Explicit formulation of different receptor–G-protein interactions and effector regulation

Jyrki P. Kukkonen

Department of Neuroscience, Unit of Physiology, Uppsala University, BMC, P.O. Box 572, SE-75123 Uppsala, Sweden

Received on September 15, 2003; revised on March 17, 2004; accepted on April 2, 2004
Advance Access publication April 15, 2004

ABSTRACT

Motivation: G-protein-coupled receptors (GPCRs) can create different intracellular signals depending on which G-proteins they couple to and which intracellular signal-integrators, such as adenylyl cyclases, are expressed in the cell. One and the same GPCR can activate multiple G-protein species, generating signals, which either inhibit or amplify each other. Because of this complexity, extraction of mechanistic information from concentration–response curves is not straightforward.

Results: To tackle this problem, I describe in this paper explicit equations for GPCR-interaction with two G-protein species by different possible mechanisms, and also an equation for the regulation of an effector enzyme by the activated G-proteins or their effectors. Arithmetic solutions to these equations are presented, which resulted in the equations being applicable in a spreadsheet program environment. These equations are useful in simulations to analyze results, to design experiments and to test hypotheses. Some examples of this are presented in this study.

Contact: jyrki.kukkonen@fysiologi.uu.se

INTRODUCTION

G-protein-coupled receptors (GPCRs) transfer signals into the cells via heterotrimeric G-proteins, though recently even G-protein-independent signaling has been shown to occur (Heuss and Gerber, 2000). There are 17 genes for Ga-subunits, 5 for Gβ-subunits and 12 for Gγ-subunits known in man (and in other mammals) (Hur and Kim, 2002). Different subunits have different ability to interact with different receptors and each other, and to activate different signaling pathways (see, e.g. Yan et al., 1996; Daaka et al., 1997). It has been recently recognized that single GPCR species may be capable of interacting with several G-protein species. These different G-protein pathways often regulate the same intracellular effector, at more or less proximal level. A special case of the former is the well-recognized opposite regulation of adenylyl cyclase by Ga and Gβ, seen to occur for several GPCRs (Kukkonen et al., 2001), but even complex interactions are likely to occur. A central question is how we would be able to detect the activation of multiple pathways, i.e. how this is reflected in the attainable effector data. We have recently published an equilibrium analysis of receptor interaction with two G-proteins using different suggested models of interaction between them (Kukkonen et al., 2001). The models (Kukkonen et al., 2001) include (i) the shuttling model (see, e.g. Tolkovsky and Levitzki, 1981), in which G-proteins are free to diffuse in the membrane, get activated by the encounter with an activated receptor and diffuse again away to the effector (Fig. 1A); (ii) the complexing model (see, e.g. Chidiac, 1998), in which the G-proteins diffuse freely when inactive but when activated by the receptor they make up a ternary complex of the receptor, G-protein and effector (Fig. 1B); and (iii) two different precoupled models (see, e.g. Neubig, 1994), in which the receptor is precomplexed with a G-protein in the absence of the ligand and the G-protein stays complexed with the receptor (Fig. 1C). The analysis was performed to investigate the intuitive arguments presented for and against the different models. The results showed, that the different models are not as different in behavior as intuitively thought. In the present study, I present the full line-out of the derivation of the formula describing these models and some further development in them to include other possible modes of interaction. The models presented here allow the investigation of interaction on one receptor species with two G-protein species but similar formalism can be used for a higher number of G-protein species. For the readout at the effector level, I have also deduced an equation for the regulation of adenylyl cyclase with two allosteric regulators, e.g. two G-protein subunits.

SYSTEM AND METHODS

For all the calculations, Microsoft Excel 97 was used. The formula for the common solution of the third degree polynomial needed for the arithmetic solution of Equations (6), (9) and (20) was obtained from the Eric Weisstein’s World of Mathematics website (http://mathworld.wolfram.com).
Fig. 1. Different modes of interaction between receptors (R), G-proteins (G) and effectors (AC). (A) The shuttling model, where the G-protein subunits are free to diffuse in the membrane after activation. (B) The complexing model, where the G-proteins would diffuse freely when inactive but when activated by the receptor they stay complexed to the receptor. In the precoupled model (C), the receptor is precomplexed with a G-protein in the absence of the ligand and may stay complexed (C) or diffuse away from the receptor after its activation. It should be noted that G-protein subunit dissociation shown in the figure does not necessarily occur (Rebois et al., 1997; Chidiac, 1998).

