Mathematical Model Investigations of Signal Transduction via G-Protein Coupled Receptors: Trafficking and Promiscuous Coupling of Receptors

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Abstract

G-protein-coupled receptors (GPCRs) are a major class of membrane protein receptors. GPCRs bind to a variety of extracellular ligands to regulate a vast diversity of physiological responses. These receptors play a critical role in signal transduction, and are among the most important pharmacological drug targets. Upon binding of extracellular ligands, these receptor molecules couple to one or several subtypes of G protein which reside at the intracellular side of the plasma membrane to trigger intracellular signaling events. The question of how GPCRs select and activate a single or multiple G protein subtype(s) has been the topic of intense investigations. In this work, we construct a mathematical model to investigate promiscuous coupling of receptors to G proteins. The model is composed of mass action ordinary differential equations, describing ligand-receptor and receptor-G protein interactions, receptors synthesis and degradation. In constructing the model, we assume that the receptors can exist in multiple conformational states allowing for a multiple effecter pathways. A genetic algorithm (GA) has been implemented to estimate the model parameters. The numerical results show some interesting effects, which are qualitatively in good agreement with some experimental results.

Keywords: G- protein-coupled receptors, Promiscuous coupling, Trafficking, Genetic algorithm

1. Introduction

G-protein-coupled receptors (GPCRs) constitute a large and diverse family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals. GPCRs are among the most heavily investigated drug targets in the pharmaceutical industry. They account for the majority of best-selling drugs and about 40% of all prescription pharmaceuticals on the market. The inactive form of G-protein is heterotrimers composed of three subunits, α, β and γ with a molecule of guanosine diphosphate (GDP) bound to the α subunit. The biding of ligand to receptors causes them to interact with the G-protein. The interaction of this inactive G-protein with bound receptor promotes the release of GDP from the α subunit and the binding of nucleotide guanosine triphosphate (GTP) at the same site. The G-protein is then released from the receptor and dissociates into separate $\beta\gamma$ and α-GTP subunits. The α-GTP is the active form of the G-protein. The activated $\beta\gamma$ and α-GTP subunits in turn stimulate the generation of second messengers via intracellular effectors, passing on the signal by altering the activities of selected cellular proteins. Depending on the type of G-protein to which the receptor is coupled, a variety of downstream signalling pathways can be activated [1, 2].

The idea that a receptor can adopt more than one active state was derived from the concept of agonist-directed trafficking of a receptor stimulation to explain the ability of structurally diverse agonists to activate different G-protein-mediated signaling [3-5]. According to this model, each agonist is able to promote its own specific active receptor state, leading to an unlimited number of receptor conformations. In contrast, Leff *et al.* (1997) suggested a three-state model where the receptor might exist in three states, an inactive (R) and two active formations (R^*, R^{**}), accounting for multiple G-protein coupling but limiting the number of active conformations [6]. Chen *et al.* [7] proposed a mathematical model in which the receptor can exist in four conformational states, one inactive and three active states, including the role of G-protein activation.

Another prominent behavior of GPCRs is internalization from the cell surface to the interior of the cell. While being natural activity of receptors linked to signaling, internalization may be therapeutically useful activity in itself. Ligands that selectively induce receptor internalization may have utility in the prevention of HIV-1 infection. This is because internalization may remove critical co-receptors for membrane fusion and subsequent HIV-1 infection [8, 9]. In fact, this approach may be superior to blocking the HIV-1 infection. The focus of our present work is to study the effect of receptors trafficking, including receptors internalization, receptors synthesis, recycling of receptors and receptors degradations by extending a mathematical model proposed in [7].

There are some quantitative pharmacological terms that are useful for our present analysis. Efficacy is defined as the ability of a drug to produce a stimulus, indicated by the maximum effect that can be produced by that drug. Potency, commonly expressed as the *EC50*, refers to the concentration

or amount of an agonist needed to produce a 50% of the maximum effect of that agonist. A Full agonist is a ligand that binds to a receptor and leads to a maximum biological response in the system under study while a partial agonist is an agonist that does not elicit as large an effect as a full agonist. An antagonist is a ligand that binds to a receptor, does not produce a biological response, and blocking the actions of agonists. An inverse agonist means a ligand that binds to a receptor and reduces the constitutive activity of the receptor, thereby producing an effect opposite to that of an agonist.

