeable effect on G-protein activation or receptor phosphorylation.

<sup>b</sup>the physiologic range of  $k_{\text{off-ag}}$  and  $k_{\text{off-antag}}$  is  $0.0001$  to  $100 s^{-1}$ . We use some values of  $k_{\text{off-ag}}$  and  $k_{\text{off-antag}}$  larger than  $100/\text{sec}$ , but expect that these values would not occur in experimental systems.

<sup>c</sup>Contreras *et al.*, 1986; Neubig *et al.*, 1988; Waelbroeck *et al.*, 1991; Kapur and Seeman, 2000

<sup>d</sup>Woolf and Linderman, 2003

<sup>e</sup>Zhong *et al.*, 2003

<sup>f</sup>Lee *et al.*, 1993; Sako and Kusumi, 1994

<sup>g</sup>Mahama and Linderman, 1995; Pardo *et al.* 1997

<sup>h</sup>Pardo *et al.* 1997