adenylyl cyclase model was derived according to the rapid equilibrium model for enzyme kinetics (Segel, 1975). The user of a spreadsheet program should be aware of the limited precision that may cause problems for some parameter sets. These errors are, however, readily visible.

for the agonist:

\[
[B] = \frac{[\text{agonist}] \times [R_{\text{total}}]}{[\text{agonist}] + K_d} = [R_{\text{act-tot}}] \tag{1}
\]

THE FORMULA

Interaction of one receptor with two G-proteins

To simulate receptor interaction with one or two G-proteins, I used the normal law-of-mass action-based binding equation for the agonist:

The number of activated receptors ([R_{\text{act-tot}}]) is here equal to the receptor occupancy ([B]). The activated receptor concentration of free, i.e. non-G-protein bound R_{act}, [R_{\text{act-f}}]< the total activated receptor pool, [R_{\text{act-tot}}]: see Equation (3).
is acting as an ‘agonist’ on two different ‘receptors’ (G-proteins).

\[
[G_{1\text{-act}}] = \frac{[R_{\text{act-f}}] \times [G_{1\text{-total}}]}{[R_{\text{act-f}}] + EC_{50\text{-G1}}},
\]

\[
[G_{2\text{-act}}] = \frac{[R_{\text{act-f}}] \times [G_{2\text{-total}}]}{[R_{\text{act-f}}] + EC_{50\text{-G2}}},
\]

\[(G_{1\text{-act}})\text{ and } [G_{2\text{-act}}]\text{ are the concentrations of the activated G-proteins; } [G_{1\text{-total}}]\text{ and } [G_{2\text{-total}}] \text{ are the total concentrations of these G-proteins; } EC_{50\text{-G1}} \text{ and } EC_{50\text{-G2}} \text{ are the half-maximal concentrations of the activated receptor required to activate these G-proteins. Similar formalism can be used for an infinite number of different G-proteins.}

**Shuttling model**

The shuttling model (Fig. 1A) represents one of the very extremes of the imaginable receptor–G-protein interaction. Both G-proteins interact completely independently with the receptor and the whole activated receptor pool is accessible to the G-proteins. This would require either a rapid activation of the G-protein and diffusion to and away from the receptor together with a long-lasting effect on the effector or an activated receptor pool in large excess to the available G-protein pool under all conditions. All these conditions would lead to \([R_{\text{act-f}}] \equiv [R_{\text{act-tot}}]\). If we thus make \([R_{\text{act-f}}] = [R_{\text{act-tot}}]\), the output of the shuttling model can be calculated directly from Equations (1) and (2). Though the shuttling model can be considered rather artificial, it can be useful to include in the testing because it represents one of the extreme conditions. The behavior of the shuttling model is visualized in Figure 2A. Since the G-proteins interact with the receptor independently of each other, increased amounts of one G-protein (G_i) do not in any way affect the activation of the other G-protein (G_j) (left panel) and an increase in G-protein level increases the efficacy infinitely (right panel).

**Complexing model**

The complexing model (Fig. 1B) represents the other extreme of the interaction: only one G-protein can interact with the receptor since each and every activated G-protein chelates the activated receptor to it. Therefore, the activated receptor number can become limiting for the response. Thus, to simulate maximally limited \([R_{\text{act-f}}]\), it was derived according to

\[
[R_{\text{act-tot}}] = [R_{\text{act-f}}] + [R_{\text{act-b}}],
\]

\(([R_{\text{act-b}}]\text{ is the concentration of the G-protein-bound } R_{\text{act}})\). Since the activated G-proteins stay complexed to the receptor after their activation, then

\[
[R_{\text{act-b}}] = [G_{1\text{-act}}] + [G_{2\text{-act}}],
\]

and we can replace Equation (2) in Equation (3) yielding

\[
[R_{\text{act-tot}}] = [R_{\text{act-f}}] + [R_{\text{act-b}}] \leftrightarrow [R_{\text{act-tot}}] = [R_{\text{act-f}}] + [G_{1\text{-act}}] + [G_{2\text{-act}}] \\
\]

\[\leftrightarrow [R_{\text{act-tot}}] = [R_{\text{act-f}}] + \frac{[R_{\text{act-f}}] \times [G_{1\text{-total}}]}{[R_{\text{act-f}}] + EC_{50\text{-G1}}}
\]

\[+ \frac{[R_{\text{act-f}}] \times [G_{2\text{-total}}]}{[R_{\text{act-f}}] + EC_{50\text{-G2}}}.\]