The genetic algorithm (GA) is an effective stochastic global search algorithm that is inspired by the evolutionary features of biological systems [10]. It has been successfully applied to various problems, such as function optimizations and parameter estimation in biochemical pathways [11-13]. As kinetic reaction rates in the signalling processes measure in reliable *in vivo* and *in vitro* experiments is currently limited to a mall number of known values. In this paper, we also apply the GA to estimate the parameter values in our model. With the parameter values obtained by using GA, the predictions of the model are then compared with the experimental results obtained by the authors of [5].

2. The Mathematical Model

2.1 Model Construction

We extended the model suggested in [7], in which receptors are allowed to exist in multiple conformational states, to include the receptors synthesis, degradation and trafficking. In the absence of agonists, the receptors can exist in four different conformational states, one inactive or resting R and three active R^{j*} states, where the superscript j (and subscript j below) henceforth takes the value of 1, 2 and 3 unless stated otherwise. Each receptor can bind to a different G-protein subtype G_j as shown in Fig. 1(a). The inactive receptors, R, are convert into an active state R^{j*} with rate constants L_j^+. The parameters K^+ and K^- represent ligand association and dissociation rates with receptor R, respectively. The effect of the agonist directed trafficking of receptor stimulus proposed in [3] is indicated by the value of the parameters μ_j, corresponding to the effect on the various G_j-linked pathways. The numbers of ligand-bound receptors are denoted by R_A while the activated ligand-bound receptor numbers are denoted by R_A^{j*}. The ligand dissociation rates from the ligand-bound activated receptors, R_A^{j*}, are assumed to be K^- / μ_j and the deactivation rates of R_A^{j*} to R_A are L_j^- / μ_j, both of which are ligand-dependent. With one effector pathway, an agonist or partial agonist which preferentially binds to an active receptor has $\mu_j > 1$, while an antagonist which binds equally well to both active and inactive receptor has $\mu = 1$ and an inverse agonist which is more likely to bind

