



Collision coupling, crosstalk, and compartmentalization in G-protein coupled receptor systems: Can a single model explain disparate results?

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ABSTRACT

The collision coupling model describes interactions between receptors and G-proteins as first requiring the molecules to find each other by diffusion. A variety of experimental data on G-protein activation have been interpreted as suggesting (or not) the compartmentalization of receptors and/or G-proteins in addition to a collision coupling mechanism. In this work, we use a mathematical model of G-protein activation via collision coupling but without compartmentalization to demonstrate that these disparate observations do not imply the existence of such compartments. In experiments with GTP analogs (commonly GTP γ S), the extent of G-protein activation is predicted to be a function of both receptor number and the rate of GTP analog hydrolysis. The sensitivity of G-protein activation to receptor number is shown to be dependent upon the assay used, with the sensitivity of phosphate production assays (GTPase) > GTP γ S-binding assays > cAMP inhibition assays. Finally, the amount of competition or crosstalk between receptor species activating the same type of G-proteins is predicted to depend on receptor and G-protein number, but in some (common) experimental regimes this dependence is expected to be minimal. Taken together, these observations suggest that the collision coupling model, without compartments of receptors and/or G-proteins, is sufficient to explain a variety of observations in literature data.

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1. Introduction

The ability of the G-protein-coupled signal transduction system to detect a stimulus depends on interactions between receptors and G-proteins. Such interactions have been described by the collision coupling model (Tolkovsky and Levitzki, 1978), reviewed in Lauffenburger and Linderman (1993), which allows for receptors and G-proteins to diffuse in the membrane and interact if the correct recognition surfaces are present when they collide. When multiple receptor types can activate the same G-proteins, the collision coupling model predicts that there is competition between the receptor types for the same G-protein pool (e.g. Graeser and Neubig, 1993). Such competition is one route to what has broadly been termed crosstalk, or the influence of activation of one receptor type on signaling through a second receptor type. In this paper, we explore the predictions of the collision coupling model for crosstalk and comment on the interpretation of experimental data that has been used previously to suggest there is compartmentalization of receptors and G-proteins.

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Several experimental techniques can be used to measure G-protein activation. Activation of G-proteins can be assayed by measuring binding of the slowly hydrolyzable (commonly referred to as “non-hydrolyzable”) GTP analogs GTP γ S (as [³⁵S]GTP γ S) or GppNHp (as [³H]GppNHp) to determine the rate of G-protein activation and the number of active G-proteins at steady state (Harrison and Traynor, 2003). GTPase experiments measure the rate at which GTP is hydrolyzed to GDP; the number of active GTP-bound G-proteins is directly proportional to the rate of phosphate production. Other methods to assess G-protein activation include measurement of downstream players, e.g. cAMP production, and a FRET-based method to detect separation of α and $\beta\gamma$ subunits (Azpiazu and Gautam, 2004).

In experiments with a single receptor type, receptor number may be decreased and the maximum level of G-protein activation (the extent of reaction) measured. If the extent of reaction is independent of the receptor concentration, this is commonly interpreted to mean that receptors are free to diffuse on the membrane, i.e. there is no compartmentalization that limits the access of receptors to the full complement of G-proteins. For example, in an early study of the β -adrenergic receptor and adenylyl cyclase (AC) activation in turkey erythrocytes, when the number of receptors was decreased the extent of cAMP production did not change (Tolkovsky and Levitzki, 1978). Fantozzi et al.

(1981) made a similar observation that the extent of cAMP inhibition did not change in NG108-15 cells when opioid receptor number was reduced. This insensitivity in cAMP inhibition to changing receptor concentration means there is a receptor reserve (Brown and Goldstein, 1986).

In other reports, however, the amount of G-protein activation is sensitive to receptor number (Costa et al., 1988; Newman-Tancredi et al., 1999; Remmers et al., 2000; Traynor et al., 2002). For example, upon reducing the number of opioid receptors heterologously expressed in digitonin-permeabilized C6 glioma cells, the degree of G-protein activation decreased (Alt et al., 2001). This has been interpreted to mean that here compartmentalization limits the access of receptors to G-proteins, suggesting that systems may behave quite differently. Indeed, submicroscopic corrals, which may be formed by actin filaments near the membrane (Ritchie et al., 2005), lipid domains or rafts (Daumas et al., 2003) or by other mechanisms (Saxton, 2005) affect diffusion in the membrane and perhaps could play a role in segregating membrane components.

To further test for compartmentalization of receptors and G-proteins and as a quantitative measure of crosstalk, investigators have used multiple agonists that bind to different receptors but activate the same class of G-proteins. If the agonist-occupied receptor species are free to diffuse in the membrane, they will compete for G-proteins, creating crosstalk. Such competition is seen when measuring GTP γ S binding in SH-SY5Y (neuroblastoma) cell membranes: activation of both endogenous μ - and δ -opioid receptors results in a maximal level of GTP γ S binding equal to activation of just μ -opioid receptors alone (Alt et al., 2002). Similar observations of GTP γ S binding are made for δ -opioid and CB1 cannabinoid receptors exogenously expressed in COS-7 cell membranes (Shapira et al., 2000). As measured by Ca²⁺ current, cannabinoid and adrenergic receptors in superior cervical ganglion neurons cells compete to activate G-proteins (Vasquez and Lewis, 1999). Further, measurements of cAMP production after activation of prostaglandin and adrenergic receptors in frog erythrocytes (Pike and Lefkowitz, 1981) and glucagon and adrenergic receptors in rat and hamster adipocytes (Murayama and Ui, 1984) also show that the two receptor types share and compete for a common pool of G-proteins. These results are in agreement with the expectation of receptors freely diffusing on the membrane in a collision coupling model.