This equation can be solved numerically for \([R_{\text{act-f}}]\) or it can be rewritten as a third degree polynomial,

\[
[R_{\text{act-f}}]^3 + a_2[R_{\text{act-f}}]^2 + a_1[R_{\text{act-f}}] + a_0 = 0,
\]

where

\[a_2 = EC_{50\text{-G1}} + EC_{50\text{-G2}} + [G_{1\text{-total}}] + [G_{2\text{-total}}] - [R_{\text{act-tot}}]
\]

\[a_1 = EC_{50\text{-G1}}EC_{50\text{-G2}} + [G_{1\text{-total}}]EC_{50\text{-G2}} + [G_{2\text{-total}}]EC_{50\text{-G1}}
\]

\[\quad - (EC_{50\text{-G1}} + EC_{50\text{-G2}})[R_{\text{act-tot}}]
\]

\[a_0 = -EC_{50\text{-G1}}EC_{50\text{-G2}}[R_{\text{act-tot}}]\]

which can be solved arithmetically (as done here). The degree of the polynomial increases by one for each G-protein species and use of an iterative approach becomes imperative. The complexing model flows Equation (1) \rightarrow Equation (6) \rightarrow Equation (2).

The behavior of the complexing model is visualized in Figure 2B. Increase in the expression level of one G-protein (G_i) effectively blocks the interaction of the receptor with the other G-protein (G_j) (left panel). The activated G-proteins effectively sequester the receptors and therefore even further increase in efficacy is ultimately blocked (right panel; compare with the shuttling model in Fig. 2A).

**Modeling the intermediate ‘complexing’: partially shuttling model**

As discussed above, the shuttling model is a very extreme theoretical model and even the complexing model, which represents the other extreme, may represent only one physiologically feasible situation. The time for a receptor to activate a G-protein is likely to be much less than the time the G-protein stays active. Therefore, unless the receptor sequesters G-proteins, a receptor would have time to activate a number of G-proteins before it is re-occupied by the ‘first’ G-protein, assuming that G-proteins could diffuse rapidly enough in the close vicinity of the receptor. This might be a reasonable view on the behavior of G-proteins in signaling microdomains (reviewed in Neubig, 1994; Hur and Kim, 2002) and actually in any systems where diffusion is limited. A straightforward way to simulate such conditions in our model is to reduce the number of receptors chelated by a G-protein. This number is represented by \(F\). Equations (4) and (5) thus
become

\[ [R_{\text{act-b}}] = F_{G_1}[G_{1-\text{act}}] + F_{G_2}[G_{2-\text{act}}], \]  \hspace{1cm} (7)

\[ [R_{\text{act-tot}}] = [R_{\text{act-f}}] + F_{G_1} \frac{[R_{\text{act-f}}] \times [G_{1-\text{total}}]}{[R_{\text{act-f}}] + \text{EC}_{50-G_1}} + F_{G_2} \frac{[R_{\text{act-f}}] \times [G_{2-\text{total}}]}{[R_{\text{act-f}}] + \text{EC}_{50-G_2}}. \]  \hspace{1cm} (8)

Thus, for instance, a \( F \) of 0.1 indicates that a receptor is able to activate 10 G-proteins during the GTP-hydrolysis cycle. By using separate values for the G-proteins \( G_1 \) and \( G_2 \), \( F_{G_1} \) and \( F_{G_2} \), different G-protein turn-over numbers can be simulated. In analogy with Equation (6),

\[ [R_{\text{act-f}}]^3 + a_2[R_{\text{act-f}}]^2 + a_1[R_{\text{act-f}}] + a_0 = 0, \]  \hspace{1cm} (9)