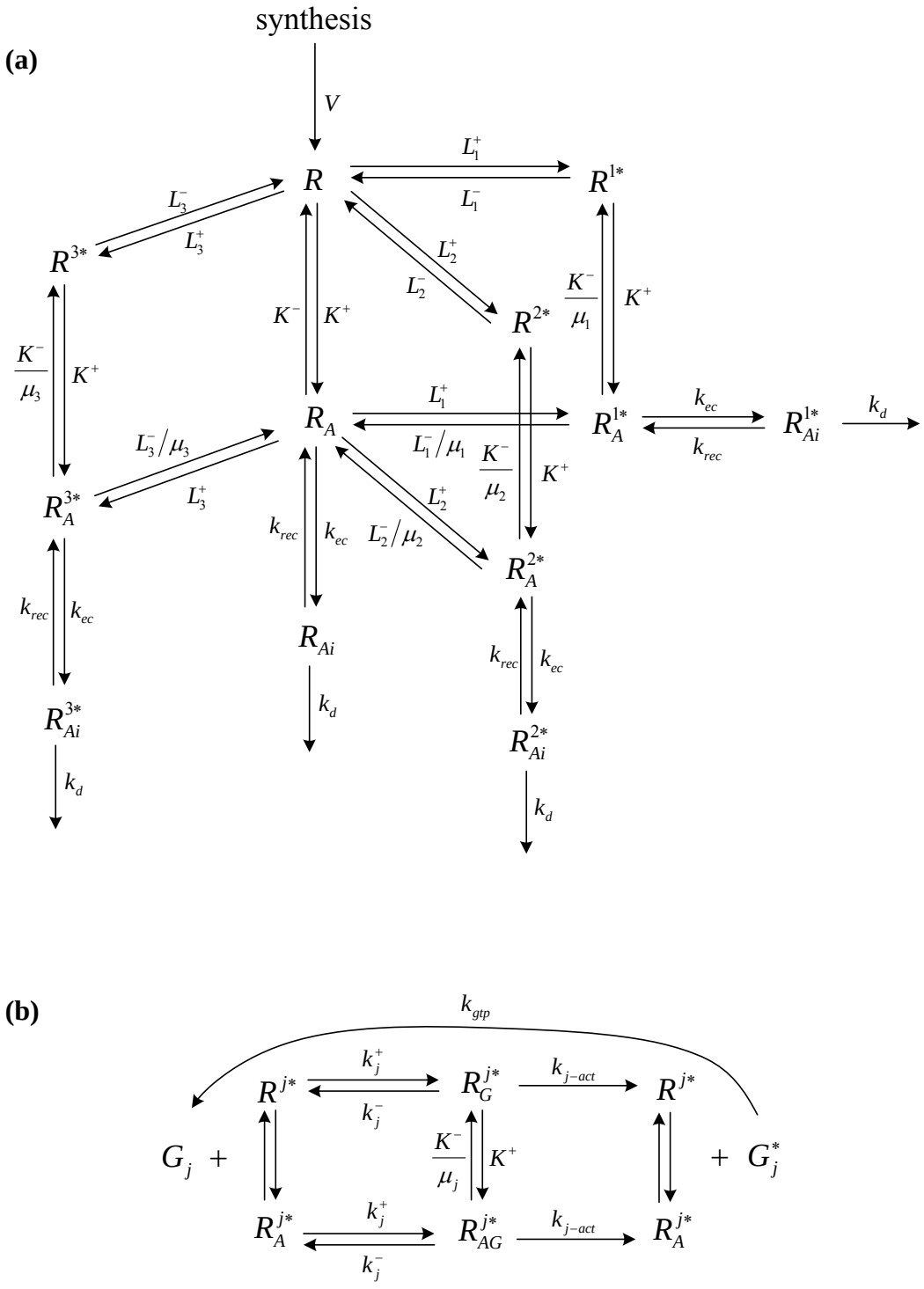

Figure 1. Extended model structure of (a) receptor-ligand binding with multiple receptor conformation. The free receptors are synthesized with rate V while the ligand-receptor complex internalize with rate k_{ec} and the internalized ligand-receptor complex degrade with rate k_d. (b) G-protein activation of the G_j-linked pathways, assuming R^{j*} and R_A^{j*} associate or dissociate with G-protein at the same rate.

to an inactive receptor has $\mu < 1$. This, however, may not always be the case for a system with multiple effector pathways. For example, an agonist with $\mu_j > 1$ for all j and, say, $\mu_1 \gg \mu_2, \mu_3$, can increase the number of active receptors in G_1-linked pathway but reduce the number of active receptors in other conformations and hence behaves as an agonist for one pathway and an inverse agonist for others [6, 7]. The rate constants K^+, K^- and μ_j are thus ligand-dependent.

In the model suggested by Chen *et. al.* [7], they assumed that the number and location of receptors on the cell surface are constant, *i e.*, that no significant synthesis, degradation, internalization, or recycling of receptors occur over the time frame for which the model applies. Under normal physiological conditions, however, these dynamic trafficking events take place concurrently with receptor-ligand biding [14-19]. The important feature of our model is the receptor synthesis, degradation and trafficking. The rate of new receptor synthesis and expression on the cell surface as free receptors is V. Rate constant describing the internalization of receptor-ligand complex is k_{ec}. k_{rec} represents the rate constant for transport of material via vesicles from the endosome back to the cell surface [20] and k_d represents a rate constant for the routing of receptor-ligand complex from the endosome to the lysosome, and degradation in the lysosome. Fig. 1(b) shows the activation of G-protein by the activated receptors. Both ligand-bound and nonligand-bound activated receptors, denoted by R_A^{j*} and R^{j*} respectively, associate with and dissociate from G-protein with rate constants k_j^+ and k_j^- for G_j. The inactive form of the G-protein consists of α, β and γ subunits with a molecule of GDP bound to the α subunit (G_α). The interaction of this inactive G-protein with an activated receptor promotes the release of GDP from the α subunit and the biding of GTP at the same site. We have assumed that the dissociation of GDP and association of GTP happen instantaneously and the activated receptor activates the G-protein with rate constant k_{j-act}. Active G-proteins are returned to their inactive state upon hydrolysis of GTP by the GTPase activity found in the α subunit itself, and the α-GDP and $\beta\gamma$ subunits ($G_{\beta\gamma}$) can then recombine. In our model, we assume that the inactivation of G-protein is so fast for our time scale, and thus we consider it to be a one-step process with rate constant k_{gtp}. Note that only the α-GTP subunits (G_α) are considered as the activated G-protein in our model.