However, in other experimental systems, receptors that activate the same class of G-proteins do not compete with each other and do not display crosstalk. In NG108-15 (neuroblastoma-glioma) cells, endogenous α -adrenergic, muscarinic and δ -opioid receptors all couple to inhibitory G-proteins but simultaneous activation of opioid and muscarinic receptors shows no crosstalk when measured at the level of agonist affinity (a proxy for G-protein activation) (Graeser and Neubig, 1993). In SK-N-SH cells (a parent cell line of SH-SY5Y cells described above), no crosstalk is observed between endogenous μ - and δ -opioid and cannabinoid receptors for GTP γ S binding (Shapira et al., 2000); the activation of both receptor types produces as much GTP γ S binding as the sum of the amount produced when each receptor type is activated alone. To explain these results it has been proposed that the different receptor types in these cell systems are to some degree segregated from each other in membrane compartments and compartmentalized with their cognate G-proteins.

We questioned whether the disparate results described above are the result of different situations, namely systems in which compartmentalization of receptors is present and systems in which it is not, or whether the results could be explained by a single model. We hypothesized that compartmentalization is not necessary to explain the sensitivity of activation to receptor number nor the absence of crosstalk in some systems. To test this

hypothesis, we used a mathematical model of the G-protein activation cycle under conditions of collision coupling but with no compartmentalization. Key model parameters include the number of cell surface receptors and G-proteins and the rate constant for hydrolysis of GTP or GTP analog. By varying these parameters, we are able to explain the range of behaviors seen experimentally without the need to invoke membrane compartments.

2. Methods

2.1. Mathematical model

Our model for the G-protein activation cycle is shown in Fig. 1 and is based on previous models of G-protein activation (Riccobene et al., 1998; Yi et al., 2003; Zhong et al., 2003). Receptors and G-proteins diffuse freely in the membrane and bind with rate constant k_+ , the value of which depends on the diffusivity of the molecules and the probability that a collision between the two is productive. Thus, this model uses a collision coupling mechanism for activation of G-proteins by bound receptors. There is no compartmentalization of molecules in the model; receptors and G-proteins are assumed to have free access to each other.

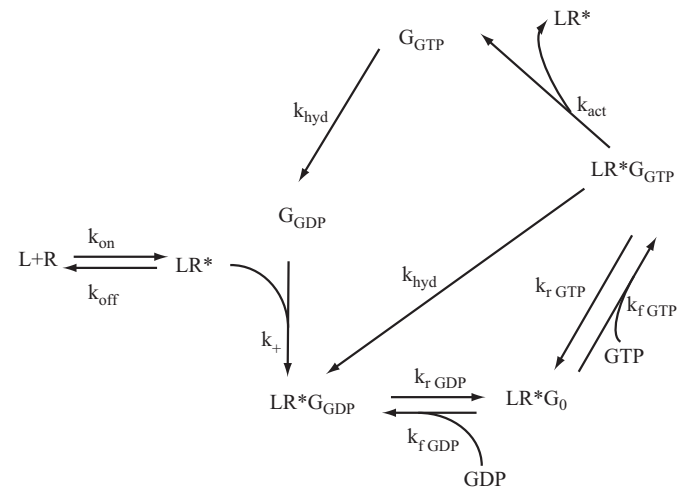


Fig. 1. G-protein activation model, based on Riccobene et al. (1998), Yi et al. (2003), and Zhong et al. (2003). Agonist (L) binds to receptor (R) to form an active agonist-receptor complex (LR^*) with rate constants k_{on} ($M^{-1}s^{-1}$) and k_{off} (s^{-1}). Because the model predictions will be compared with data on systems not known for significant constitutive activity, constitutive activity is not included in the model. The active agonist-receptor complex binds to inactive GDP-bound G-proteins (G_{GDP}) with rate constant k_+ ($M^{-1}s^{-1}$). The value of k_+ depends on the diffusivity of the agonist-receptor complex and G-protein and the probability that a collision between the two is productive. GDP dissociates from the G-protein to create an empty G-protein state (LR^*G_0) with rate constant k_{rGDP} (s^{-1}) which then forms an active GTP bound complex (LR^*G_{GTP}) with rate constant k_{fGTP} ($M^{-1}s^{-1}$). The empty G-protein has an affinity for both GTP and GDP, but a much higher affinity for GTP. However, when $[GDP]$ is very high the reaction is driven towards forming the GDP complex (this is a common experimental condition in GTP γ S experiments to create a large signal to detect over the experimental noise). The agonist-receptor-GTP-G-protein complex dissociates into α -GTP (here denoted as G_{GTP} , $\beta\gamma$ (which we do not track explicitly), and receptor with rate constant k_{act} (s^{-1}). Hydrolysis of the GTP-bound G-protein (either alone or in complex with LR^*) occurs with rate constant k_{hyd} (s^{-1}) and the GDP-bound α subunit quickly recombines with the $\beta\gamma$ subunit to reform the inactive G_{GDP} bound state. The recombination of α and $\beta\gamma$ subunits is not explicitly modeled because this reaction is not thought to be limiting (estimates of the reaction rate are $\sim 6 \times 10^{10} M^{-1}s^{-1}$ (Yi et al., 2003), much faster than the other reactions in the G-protein activation cycle).

The G-protein activation model is described by mass action kinetics with the following differential equations:

$$d[L]/dt = k_{off}[LR^*] - k_{on}[L][R] \quad (1)$$

$$d[LR^*]/dt = k_{on}[L][R] + k_{act}[LR^*G_{GTP}] - k_{off}[LR^*] - k_{+}[LR^*][G_{GDP}] \quad (2)$$

$$d[LR^*G_{GDP}]/dt = k_{+}[LR^*][G_{GDP}] + k_{hyd}[LR^*G_{GTP}] + k_{fGDP}[LR^*G_0][GDP] - k_{rGDP}[LR^*G_{GDP}] \quad (3)$$

$$d[LR^*G_0]/dt = k_{rGDP}[LR^*G_{GDP}] + k_{rGTP}[LR^*G_{GTP}] - k_{fGDP}[LR^*G_0][GDP] - k_{fGTP}[LR^*G_0][GTP] \quad (4)$$

$$d[LR^*G_{GTP}]/dt = k_{fGTP}[LR^*G_0][GTP] - (k_{rGTP} + k_{hyd} + k_{act})[LR^*G_{GTP}] \quad (5)$$

$$d[G_{GTP}]/dt = k_{act}[LR^*G_{GTP}] - k_{hyd}[G_{GTP}] \quad (6)$$

$$d[G_{GDP}]/dt = k_{hyd}[G_{GTP}] - k_{+}[G_{GDP}][LR^*] \quad (7)$$

Definitions of rate constants are given in Table 1 and species (L , R , LR^* , etc.) are as defined in Fig. 1. Versions of the model that include the effects of GTPase-activating proteins (GAPs) in a manner similar to Bornheimer et al. (2004), or that include the very slow dissociation of GTP from free G-protein to leave the “empty” G state (Traynor et al., 2002), predict trends similar to those described in Section 3 (data not shown).