Fig. 2. Continued
Receptor–G-protein–effector interaction

Fig. 2. Manifestations of the different modes of interaction between receptors and G-proteins at different G-protein levels. On the left the maximum G-protein activation and on the right the adenylyl cyclase response. The parameters are: $[R_{\text{tot}}] = 1$, $K_d = 0.01$, $[G_{i-tot}] = 100$, $[G_{s-tot}] = 1 - 10000$ (as indicated in the figures), $EC_{50,G_i} = EC_{50,G_s} = 1$, $V = 100$, $\sigma = 0.1$, $K_{G_i} = K_{G_s} = 1$, $a(G\alpha) = 10$, $b(G\alpha) = 0.1$, $c(G\alpha_s$ and $G\alpha_i) = 2$, $\alpha = \beta = \gamma = \delta = 1$. (C) $F_{G_i} = F_{G_s} = 0.1$. (D) and/or (E) $p = 0.5$, $k = 1$ and $K_{R_{G_i}} - G_1 = K_{R_{G_s}} - G_2 = 1$.

The behavior of the partially shuttling model with $F = 0.1$ at different G-protein expression levels is shown in Figure 2C. Like the shuttling model, there is a clear right-shift in the concentration response curves (i.e., increase in potency; right panel) but there is a limit for the shift unlike the shuttling model. Giving $F$ all the values in the range of 0–1 simulates all the situations between complexing and shuttling models. $F_{G_i} = F_{G_s} = 1$ gives the previous formula for complexing model [Equation (6)] and $F_{G_i} = F_{G_s} = 0$ reduces $F$-dependent terms from Equation (9) so that the only allowed solution is $[R_{act,i}] = [R_{act-tot}]$ giving the shuttling model. The fluent transition between different models is shown in Figure 3 with respect to the cAMP response. Please also observe that the experimental data in Figure 5 was best fit using a partially shuttling model (see below).

**Precoupled model**

The precoupled model (Fig. 1C) implies that the G-proteins may be prebound to the receptors even in the absence of stimulation. Theoretically, after the receptor activation, the G-proteins can either diffuse away to the effector as in the shuttling model or stay bound as in the complexing model. Only the latter (Fig. 1C) is meaningful in living cells and only modeling of that has thus been performed.

If the receptor is thought to be able to exist in different conformations preferentially interacting with each G-protein type, two different schemes can be imagined: (1) the G-proteins are passive, thus not affecting the receptors inherent ability to adopt conformational equilibrium between these
Fig. 3. Fluent transformation of the complexing model to the shuttling model in partially shuttling model. The behavior is visualized with respect to the effect on adenylyl cyclase. In figure, the degree of complexing, according to the factors \( F_{G_1} \) and \( F_{G_2} \) in Equation (8), is changed from 1, which is equal to the complexing model, stepwise to 0, which is equal to the shuttling model. \( F_{G_1} = F_{G_2} \) (indicated in the figure). \( R_{\text{tot}} = 1, K_a = 0.01, [G_{\text{tot}}] = [G_{\text{tot}}] = 100, EC_{50,G_1} = 0.1, EC_{50,G_2} = 3, V = 100, \sigma = 0.1, K_G = K_{G_1} = 1, a(G_G) = 10, b(G_G) = 0.1, c(G_G, and G_G) = 2, \alpha = \beta = \gamma = \delta = 1.

states, and (2) the G-proteins actively affect the equilibrium. Here, we call these the precoupled G-protein-independent respectively precoupled G-protein-affected models.

In both, the total number of receptors, \( R_{\text{tot}} \), is equal to the sum of the receptors in configurations that can interact with each G-protein.

\[
R_{\text{tot}} = [R_{G_1-\text{tot}}] + [R_{G_2-\text{tot}}].
\] (10)

Also, the total number of receptors is the sum of G-protein-bound and free receptors.

\[
R_{\text{tot}} = [R_{G_1 - G_1}] + [R_{G_2 - G_2}] + [R_{f}].
\] (11)

Whether a receptor is bound with a G-protein is dependent on the respective affinities and receptor and G-protein levels. Since the free receptors can be in a conformation that prefers either \( G_1 \) or \( G_2 \),

\[
[R_f] = [R_{G_1-f}] + [R_{G_2-f}].
\] (12)

Equation (11) evolves to

\[
[R_{\text{tot}}] = [R_{G_1 - G_1}] + [R_{G_2 - G_2}] + [R_{G_1-f}] + [R_{G_2-f}].
\] (13)