Based on the model structure in Fig.1, the law of mass action leads to the following set of coupled ordinary differential equations.

$$\frac{dR}{dt} = K^- R_A - \sum_{n=1}^{3} L_n^+ R - K^+ AR + \sum_{n=1}^{3} L_n^- R^{n*} + V, \tag{2.1}$$

$$\frac{dR^{j*}}{dt} = L_j^+ R - \left(L_j^- + K^+ A\right) R^{j*} + \frac{K^-}{\mu_j} R_A^{j*} - k_j^+ G_j R^{j*} + \left(k_j^- + k_{j-act}\right) R_G^{j*}, \tag{2.2}$$

$$\frac{dR_A^{j*}}{dt} = K^+ A R^{j*} - \left(\frac{L_j^-}{\mu_j} + \frac{K^-}{\mu_j}\right) R_A^{j*} + L_j^+ R_A - k_j^+ G_j R_A^{j*} + \left(k_j^- + k_{j-act}\right) R_{AG}^{j*} - k_{ec} R_A^{j*} + k_{rec} R_{Ai}^{j*}, \tag{2.3}$$

$$\frac{dR_{AG}^{j*}}{dt} = k_j^+ G_j R_A^{j*} - \left(k_j^- + k_{j-act}\right) R_{AG}^{j*} + K^+ A R_G^{j*} - \frac{K^-}{\mu_j} R_{AG}^{j*}, \tag{2.4}$$

$$\frac{dR_G^{j*}}{dt} = k_j^+ G_j R^{j*} - \left(k_j^- + k_{j-act}\right) R_G^{j*} - K^+ A R_G^{j*} + \frac{K^-}{\mu_j} R_{AG}^{j*}, \tag{2.5}$$

$$\frac{dG_j^*}{dt} = k_{j-act}\left(R_G^{j*} + R_{AG}^{j*}\right) - k_{gtp} G_{\beta\gamma} G_j^*, \tag{2.6}$$

$$\frac{dR_{Ai}}{dt} = k_{ec} R_A - k_d R_{Ai} - k_{rec} R_{Ai}, \tag{2.7}$$

$$\frac{dR_{Ai}^{j*}}{dt} = k_{ec} R_A^{j*} - k_d R_{Ai}^{j*} - k_{rec} R_{Ai}^{j*}, \tag{2.8}$$

where $G_{\beta\gamma} = G_1^* + G_2^* + G_3^*$ and

$$R_A = R_0 - R - R_{Ai} - \sum_{n=1}^{3}\left(R^{n*} + R_A^{n*} + R_{AG}^{n*} + R_G^{n*} + R_{Ai}^{n*}\right), \tag{2.9}$$

$$G_j = g_j - G_j^* - R_{AG}^{j*} - R_G^{j*}. \tag{2.10}$$

In order to obtain the model equations, we assume that the total number of G-protein subtype on the cell surface and the concentration of ligand, denoted by A, remain constant. Moreover, we also assume that the number of newly synthesized receptors is approximately equal to the number of degraded receptors so that the total number of receptors is conserved. The parameters g_j's and R_0 are the total number of each G-protein subtype and that of receptors, respectively. The notation R_G^{j*} and R_{AG}^{j*} denote the number of G_j-precoupled active receptors and of ligand-receptor-G_j complexes. The initial conditions for the system of equations are as following

$$R = R_0, \quad G_j = g_j, \quad \text{at } t = 0, \tag{2.11}$$

with the concentrations of all other species being zero at $t = 0$.

2.2 Nondimensionalization

We now, in the same manner as in [7], proceed to carry out nondimensionalization by the following rescaling,

$$t=\bar{t}/K^-,\qquad R=R_0\bar{R},\qquad R_A=R_0\bar{R}_A,$$
$$G_{\beta\gamma}=G_0\bar{G}_{\beta\gamma},\qquad G_j^*=G_0\bar{G}_j^*,\qquad G_j=G_0\bar{G}_j,$$
$$A=a_0\bar{A},\qquad R^{j*}=R_0\bar{R}^{j*},\qquad R_A^{j*}=R_0\bar{R}_A^{j*},$$
$$R_{AG}^{j*}=R_0\bar{R}_{AG}^{j*},\qquad R_G^{j*}=R_0\bar{R}_G^{j*},\qquad g_j=G_0\bar{g}_j$$
$$\bar{K}^+=\frac{K^+a_0}{K^-},\qquad \bar{L}_j^+=\frac{L_j^+}{K^-},\qquad \bar{L}_j^-=\frac{L_j^-}{K^-},$$

where $G_0=g_1+g_2+g_3$ and thus $\bar{g}_1+\bar{g}_2+\bar{g}_3=1$. For the purpose of drawing the concentration-respond curve, we rescale A by a given, representative, constant a_0 rather than scaling such that $\bar{A}=1$. The constant a_0 is chosen such that $\bar{K}^+=K^+a_0/K^-=\mathrm{O}(1)$. Since the binding of G-protein to an activated receptor often leads to the activation of the G-protein, we assume that the G-protein activation rate constant k_{j-act} is very large and G-protein dissociation rate constant k_j^- is very small. Now, let we make some approximations by letting