Equations were solved using the NSolve function in Mathematica (Wolfram Research). For each of the three different types of assays simulated a different model output was needed. For simulation of a GTP analog assay, the relevant model output is the concentration of analog bound to G-protein, here denoted using the notation G_{GTP} for all analogs (Eq. (6)). For simulation of a GTPase experiment, the relevant model output is the concentration of free phosphate as calculated by

$$d[P]/dt = k_{hyd}([G_{GTP}] + [LR^*G_{GTP}]) \quad (8)$$

For simulation of a cAMP experiment, the relevant model output is the percent inhibition of cAMP production as calculated by

$$\% \text{ inhibition cAMP} = V_{max}[G_{GTP}]/(K_M + [G_{GTP}]) \quad (9)$$

More complex models of cAMP production have been used (Bhalla and Iyengar, 1999; Dougherty et al., 2005), but this simple

model provides a sufficient demonstration for our purposes of the role of receptor number and crosstalk in cAMP inhibition.

To approximately characterize the rate and extent of G-protein activation for some cases, the time course of G-protein activation ($G_{GTP}(t)$) was fit using least-squares regression to a one-phase association:

$$G_{GTP}(t) = G_{GTP,max}^*(1 - e^{-k_{obs}^*t}) \quad (10)$$

where the two fit parameters are $G_{GTP,max}$ and k_{obs}^* . $G_{GTP,max}$ is the extent of the reaction, or the maximal amount of G-protein that will be bound to GTP at steady state. For clarity, the notation $G_{GTP\gamma S,max}$ will be used for GTP γ S binding. k_{obs}^* is the observed rate constant for activation.

To quantify the amount of crosstalk between two receptor types R_1 and R_2 , we compute the sum of the activation produced by binding each receptor type alone (R_1+R_2) and that produced by binding both receptors simultaneously (R_{1+2}) and then calculate

$$\% \text{ competitive} = \left(\frac{(R_1 + R_2) - R_{1+2}}{(R_1 + R_2) - \max(R_1, R_2)} \right) \times 100\% \quad (11)$$

The maximum amount of competition (100%) occurs when adding a second receptor type produces no additional G-protein activation. When the receptors do not compete, the amount of activation produced by binding both receptors is equal to the sum of that produced by each receptor alone and the % competition is 0%.

2.2. Parameter values

Parameter values used in the simulations are given in Table 1 and are similar to those reported by Zhong et al. (2003) for α_{2a} -adrenergic receptors in CHO cells. The rate constant for coupling between receptors and G-proteins, k_{+} , depends on the concentrations of G-protein and receptor and the diffusion coefficient, D , and was estimated by

$$k_{+} = \frac{2\pi D}{\ln(b/s)} \quad \text{where } b = \sqrt{\frac{SA}{\pi[G_{GDP}(t)]}} \quad (12)$$

where s is the interaction radius of a receptor and G-protein and SA is the surface area of the cell membrane (Lauffenburger and Linderman, 1993); we used $D = 10^{-10} \text{ cm}^2 \text{ s}^{-1}$, $s = 10 \text{ nm}$, $SA = 1400 \mu\text{m}^2$. The concentration of the GDP-bound heterotrimeric G-protein

Table 1
Parameter values for the G-protein activation cycle

Parameter	Definition	Value	Reference
Rate constants			
k_{on} ($\text{M}^{-1} \text{s}^{-1}$)	Ligand binding	10^6	Kenakin (1993), Lauffenburger and Linderman (1993)
k_{off} (s^{-1})	Ligand dissociation	1	Kenakin (1993), Lauffenburger and Linderman (1993)
k_{+} ($\text{M}^{-1} \text{s}^{-1}$)	LR coupling to G	Eq. (12)	
k_{fGDP} ($\text{M}^{-1} \text{s}^{-1}$)	GDP association	3×10^6	Calculated from measured values of k_r , K_D , Breivogel et al. (1998)
k_{rGDP} (s^{-1})	GDP dissociation	3	Mukhopadhyay and Ross (1999), Thomsen and Neubig (1989), and Zhong et al. (2003)
k_{fGTP} ($\text{M}^{-1} \text{s}^{-1}$)	GTP association	10^7	Mukhopadhyay and Ross (1999), Thomsen and Neubig (1989), and Zhong et al. (2003)
k_{rGTP} (s^{-1})	GTP dissociation	0.005	Traynor et al. (2002)
k_{act} (s^{-1})	G-protein release	1	Sarvazyan et al. (2002)
k_{hyd} (s^{-1})	GTP hydrolysis with RGS	30	Mukhopadhyay and Ross (1999), Zhong et al. (2003)
	GTP hydrolysis without RGS	0.02	Mukhopadhyay and Ross (1999), Zhong et al. (2003)
	GppNHp hydrolysis	0.0012	Ott and Costa (1989)
	GTP γ S hydrolysis	0.0006	Ott and Costa (1989)
Concentrations			
$[R]$ (M)	Receptor concentration	Varies	
$[G]$ (M)	G-protein concentration	Varies	

Reported values of receptor and G-protein concentrations in mol mg^{-1} were converted to approximate $\# \text{ cell}^{-1}$ (R_{TOT} , G_{TOT}) or mol vol^{-1} of solution (used in equations) using the values of 2×10^9 cells/250 mg total protein and 4×10^5 cells ml^{-1} (Holmes and Pollenz, 1997; Traynor et al., 2002).