If the likelihood of a free receptor to adopt a conformation preferring \( G_1 \) (\( R_{G_1-f} \)) is \( p \) then the likelihood of the free receptor to adopt a conformation preferring \( G_2 \) (\( R_{G_2-f} \)) is \( 1-p \). In the precoupled G-protein-independent model (see above), G-protein binding is not affecting the receptor equilibrium; thus

\[
p = \left( \frac{[R_{G_1-f}] + [R_{G_1 - G_1}]}{[R_{\text{tot}}]} \right)
\]

\[
\Leftrightarrow [R_{G_1-f}] + [R_{G_1 - G_1}] = p \times [R_{\text{tot}}].
\]

\[
1 - p = \left( \frac{[R_{G_2-f}] + [R_{G_2 - G_2}]}{[R_{\text{tot}}]} \right)
\]

\[
\Leftrightarrow [R_{G_2-f}] + [R_{G_2 - G_2}] = (1 - p) \times [R_{\text{tot}}].
\] (14)

How many of these receptors are precoupled with G-protein depends on the affinity between each receptor conformation and ‘its’ G-protein (\( K_{R_{G_1-\text{G}_1}} \) respectively \( K_{R_{G_2-\text{G}_2}} \)).

\[
[R_{G_1 - G_1}] = \frac{[G_{1-\text{tot}}] \times p \times [R_{\text{tot}}]}{[G_{1-f}] + K_{R_{G_1-\text{G}_1}}}
\]

\[
\Leftrightarrow [R_{G_1 - G_1}] = \frac{[G_{1-\text{tot}}] - [R_{G_1 - G_1}] \times p \times [R_{\text{tot}}]}{[G_{1-f}] + K_{R_{G_1-\text{G}_1}}}
\]

\[
[R_{G_2 - G_2}] = \frac{[G_{2-\text{tot}}] \times (1 - p) \times [R_{\text{tot}}]}{[G_{2-f}] + K_{R_{G_2-\text{G}_2}}}
\]

\[
\Leftrightarrow [R_{G_2 - G_2}] = \frac{[G_{2-\text{tot}}] - [R_{G_2 - G_2}] \times (1 - p) \times [R_{\text{tot}}]}{[G_{2-f}] + K_{R_{G_2-\text{G}_2}}}.
\] (15)

These can also be expressed as independent second degree polynomials

\[
[R_{G_1 - G_1}]^2 + b_1[R_{G_1 - G_1}] + b_0 = 0.
\] (16)

\[
[R_{G_2 - G_2}]^2 + c_1[R_{G_2 - G_2}] + c_0 = 0.
\]

where

\[
b_1 = -([G_{1-\text{tot}}] + p \times [R_{\text{tot}}] + K_{R_{G_1-\text{G}_1}})
\]

\[
b_0 = [G_{1-\text{tot}}] \times p \times [R_{\text{tot}}]
\]

\[
c_1 = -([G_{2-\text{tot}}] + (1 - p) \times [R_{\text{tot}}] + K_{R_{G_2-\text{G}_2}})
\]

\[
c_0 = [G_{2-\text{tot}}] \times (1 - p) \times [R_{\text{tot}}]
\]

and are thus solved in a straightforward fashion.

In the precoupled G-protein-affected model (see above), G-protein binding affects the receptors’ equilibrium and \( p \) is not constant at different G-protein concentrations. For instance, a high overexpression of one G-protein species converts all the receptors into the conformation preferring this species.