$$\varepsilon=\frac{K^-}{k_{1-act}},\quad \bar{k}_{j-act}=\frac{k_{j-act}}{k_{1-act}},\quad \bar{k}_j^-=\frac{k_j^-k_{1-act}}{\left(K^-\right)^2},\quad \bar{k}_{ec}=\frac{k_{ec}}{K^-},\quad N=\frac{R_0}{G_0},$$
$$\bar{k}_{gtp}=\frac{k_{gtp}G_0}{K^-},\quad \bar{k}_j^+=\frac{k_j^+G_0}{K^-},\quad \bar{V}=\frac{V}{R_0K^-},\quad \bar{k}_{rec}=\frac{k_{rec}}{K^-},\quad \bar{k}_d=\frac{k_d}{K^-},$$

where $\varepsilon \ll 1$. The overbars will be dropped henceforth for brevity. The nondimensionalized system of equations is then

$$\frac{dR}{dt}=R_A-\sum_{n=1}^{3}L_n^+R-K^+AR+\sum_{n=1}^{3}L_n^-R^{n*}+V, \tag{2.12}$$

$$\frac{dR^{j*}}{dt}=L_j^+R-\left(L_j^-+K^+A\right)R^{j*}+\frac{R_A^{j*}}{\mu_j}-k_j^+G_jR^{j*}+\left(\varepsilon k_j^-+\frac{k_{j-act}}{\varepsilon}\right)R_G^{j*}, \tag{2.13}$$

$$\frac{dR_A^{j*}}{dt}=K^+AR^{j*}-\left(\frac{L_j^-}{\mu_j}+\frac{1}{\mu_j}\right)R_A^{j*}+L_j^+R_A-k_j^+G_jR_A^{j*}+\left(\varepsilon k_j^-+\frac{k_{j-act}}{\varepsilon}\right)R_{AG}^{j*}-k_{ec}R_A^{j*}+k_{rec}R_{Ai}^{j*}, \tag{2.14}$$

$$\frac{dR_{AG}^{j*}}{dt}=k_j^+G_jR_A^{j*}-\left(\varepsilon k_j^-+\frac{k_{j-act}}{\varepsilon}\right)R_{AG}^{j*}+K^+AR_G^{j*}-\frac{R_{AG}^{j*}}{\mu_j}, \tag{2.15}$$

$$\frac{dR_G^{j*}}{dt}=k_j^+G_jR^{j*}-\left(\varepsilon k_j^-+\frac{k_{j-act}}{\varepsilon}\right)R_G^{j*}-K^+AR_G^{j*}+\frac{R_{AG}^{j*}}{\mu_j}, \tag{2.16}$$

$$\frac{dG_j^*}{dt}=N\frac{k_{j-act}}{\varepsilon}\left(R_G^{j*}+R_{AG}^{j*}\right)-k_{gtp}G_{\beta\gamma}G_j^*, \tag{2.17}$$

$$\frac{dR_{Ai}}{dt}=k_{ec}R_A-k_dR_{Ai}-k_{rec}R_{Ai}, \tag{2.18}$$

$$\frac{dR_{Ai}^{j*}}{dt} = k_{ec}R_A^{j*} - k_d R_{Ai}^{j*} - k_{rec}R_{Ai}^{j*}, \tag{2.19}$$

and

$$R_A = 1 - R - R_{Ai} - \sum_{n=1}^{3}\left(R^{n*} + R_A^{n*} + R_{AG}^{n*} + R_G^{n*} + R_{Ai}^{n*}\right), \tag{2.20}$$

$$G_j = g_j - G_j^* - NR_{AG}^{j*} - NR_G^{j*}. \tag{2.21}$$

The initial conditions are $R = 1,\ G_j = g_j$ with $g_1 + g_2 + g_3 = 1$, with other species having zero initial concentration.

Since we have assumed that k_{j-act} is very large, it is reasonable to rescale R_G^{j*} and R_{AG}^{j*} by letting

$$R_G^{j*} = \varepsilon\tilde{R}_G^{j*} \text{ and } R_{AG}^{j*} = \varepsilon\tilde{R}_{AG}^{j*}. \tag{2.22}$$