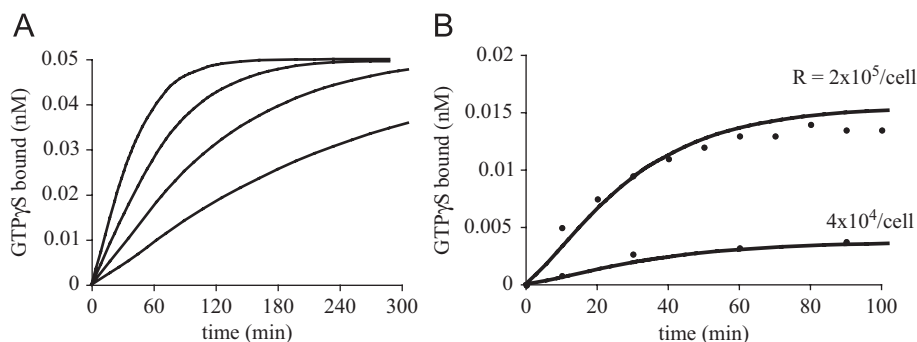


Fig. 2. GTP γ S binding for different numbers of receptors: (A) Model predictions with no GTP γ S hydrolysis ($k_{hyd} = 0$) and total receptor number R_{TOT} varied from 2×10^4 – 4×10^5 cell $^{-1}$. For all receptor numbers, the maximum degree of G-protein analog binding occurs at $G_{GTP\gamma S, max} = 0.05$ nM. The observed rate constant k_{obs} depends on the receptor concentration and ranges from 0.003 to 0.055 min $^{-1}$. These values are based on the physiological parameter values reported in Table 1; we note that for other parameter choices the level of the plateau is determined not only by the amount of GTP γ S present but is also by the rate constants for GTP γ S binding and dissociation. (B) Model simulations with slow GTP hydrolysis ($k_{hyd} = 0.0006$ s $^{-1}$) are shown as smooth curves. For total receptor number $R = 4 \times 10^4$ cell $^{-1}$, $G_{GTP\gamma S, max} = 0.004$ nM and $k_{obs} = 0.020$ min $^{-1}$. For total receptor number $R = 2 \times 10^5$ cell $^{-1}$, $G_{GTP\gamma S, max} = 0.017$ nM and $k_{obs} = 0.028$ min $^{-1}$. Experimental data of Alt et al. (2001) using digitonin-permeabilized C6 rat glioma cells transfected with μ -opioid receptors are shown as filled circles for comparison (conversion of % bound to nM with values reported in Traynor et al., 2002). For both panels, remaining parameter values are as listed in Table 1 with $G_{TOT} = 7 \times 10^5$ cell $^{-1}$.

(G_{GDP}) is a function of time and the model recalculates the value of b at each time step based on the concentration of G_{GDP} at that time. Improved estimates of k_+ can be obtained from Monte Carlo simulations of receptor and G-protein diffusion and collision in the membrane (Shea et al., 1997; Shea and Linderman, 1998), but those do not affect the conclusions drawn here.

The value of the rate constant for GTP hydrolysis, k_{hyd} , was taken from Zhong et al. (2003) and Mukhopadhyay and Ross (1999). The GTP analogs GTP γ S and GppNHp are commonly termed non-hydrolysable because of their long half-life, but they are in fact slowly hydrolyzed. Ott and Costa (1989) measured GTP analog half-life ($t_{1/2}$) for GTP γ S and GppNHp. Assuming a first-order process, these lifetimes can be converted to hydrolysis rate constants according to

$$k_{hyd} = \frac{\ln 2}{t_{1/2}} \quad (13)$$

Numbers of receptors and G-proteins per cell may vary widely between cell lines and between transfected and endogenous systems. Values used here are estimated from available data and are useful in demonstrating trends and qualitative agreement of model and data; more measurements of these key parameters are needed for quantitative testing.

3. Results and discussion

With our model of G-protein activation, we simulate three types of assays that measure G-protein activation: GTP γ S binding, phosphate production from GTP (GTPase), and inhibition of cAMP.

3.1. GTP analog hydrolysis has a major impact on the extent of reaction

We consider first experiments in which the GTP analog GTP γ S is used to monitor G-protein activation. We use our model describing free access of receptor and G-protein (Fig. 1) to predict the amount of GTP γ S bound when the GTP hydrolysis rate constant (k_{hyd}) is zero, the common assumption when using the “non-hydrolysable” GTP analog GTP γ S. In this case, the model predicts that changes in receptor number change the observed rate constant for the reaction but not the maximal amount of binding ($G_{GTP\gamma S, max}$) (Fig. 2A). For the parameter values used (Table 1, $k_{hyd} = 0$, total concentration of GTP γ S = 0.05 nM), $G_{GTP\gamma S, max}$ is 0.05 nM for all receptor concentrations but k_{obs}

increases from 0.003 min $^{-1}$ at the lowest receptor concentration ($R_{TOT} = 2 \times 10^4$ cell $^{-1}$) to 0.027 min $^{-1}$ at the highest receptor concentration ($R_{TOT} = 4 \times 10^5$ cell $^{-1}$). The observed overall rate constant k_{obs} increases with receptor number because it is the product of the intrinsic rate constant and the concentrations of agonist-bound receptors and G-proteins. For the parameter values used here, G-protein activation reaches a steady state when the limiting reagent GTP γ S is consumed; a higher concentration of GTP γ S will label more G-proteins in both the model and in experiments (Remmers et al., 2000). These model predictions conflict with the observation in many systems that the extent of reaction decreases with decreasing receptor number (Graeser and Neubig, 1993; Remmers et al., 2000; Alt et al., 2001; Traynor et al., 2002), suggesting that the collision coupling model as formulated here is incorrect.

However, the rate constant for GTP γ S hydrolysis is not zero and in fact has a low but measurable value (Table 1). With our model, we next calculate the effect of using the slow measured rate constant of GTP γ S hydrolysis to observe the impact hydrolysis has on the extent of reaction, using experimental values for receptor number from Alt et al. (2001). As shown in Fig. 2B, both the maximal amount of GTP γ S bound ($G_{GTP\gamma S, max}$) and the overall observed rate constant for the reaction (k_{obs}) increase with increasing receptor number. For comparison on the same plot are the experimental data of Alt et al. (2001) showing the effect of opioid receptor number on G-protein activation.