According to Equation (13), the total number of receptors is yet constant. Constant is also the relationship of the free receptor forms to each other, \( [R_{G_1-f}]/[R_{G_2-f}] \), because of the way of definition of the conformational preference and the law of mass action. Determination of receptors in G-protein bound states (\( [R_{G_1 - G_1}] \) and \( [R_{G_2 - G_2}] \)) is of highest
importance, since this determines the profile of the response. Though the receptors interact with the G-proteins as in Equation (15), the equation cannot be solved from the polynomial (16), since \( R_{G_1, tot} = (R_{G_1} - G_1) + [R_{G_1} \cdot f] \) and \( R_{G_2, tot} = (R_{G_2} - G_2) + [R_{G_2} \cdot f] \) are now dependent on each other and each one is affected by the G-protein binding. In mathematical terms,\[
\frac{[R_{G_1} \cdot f]}{[R_{G_2} \cdot f]} = k \text{(constant)} \iff [R_{G_1} \cdot f] = k \times [R_{G_2} \cdot f]. \tag{17}
\]
and (20) will be used in the place of (17, 18),\[
[R_{tot}] = [R_{G_1} - G_1] + [R_{G_2} - G_2] + [R_{G_1} \cdot f] + [R_{G_2} \cdot f]
\]
\[\iff [R_{tot}] = \frac{[R_{G_1} \cdot f] \times [G_1 \cdot tot]}{[R_{G_1} \cdot f] + K_{R_0} - G_1} + \frac{[R_{G_2} \cdot f] \times [G_2 \cdot tot]}{[R_{G_2} \cdot f] + K_{R_0} - G_2} + [R_{G_1} \cdot f] + [R_{G_2} \cdot f]. \tag{18}\]
Replacement of Equation (17) in Equation (18) gives\[
[R_{tot}] = k \times [R_{G_1} \cdot f] \times [G_1 \cdot tot] + [R_{G_1} \cdot f] \times [G_2 \cdot tot]
\]
\[= [R_{G_1} \cdot f] \times [G_1 \cdot tot] + [R_{G_1} \cdot f] \times [G_2 \cdot tot] \]
\[+ k \times [R_{G_2} \cdot f] + [R_{G_2} \cdot f]. \tag{19}\]
which can be rewritten as a third-degree polynomial\[
c_3[R_{G_1} \cdot f]^3 + c_2[R_{G_1} \cdot f]^2 + c_1[R_{G_1} \cdot f] + c_0 = 0 \tag{20}\]
where\[
c_3 = k^2 + k
\]
\[c_2 = (k + 1)K_{R_0} - G_1 + (k^2 + k)K_{R_0} - G_2
\]+ \([G_1 \cdot tot] + [G_2 \cdot tot] - [R_{tot}])k
\]
\[c_1 = (k + 1)K_{R_0} - G_1 K_{R_0} - G_2 + K_{R_0} - G_1 [G_2 \cdot tot]
\]
\[+ K_{R_0} - G_2 [G_1 \cdot tot] k - (K_{R_0} - G_1 + K_{R_0} - G_2) [R_{tot}]
\]
\[c_0 = -K_{R_0} - G_1 K_{R_0} - G_2 [R_{tot}]
\]
From the \([R_{G_1} \cdot f] \) all other parameters \([R_{G_1} \cdot f], [R_{G_1} - G_1], [R_{G_1} - G_2]) \) can be solved using the relationships in Equations (17) and (18).

For both independent and interacting models, \([R_{G_1} - G_1] \) and \([R_{G_2} - G_2] \) from Equations (16) respectively (17, 18) and (20) will be used in the place of \([R_{tot}] \) in Equation (1).

Two separate Equations (1), (2) and (6) are thus needed but they are simpler since each one only deals with one G-protein species.

The difference between the two ways of imagining the pre-coupled receptor model is illustrated in Figures 2D and E. In the G-protein-independent model, increased expression in one G-protein increases activation of this G-protein to a certain limit set by the total receptor number, as in the complexing model, and by the receptors in the correct conformation. However, more like the shuttling model, the activation of the other G-protein is not affected since the G-proteins cannot change the receptor equilibrium. In the G-protein-affected model, the increased level of one G-protein eventually shifts all the receptors to the conformation preferring this G-protein, very much like the complexing model. Please observe that the maximum number of G-proteins activated by the receptor remains below the number in the complexing model. This is because even in the very high G-protein excess only the pre-bound G-proteins can be activated; in the case of Figure 2D and E the EC50-G1 = EC50-G2 = 1 limits the maximum to 0.5 \times [R_{act-tot}].