Substituting (2.22) in (2.15), (2.16), (2.17), (2.20) and (2.21) leads to

$$\frac{d\tilde{R}_{AG}^{j*}}{dt} = \frac{k_j^+}{\varepsilon}G_j R_A^{j*} - \left(\varepsilon k_j^- + \frac{k_{j-act}}{\varepsilon}\right)\tilde{R}_{AG}^{j*} + K^+A\tilde{R}_G^{j*} - \frac{\tilde{R}_{AG}^{j*}}{\mu_j}, \tag{2.23}$$

$$\frac{d\tilde{R}_G^{j*}}{dt} = \frac{k_j^+}{\varepsilon}G_j R^{j*} - \left(\varepsilon k_j^- + \frac{k_{j-act}}{\varepsilon}\right)\tilde{R}_G^{j*} - K^+A\tilde{R}_G^{j*} + \frac{\tilde{R}_{AG}^{j*}}{\mu_j}, \tag{2.24}$$

$$\frac{dG_j^*}{dt} = Nk_{j-act}\left(\tilde{R}_G^{j*} + \tilde{R}_{AG}^{j*}\right) - k_{gtp}G_{\beta\gamma}G_j^*, \tag{2.25}$$

$$R_A = 1 - R - R_{Ai} - \sum_{n=1}^{3}\left(R^{n*} + R_A^{n*} + \varepsilon\tilde{R}_{AG}^{n*} + \varepsilon\tilde{R}_G^{n*} + R_{Ai}^{n*}\right), \tag{2.26}$$

$$G_j = g_j - G_j^* - \varepsilon N\tilde{R}_{AG}^{j*} - \varepsilon N\tilde{R}_G^{j*}. \tag{2.27}$$

To simplify the calculation, we will also assume that the concentration of $G_{\beta\gamma}$ is constant instead of varying with G_j^*. In the limit $\varepsilon \to 0$, with quasi-steady state analysis, equations (2.23) and (2.24) yield

$$\tilde{R}_{AG}^{j*} = \frac{k_j^+G_j}{k_{j-act}}R_A^{j*}, \qquad \tilde{R}_G^{j*} = \frac{k_j^+G_j}{k_{j-act}}R^{j*}, \tag{2.28}$$

Using (2.28) to eliminate R_G^{j*} and R_{AG}^{j*} in (2.13) and (2.14) and substituting (2.28) in (2.25), our system of equations becomes

$$\frac{dR}{dt} = R_A - \sum_{n=1}^{3}L_n^+R - K^+AR + \sum_{n=1}^{3}L_n^-R^{n*} + V \tag{2.29}$$

$$\frac{dR^{j*}}{dt} = L_j^+R - \left(L_j^- + K^+A\right)R^{j*} + \frac{R_A^{j*}}{\mu_j} \tag{2.30}$$

$$\frac{dR_A^{j*}}{dt} = K^+AR^{j*} + L_j^+R_A - \left(\frac{L_j^-}{\mu_j} + \frac{1}{\mu_j}\right)R_A^{j*} - k_{ec}R_A^{j*} + k_{rec}R_{Ai}^{j*} \tag{2.31}$$

$$\frac{dR_{Ai}}{dt} = k_{ec}R_A - k_d R_{Ai} - k_{rec}R_{Ai} \tag{2.32}$$

$$\frac{dR_{Ai}^{j*}}{dt} = k_{ec}R_A^{j*} - k_d R_{Ai}^{j*} - k_{rec}R_{Ai}^{j*} \tag{2.33}$$

where

$$\begin{aligned} R_A &= 1 - R - R_{Ai} - \sum_{n=1}^{3}\left(R^{n*} + R_A^{n*} + R_{Ai}^{n*}\right) \\ G_j^* &= \frac{H_j g_j}{1+H_j}, \qquad G_j = g_j - G_j^* \\ H_j &\equiv \frac{Nk_j^+}{k_{gtp}G_{\beta\gamma}}\left(R_A^{j*} + R^{j*}\right). \end{aligned} \tag{2.34}$$

Equations (2.29) – (2.34) are quasi-steady state equations where $d\tilde{R}_{AG}^{j*}/dt$, $d\tilde{R}_G^{j*}/dt$ and dG_j^*/dt have already been set to zero. These equations can now be used to describe the steady state of ligand-receptors-G-proteins binding, if all of a parameter values were known.

3. Parameters Estimations

Our model equations (2.29)-(2-34) contain 18 parameters, L_j^+, L_j^-, μ_j, k_j^+, K^+, $k_{gtp}G_{\beta\gamma}$, V, k_{ec}, k_{rec} and k_d for $j = 1,2,3$, but the experimental data available from the literatures are limited to only a few parameters and only some types of receptors [21]. When only a few kinetic parameters are available to implement a model of the signal transductions, one might resort to attempting a theoretical estimate of these values. The attempt could be performed, in principle, by using an "inverse problem" approach, *i e.* by optimizing the unknown parameters of a reaction's model in order to obtain the best possible agreement between simulated and experimental data [11-13]. In the present work we will use genetic algorithm (GA) to estimate these unknown parameters.