The simulation result of Fig. 2B is different—both qualitatively and quantitatively from that of Fig. 2A because the structure of the reaction network is changed. When the hydrolysis rate constant (k_{hyd}) is set to zero, and because GTP γ S is present in low concentrations but has a high affinity for LR^*G_0 , the simulation shows that essentially all GTP γ S ultimately becomes bound to G-protein regardless of the receptor number, although the rate at which it all becomes bound does depend on receptor number. However, when the hydrolysis rate constant is non-zero, an additional pathway, the hydrolysis reaction, acts to remove bound GTP γ S from the system. The plateau in bound GTP γ S reached in Fig. 2B reflects a balance in the rates of receptor and G-protein association (which, as in the previous case, is a function of receptor number) and GTP γ S dissociation and hydrolysis (Traynor et al., 2002; Breivogel et al., 1998).¹ Thus, when GTP analog hydrolysis is included, the collision coupled model (Fig. 1) can

¹ Assuming GTP γ S present in excess.

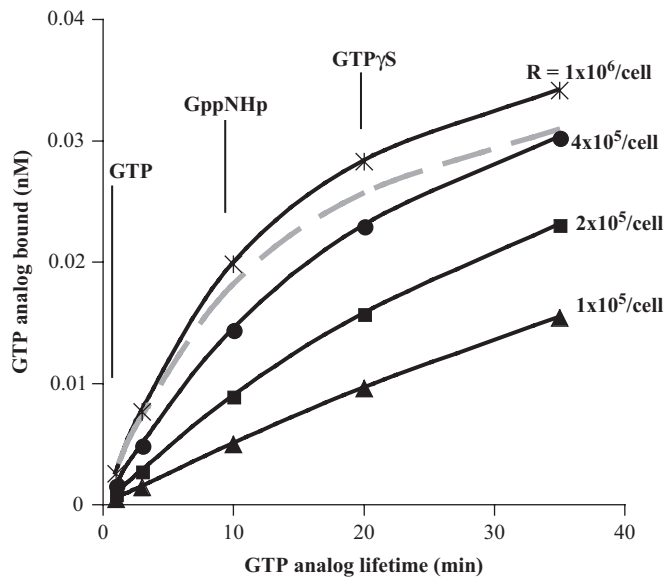


Fig. 3. Extent of GTP analog binding and the GTP analog hydrolysis rate constant. The hydrolysis rate constant k_{hyd} (value indicated for each analog by a vertical line under the analog name) is inversely related to the analog lifetime. Increasing GTP analog lifetime or receptor number increases the extent GTP analog binding. The dashed line shows 90% of the maximum response (at $R_{TOT} = 5 \times 10^5 \text{ cell}^{-1}$). $G_{TOT} = 7 \times 10^5 \text{ cell}^{-1}$ and other parameter values are as listed in Table 1.

account for the decrease in extent of G-protein activation due to decreased receptor number and it is not necessary to postulate the existence of compartments to explain the experimental data.

The maximum degree of G-protein analog binding that occurs depends on the GTP analog hydrolysis rate constant (Fig. 3). As the hydrolysis rate constant increases (or the analog lifetime decreases), the maximum degree of G-protein analog binding decreases. To test this prediction, one might use the GTP analog GppNHp, which has a hydrolysis rate constant intermediate between that of GTP γ S and GTP. For example, for the conditions used in Fig. 3 (and representative of a GTP γ S experiment) the maximum degree of GppNHp binding is predicted to be approximately two-thirds that which would be obtained using GTP γ S. The maximum degree of binding by a G-protein analog that could not be hydrolyzed would be limited by the concentration of G-protein analog, and varying receptor number would vary the observed rate constant k_{obs} but not the maximum degree of binding (recall Fig. 2A).

3.2. Sensitivity of G-protein activation to receptor number depends on the assay used

In Figs. 2 and 3, we examined the predictions of the collision coupled model when a GTP analog is used to monitor G-protein activation. We now turn to experiments that quantify G-protein activation in other ways. We model the production of phosphate from hydrolysis of GTP to GDP as a function of time and in Fig. 4A show strong agreement between simulation results (solid line) and experimental data from Clark et al. (2003). The model can also reproduce the inhibition of cAMP production by AC over a range of concentrations of the μ -opioid agonist DAMGO (Fig. 4B) seen in a variety of different systems (Carter and Medzihradsky, 1993; Liu and Prather, 2001; Gharagozlou et al., 2003; Mandyam et al., 2003; Clark and Traynor, 2005).

Thus, our model can be used in appropriate form to represent G-protein activation as assayed by GTP analogs, phosphate production, or cAMP production. We are now able to predict and compare the impact of receptor concentration on G-protein

activation in each of the three assays for a constant G-protein concentration. In Fig. 3, we show that although in general increasing receptor number leads to an increase in the maximal response ($G_{GTP\gamma S, max}$), increasing receptor number beyond $\sim 5 \times 10^5 \text{ receptors cell}^{-1}$ gives only a marginal increase in $G_{GTP\gamma S, max}$. Similarly, in Figs. 4C and D, the effect of receptor number on the rate of phosphate production and on percent inhibition of cAMP production are shown. In these assays, too, increasing receptor number increases the response but once a high enough receptor number is reached the effect saturates. For assays of the rate of phosphate production, 90% of the maximum (dashed line) occurs $\sim 6 \times 10^6 \text{ receptors cell}^{-1}$; for assays of inhibition of cAMP production 90% of the maximum (dashed line) occurs $\sim 1 \times 10^5 \text{ receptors cell}^{-1}$.