Separation between the different receptor models
As in part demonstrated above or before (Kukkonen et al., 2001), some simple behavioral differences can be identified between each receptor model. Increased receptor expression levels result in enhanced efficacy (most clearly seen as a shift of the EC50), independently of the receptor model chosen. However, if the G-protein number becomes limiting, which though may be difficult to obtain in a physiological system, the further enhancement of the signaling of the precoupled receptors ceases, since only the receptors with precoupled G-proteins can signal. This separates the precoupled models from the shuttling, partially shuttling and complexing models. To separate between the latter models, G-protein expression levels should be manipulated. Shutting model gives an unlimitedly enhanced efficacy (shift in the EC50 with possible increase in the maximum) upon increased G-protein expression, whereas the complexing model does not result in the shift of the EC50 (some enhancement of the maximum response can be seen). In the case of the partially shutting model, the more ‘shuttling’ the model is the more it reminds of the shutting model, but there is always a limit for the shift in the EC50 unlike the shutting model. Thus, after an initial shift, the further enhancement ceases. Despite the usefulness of these criteria, simulations are always recommended in order to avoid erroneous conclusions caused by, e.g. diffusional limits or steric hindrance, signal amplification processes and interaction of the receptor with several G-proteins (see also the Discussion section).

Development of the adenylyl cyclase model
Mammalian membrane-bound adenylyl cyclases comprises nine subtypes (Tang and Hurley, 1998). The cytosolic regions C1a and C2a display the catalytic activity, and they have to be brought to close proximity and correct orientation for the conversion of ATP into cAMP. Allosteric regulators (e.g. Goα, Goγ, Gβγ, Ca2+) are thought to enhance/interfere with this co-ordination (Tang and Hurley, 1998). Multiple regulators can simultaneously regulate the activity of a single adenylyl
PKC-phosphorylation, Ca$^{2+}$ ATP is essentially irreversible due to the PPi hydrolysis, the task model can be produced. Since cAMP production from Elsevier.

lase, one substrate (ATP) and two allosteric regulators (G1 and regulation, a quaternary complex model for one adenylyl cyclase subtype. To simulate adenylyl cyclase activity and di, any two allosteric regulators such as G$i$ and G$s$, was used as a base (Fig. 4). With this a simple yet versatile model can be produced. Since cAMP production from ATP is essentially irreversible due to the PPi hydrolysis, the rapid equilibrium model for enzyme kinetics (Segel, 1975) was used for the derivation of Equation (21)

$$v = \frac{V\sigma}{\sigma_1} = \frac{V\sigma}{\sigma_1} = \frac{V\sigma}{\sigma_1} = \frac{V\sigma}{\sigma_1}$$

where $V =$ maximum velocity $= k_p \times [\text{adenylyl cyclase}]_{\text{total}}$, $\sigma =$ [substrate (ATP)]/[K$S$; $\zeta_1 = [G_1]\text{-act}/K_{G_1}$ and $\zeta_2 = [G_2]\text{-act}/K_{G_2}$. It should be noted that similar equations may be used in other enzyme kinetic studies; in spite of the careful review of published papers and available books, I could not able to find any and had to derive one by myself. This equation allows manipulation of multiple parameters associated with the binding ($\alpha$, $\beta$, $\gamma$ and $\delta$) and enzyme activation (a, b and c). The constants for the binding equations are dissociation constants, therefore, e.g. $\alpha < 1$ indicates stimulation of binding of G1 and S by one another; this could be used for instance in simulation of the mutual enhancement of binding by G$i$ and forskolin (Sutkowski et al., 1994). Any positive or negative allosteric regulator of adenylyl cyclase can be taken as $G_1\text{-act}$ and $G_2\text{-act}$, for instance $G_i\text{-act}$ and $G_s\text{-act}$, as in most of the simulations [Figs 1–2; see also Kukkonen et al. (2001)].