The genetic algorithm is an effective stochastic global search algorithm that mimics biological evolution [10]. As it is robust, *i e.* it uses only objective function information and not other auxiliary information, it has been successfully applied to various problems, such as function optimization and combinatorial optimization, especially when a rigorous mathematical model is too complicated to be practically implemented [22]. In our problem, the input to the GA is a set (called a "population") of vectors (called "individuals") whose elements (called "genomes") are the values of those 18 parameters. A fitness function is defined to be the distance $f(\mathbf{x})$ measured between experimental and predicted values of the steady state activated G-proteins concentration,

$$f(\mathbf{x}) = \left(\sum_{i=1}^{n}\sum_{j=1}^{m}\left\{ \frac{\left| y_{\text{pred}}(i) - y_{\text{exp}}(i) \right|}{\left| y_{\text{exp}}(i) \right|} \right\}_j \right)^2, \tag{3.1}$$

where n is the number of data points for each experiment, m is the number of G-protein subtypes, $y_{\exp}$ represents the known experimental data, and y_{pred} is simulated data of the steady state activated G-proteins concentration obtained by using GA. The purpose of the GA is to produce successive populations of individuals which are generated with the aim of increasing the fitness of their individuals, i.e. their ability to solve the optimization problem by decreasing the distance $f(\mathbf{x})$ between simulated data and experimental data. The GA was performed by using 60 individuals of population and was run up to 100 generations.

4. Results and Discussion

To verify the validity of the model, with the help of GA for parameters estimation, we will qualitatively reproduce the experimental results given by Cordeaux *et al.* [5]. Note, however, that the system under investigation does not guarantee that the inverse problem has one unique solution, we could say only that we have found the good solution but that might not be the best solution. In the experiment of Cordeaux *et al*., they investigated the effect of two agonists, NECA and CPA, on adenosine A_1 receptors which can couple to three different families of G-protein, G_i, G_s and G_q, where each family of G-protein regulates specific classes of effector molecules within the cell. In this study, they found that NECA had a more efficacy (i.e., induced higher maximum response) while CPA appeared to has a higher potency (having lower *EC50*). To be consistent with our model, we thus set $G_i = G_1$, $G_q = G_2$ and $G_s = G_3$. We rescale the agonist concentrations by using $a_0 = 10^8$ M^{-1}, so that $\bar{A} = A \times 10^8$ and for the purpose of a good parameters estimation by using GA, we interpolate the experimental data in their Fig. 9, using their experimentally fitted curve and steady state solutions given in [7], from 6 points to 50 points per curve. The best fitness value for NECA is 13.1123 while that of CPA is 0.3121. The obtained parameter values for NECA and CPA are shown in table 1 with $N = 1$. For both NECA and CPA, we found that $\mu_1 > \mu_2 > \mu_3$, this result is consistent with the result obtained in. [5] and [7] so that it can be concluded that both agonists prefer the G_1-linked pathways. If we consider the trafficking event of the receptors, we could see that the receptors which bind or couple to NECA may be internalized and degraded faster than those which bind or couple to CPA. This result suggests that not only the efficacy and potency of receptors which depend on the type of agonists [3, 4], but the trafficking event of receptors may also depend on the type of agonists.

The comparison of steady state G-protein activation of these two agonists, by using the parameter values shown in Table 1., is shown in Fig. 2. They are in good agreement, at least

qualitatively, with the concentration response curves reported in [5]. From this figure, we can clearly see that CPA appears to be more potent while NECA is a more efficacious drug.

	NECA	CPA
L_1^+	18.85	19.95
L_2^+	16.10	13.65
L_3^+	0.1253	8.197
L_1^-	1498	1499
L_2^-	1457	1105
L_3^-	87.08	1312
μ_1	2116	2499
μ_2	1007	53.55
μ_3	500	32.77
k_1^+	75.41	98.98
k_2^+	79.25	68.23
k_3^+	0.7303	0.1328
K^+	0.0089	1.155
$k_{gtp}G_{\beta\gamma}$	81.22	78.07
V	0.0001	0.0021
k_{ec}	0.0095	53.00
k_d	0.00004	911.7
k_{rec}	453.3	2494

Table 1. The parameter values for agonists NECA and CPA obtained by using genetic algorithm.