These results suggest that the degree of dependence of G-protein activation on receptor number depends on the assay used; for example, a greater dependence might be observed in GTP analog assays than in cAMP assays. Indeed this is the case. Data showing a sensitivity of G-protein activation to receptor number have been reported from many GTP analog assays, as described above (Remmers et al., 2000; Alt et al., 2001; Traynor et al., 2002). Yet data showing no dependence of G-protein activation on receptor number have also been reported from cAMP assays (Tolkovsky and Levitzki, 1978; Fantozzi et al., 1981). Because the dependence on receptor number saturates at different receptor numbers for the different assays, these reports are all consistent with the collision coupled model (i.e. with no compartmentalization). In other words, spare receptors or a receptor reserve might be observed in a particular system (with a particular receptor number) for cAMP production but not GTP γ S binding.

3.3. Receptor and G-protein concentrations influence receptor crosstalk

An implication of the collision coupled model is that receptor types which activate the same class of G-proteins will compete for those G-proteins, giving rise to crosstalk. On the other hand, if there are compartments such that not all G-proteins are accessible to a particular receptor type, then there will be reduced (or no) competition or crosstalk. With our model, we simulate GTP γ S binding or phosphate production from GTP and compare levels of activation produced by agonists binding to two receptor types with activation produced by each of those agonists alone. If there is no competition for G-proteins, activation for the pair experiment will be the same as the sum of the activation caused by the individual receptors alone. If there is competition, activation found for the pair experiment will be less than the sum. As shown in Fig. 5A and B, both behaviors are seen in experimental data in various systems (Shapira et al., 2000).

Simulations of GTP γ S binding via two receptors show both competitive and non-competitive behavior depending on the receptor concentrations (Fig. 5C and D). Receptor concentrations used are an approximation of measured values for opioid receptors in cells endogenously expressing opioid receptors or heterologously transfected with opioid receptors. The transfected cell has a high receptor concentration and the endogenous receptor concentration is many orders of magnitude smaller (Shapira et al., 2001). At a high receptor concentration ($2 \times 10^6 \text{ cell}^{-1}$ each of R_1 and R_2) the receptors compete for G-proteins. Both agonists together activate nearly the same number of G-proteins as one agonist alone and much less than the sum of the responses to both agonists if delivered individually. At a low receptor concentration ($2 \times 10^4 \text{ cell}^{-1}$ of each R_1 and R_2) the activation by both agonists together is nearly the sum of that

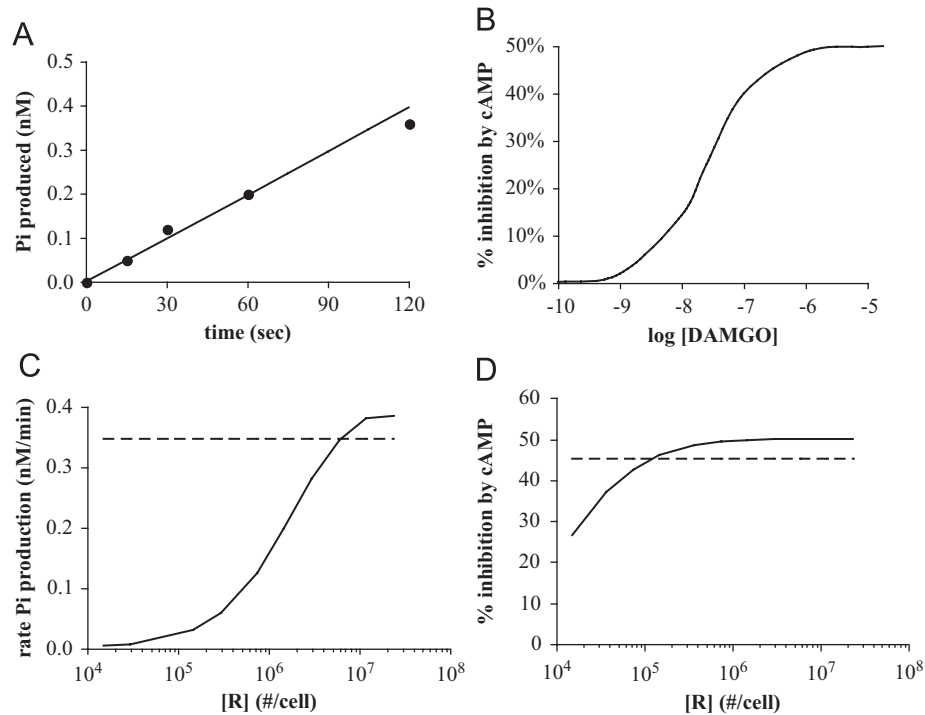


Fig. 4. Model predictions for phosphate production (GTPase assay) and inhibition of cAMP: (A) Phosphate production for $R_{TOT} = 7 \times 10^5 \text{ cell}^{-1}$. Experimental data of Clark et al. (2003) are shown as points and simulation results are shown as a smooth curve. Both data and simulations are in a system insensitive to RGS proteins ($k_{hyd} = 0.02 \text{ s}^{-1}$, see Table 1). (B) Percent inhibition of cAMP production as a function of agonist concentration. Values for the parameters V_{max} and K_M (Eq. (9)) were determined by fitting the output of Eqs. (1)–(7) and Eq. (9) to data on cAMP inhibition by μ -opioid receptors activated with the full agonist DAMGO (Gharagozlou et al., 2003). For this simulation, we used $R_{TOT} = 2 \times 10^5 \text{ receptors cell}^{-1}$, $G_{TOT} = 7 \times 10^5 \text{ G-proteins cell}^{-1}$, and other parameter values are as in Table 1. (C) Predicted rate of phosphate production for R_{TOT} ranging from 1.5×10^4 to $2.4 \times 10^7 \text{ cell}^{-1}$. For $R_{TOT} = 6 \times 10^6 \text{ cell}^{-1}$, 90% of the maximum rate is reached (dotted line). (D) Predicted inhibition of cAMP production for saturating agonist concentration (10^{-5} M) as a function of receptor number. For $R_{TOT} = 1 \times 10^5 \text{ cell}^{-1}$, 90% of the maximum inhibition is reached (dotted line). $G_{TOT} = 7 \times 10^5 \text{ cell}^{-1}$ and other parameter values are as listed in Table 1.

produced by each receptor type alone. A similar dependence on total receptor number occurs when G-protein activation is measured in a GTPase experiment (Fig. 5E and F). These results are in agreement with FRET data with tagged muscarinic receptors expressed in CHO cells that show for lower receptor concentrations multiple receptor types do not compete for G-proteins but when one receptor type is overexpressed there is competition (Azpiazu and Gautam, 2004).

Whether competition or crosstalk is seen may also depend on G-protein concentration. Graeser and Neubig (1993) report non-competitive behavior for a G-protein concentration estimated to be on the order of $7.5 \times 10^5 \text{ cell}^{-1}$ and a total receptor concentration of $7.5 \times 10^4 \text{ cell}^{-1}$ (a combination of α_2 adrenergic, muscarinic and δ -opioid receptors) in NG108-15 cells. For these conditions, our simulations predict competition is minimal (Fig. 6). Graeser and Neubig then significantly reduced the G-protein concentration but the receptors were still not competitive, leading to their suggestion of membrane compartments segregating G-proteins and receptors. Shapira et al. (2000) also observed that a significant reduction in G-protein concentration in some cases does not create competition.

In our model for a high number of G-proteins and a low number of receptors (bottom right quadrant of Fig. 6) a change in G-protein concentration has only a minimal effect on the predicted amount of competition. But, for a low number of G-proteins and a high number of receptors (top left quadrant of Fig. 6) competition between receptors is significant. In general, competition is more sensitive to receptor number than G-protein number (contours in Fig. 6 are much steeper changing R_{TOT} than G_{TOT}). To allow a comparison with the results of Graeser and Neubig (1993), we ran simulations at $R_{TOT} = 7.5 \times 10^4 \text{ cell}^{-1}$ and $G_{TOT} = 7.5 \times 10^4$ or $7.5 \times 10^5 \text{ cell}^{-1}$. The results of these two

conditions are shown in Fig. 6. At the higher G-protein number, both receptor types produce G-protein activation nearly equal to the sum of both receptor types alone (17% competitive). At the lower G-protein number, both receptor types activate almost the same relative amount of G-proteins (27% competitive). The difference between 17% and 27% competitive is likely below the detection threshold of the experimental assay. Thus, an explanation for the lack of crosstalk or competition among receptor types for the parameters tested here does not need to invoke compartmentalization of receptors.

4. Conclusions

In this work, we demonstrate using a mathematical model of collision coupling that compartmentalization of receptors and/or G-proteins within the membrane is not necessary to explain literature data on the extent of G-protein activation and receptor crosstalk. First, we show that the slow hydrolysis rate of GTP γ S, a GTP analog commonly termed “non-hydrolyzable”, plays an important role in determining the extent of G-protein activation. We predict that two different GTP analogs tested in the same experimental system will produce different extents of G-protein activation because they have different rates of hydrolysis (Fig. 3). Second, the dependence of G-protein activation on receptor number is shown to be assay dependent, with the sensitivity of phosphate production assays (GTPase assay) > (GTP γ S-binding assay) > (cAMP inhibition assay) (Figs. 3 and 4). Finally, we find that the number of receptors and G-proteins available affects receptor crosstalk in a nonlinear fashion (Fig. 6). Thus upregulation, internalization, or desensitization of receptors and G-proteins that occur on agonist occupancy of receptors will