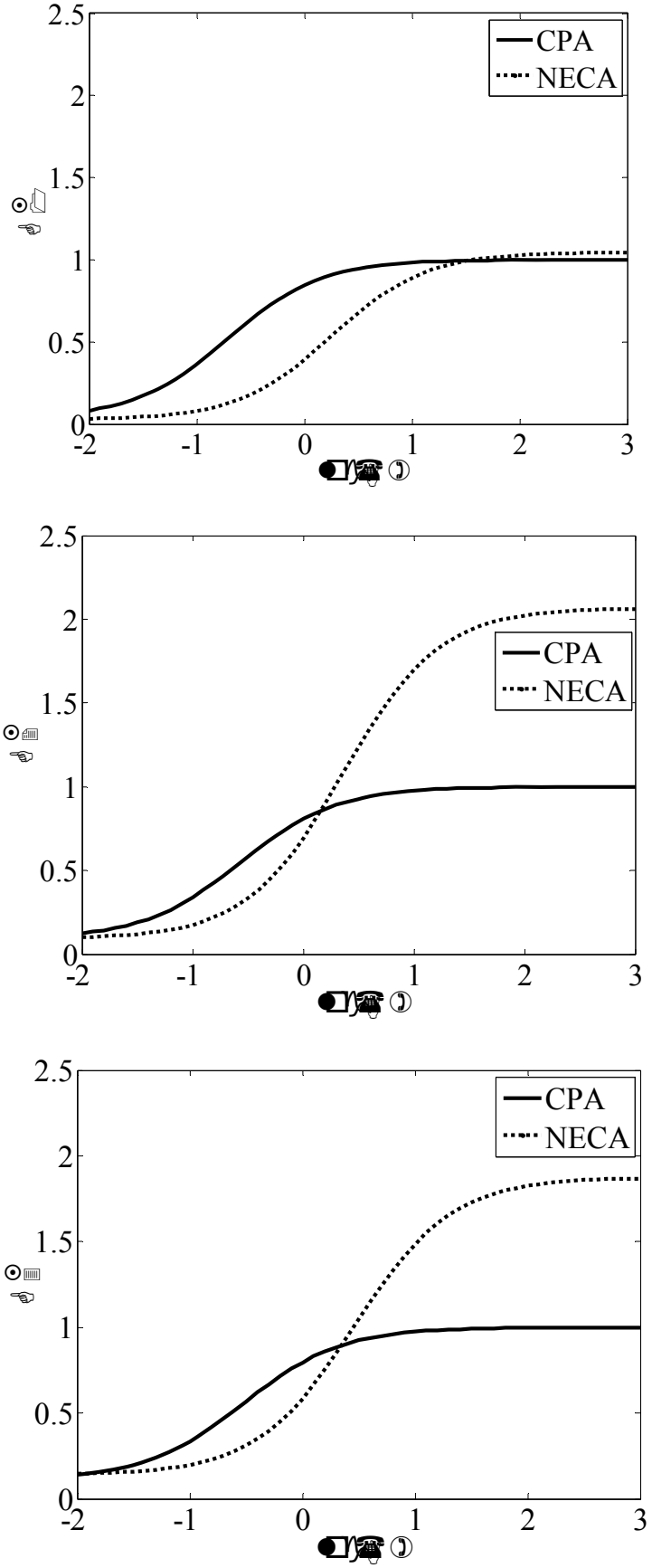

Figure 2. Simulated data of the effect of CPA and NECA, given relative to the maximum response of CPA, on G-protein activation for G_1, G_2 and G_3. The parameter values used are given in Table 1.

5. Conclusions

In this work, we have adapted a mathematical model proposed in [7] to study the trafficking events and the promiscuous coupling of receptors to G-proteins. The trafficking events we consider here include receptors synthesis, receptors endocytosis or internalization, recycling of receptors and receptors degradation. Taking the number of G-protein subtypes in the system to indicate the number of receptor conformations, our model assume four receptor states, including one resting, to account for coupling separately to G_1, G_2 and G_3. When the trafficking events of receptors are integrated into the model, we have found extra information which indicates that the trafficking events of membrane receptors may depend on the type of agonists which bind to them. The lack of kinetic interaction rates measured in reliable *in vivo* and *in vitro* experiments is currently the major limitation to the creation of complex models of signaling pathways. Thus, we have also used the genetic algorithm to estimate sets of unknown parameters. With the parameter values estimated by the genetic algorithm, the model is able to predict pathways-dependent agonist potency and efficacy as observed by Cordeaux *et al.* [5].

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