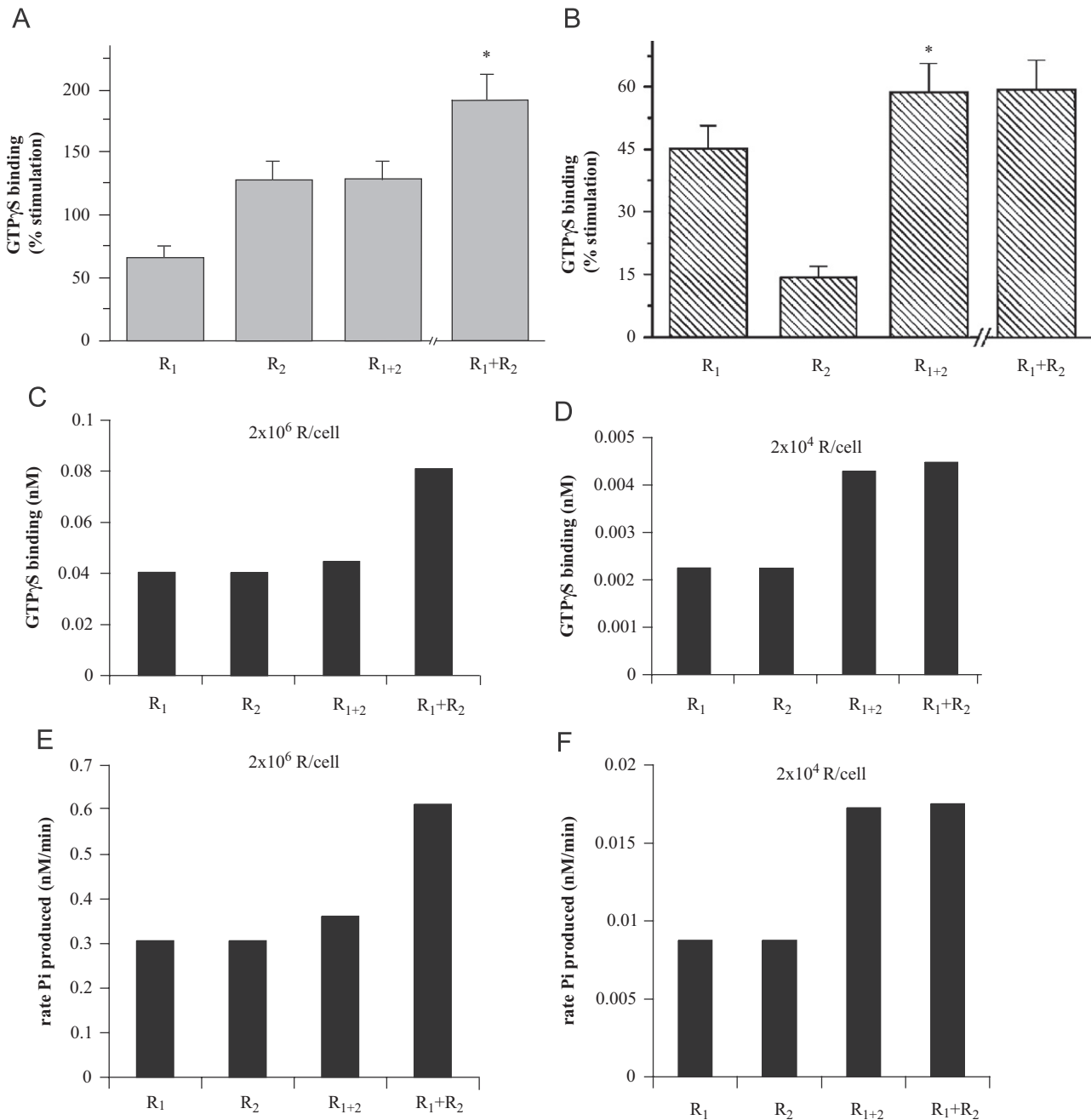


Fig. 5. Competitive and non-competitive (additive) behavior seen in multiple agonist experiments: (A) CB-1 cannabinoid and μ -opioid receptors in COS-7 cells compete for G-proteins in a GTP γ S assay (Shapira et al., 2000). (B) μ and δ -opioid receptors in N18TG2 cells do not (Shapira et al., 2000). (C) Model predictions of GTP γ S binding show receptors compete for G-proteins at high receptor numbers. Stimulation via the first receptor type ($[R_1] = 2 \times 10^6$), second receptor type ($[R_2] = 2 \times 10^6$), or both together (R_{1+2}) is shown. Also shown is the sum of the individual responses (R_1+R_2). Predicted % competition (Eq. (11)) is 90%. (D) Model predictions of GTP γ S binding show receptors do not compete for G-proteins at low receptor numbers. For stimulation via the first receptor type ($[R_1] = 2 \times 10^4$), second receptor type ($[R_2] = 2 \times 10^4$) or both together (R_{1+2}) is shown. Also shown is the sum of the individual receptors (R_1+R_2). Predicted % competition (Eq. (11)) is 9%. (E) Model predictions of phosphate production (GTPase assay) show receptors compete for G-proteins at high receptor number. For stimulation via the first receptor type ($[R_1] = 2 \times 10^6$), second receptor type ($[R_2] = 2 \times 10^6$) or both together (R_{1+2}) is shown. Also shown is the sum of the individual receptors (R_1+R_2). Predicted % competition (Eq. (11)) is 82%. (F) Model predictions of phosphate production (GTPase assay) show receptors do not compete for G-proteins at low receptor numbers. For stimulation via the first receptor type ($[R_1] = 2 \times 10^4$), second receptor type ($[R_2] = 2 \times 10^4$) or both together (R_{1+2}) is shown. Also shown is the sum of the individual receptors (R_1+R_2). Predicted % competition (Eq. (11)) is 3%. Model parameter values are as in Table 1 with $G_{TOT} = 7 \times 10^5 \text{ cell}^{-1}$.

influence the extent of cross talk *in vivo* and might be used experimentally to influence the extent of crosstalk in a particular system.

In developing models of G-protein activation, our ultimate goal is to enable quantitative hypothesis testing of signaling mechanisms. Experimental examination of signaling processes at different spatial and temporal scales, for example the motion of individual

molecules or the formation of particular signaling complexes, may require a more detailed model than that used here. Indeed, mechanisms other than variation in receptor or G-protein number, including the formation of multi-protein signaling complexes, clustering of receptors via dimerization or regulation of the numbers of other signaling proteins, may play roles in regulating crosstalk (Hall and Lefkowitz, 2002; Breitwieser, 2004; Woolf and

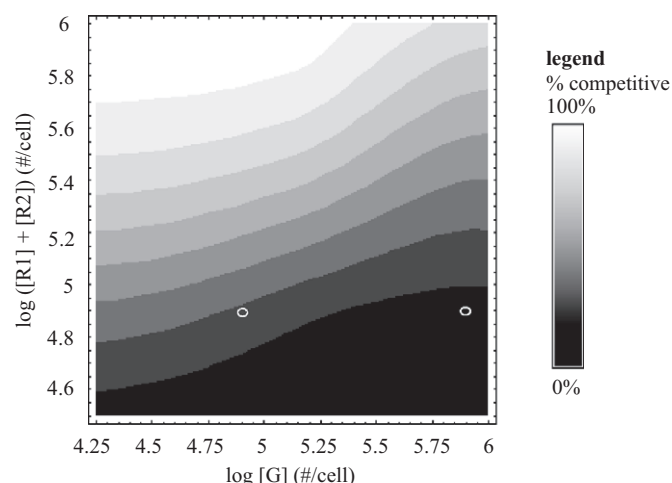


Fig. 6. Crosstalk depends in part on both receptor and G-protein concentrations: (A) Predicted % competition (Eq. (11)) for a range of total receptor ($R_1 + R_2$) and G-protein concentrations. G-protein activation is simulated via the GTP γ S assay. Predicted % competition is marked with a white circle for the conditions of Graeser and Neubig (1993) where $G_{TOT} = 7.5 \times 10^5 \text{ cell}^{-1}$, $R_{TOT} = 7.5 \times 10^4 \text{ cell}^{-1}$ and a reduced $G_{TOT} = 7.5 \times 10^4 \text{ cell}^{-1}$, $k_{hyd} = 0.0006 \text{ s}^{-1}$ and other parameter values are as listed in Table 1.

Linderman, 2004; Desai et al., 2006). While we found it was not necessary to incorporate compartments to explain the signaling data explored here, compartments may indeed exist and play a role at other levels. In recent years, there have been several reports of anomalous diffusion of proteins in the cell membrane which may be the result of membrane compartments (Daumas et al., 2003; Ritchie et al., 2005; Saxton, 2005). Membrane compartments may also have important role in initiating the formation of signaling complexes (Barnett-Norris et al., 2005). Future work may elucidate the roles that these various phenomena play in signaling.

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