In Figure 5, I have demonstrated simulation of the effects of G$i$ and G$s$ on experimental data from Näsman et al. (2001). $a_{2B}$-adrenoceptors are expressed in SF9 insect cells either alone or together with G$i$ or G$s$, as indicated in the legend. Two different $a_2$-adrenoceptor agonists, noradrenaline (Fig. 5A) and UK14,304 (Fig. 5B) are used to stimulate the receptors. UK14,304 apparently couples more efficiently to G$i$ than to G$s$. Similar values for adenylyl cyclase and G-proteins fit the data well except for the G$i$ overexpression, where different total G-protein levels have to be used for noradrenaline and UK14,304. The reason to this is unknown, but it may be related to the fact that the absolute G-protein levels were hard to estimate. The model used is the partially shuttling model, which is the only one fitting the data reasonably well. In Figure 6, I have instead demonstrated simulation of experimental data for two positive regulators, G$s$ and Ca$^{2+}$/calmodulin. Two different endogenous octopamine receptors synergistically stimulate adenylyl cyclase activity in SF9 cells via G$s$ and Ca$^{2+}$ (Näsmann et al., 2002). Although Ca$^{2+}$ alone cannot stimulate adenylyl cyclase in these cells, octopamine-elicited Ca$^{2+}$ response approximately doubles the cAMP response to octopamine-elicited G$s$ activation (Fig. 6A). In the presence of Ca$^{2+}$, when both of these receptors are activated, they can be pharmacologically separated by their different affinity for the antagonist MK-912 (Fig. 6B). The model used for simulation is the complexing model; even with other models, very good fits are obtained. This physiological system does not allow separation of the model behavior since the two signals to adenylyl cyclase arise from two different receptors and thus do not compete for the activated receptor.

**DISCUSSION**

In this study, I have mathematically formulated some general principles for interaction between receptors, G-proteins and effectors. The development of the formula was originally initiated to gain understanding of the experimental results and to test the validity of intuitive interpretation of experimental results [see, e.g. Kukkonen et al. (2001) and Näsman et al. (2001) and Figs 5 and 6]), but they would fit well for experimental design and theoretical studies as well. The models are flexible and can be adapted to many different situations as exemplified in the figures, and the principles formulated in this study should be able to find their way to wider and even more advanced models in future. For instance, the mobility of signal molecules with respect to each other as formulated in Stickle and Barber (1989, 1993) can be easily incorporated. On the other hand, some parts, in
Receptor–G-protein–effector interaction

Fig. 5. Regulation of the endogenous adenylyl cyclase in Sf9 cells by heterologous αβ2-adrenoceptors via Gαi and Gαs. The symbols represent the experimentally determined values (Näsman et al., 2001) and solid lines, the simulated values. For the cAMP experiments, heterologous αβ2-adrenoceptors were expressed in Sf9 cells either alone or together with heterologous Gα or Gαs. cAMP measurements were performed using [3H]adenine prelabeling of intact cells, and chromatographic separation of the ATP/ADP and cAMP fractions after stimulations (Näsman et al., 2001). The common simulation parameters are: \([R_{\text{tot}}]=1, [G_{\text{tot}}]=100\) (for non-co-expressed data), EC50-Gαs = 10, V = 11, σ = 0.1, KGαs = KGαi = 1, a(Gαs) = 30, b(Gαs) = 0.3, c(Gαs and Gαi) = 7.4, α = β = γ = δ = 1. Specific parameters for (A): KD = 0.00000015, \([G_{\text{tot}}]\) + Gαs = 190, \([G_{\text{tot}}]\) + Gαi = 250, EC50-Gαi = 0.1. Specific parameters for (B): KD = 0.0000012, \([G_{\text{tot}}]\) + Gαs = 195, \([G_{\text{tot}}]\) + Gαi = 400, EC50-Gαi = 0.06. The model used is the partially shuttling model with \(F_{G1} = F_{G2} = 0.03\). The experiments were performed in the presence of 10 μM forskolin. For the sake of clarity, all the curves have been normalized to the AC activity in the absence of receptor agonist.

Fig. 6. Co-activation of the endogenous adenylyl cyclase in Sf9 cells by two different endogenous octopamine receptors via Gα, respectively Ca2+. (A) Gα stimulation can be seen in the absence of extracellular Ca2+ (−Ca2+), whereas the +Ca2+-data incorporate both Gαs and Ca2+ stimulation. The circles represent the experimentally determined values (Näsman et al., 2002) and the solid lines the simulated values. cAMP measurements were performed as in Figure 5. Simulation parameters: \([R_{\text{tot}}]=1, K_{D} = 0.000028, [G_{\text{calcium}}] = [G_{\text{tot}}]=100, EC50-Gαs = EC50-Gαi = 1, V = 100, σ = 0.1, KGαs = KGαi = 1, a(Ca2+) = 1.55, b(Gαs) = 3, c(Ca2+ and Gαs) = 7, α = β = γ = δ = 1. The model used is the complexing model. For the sake of clarity, both curves in (A) have been normalized to the AC activity in the absence of receptor agonist in +Ca2+. The experiment in (B) was performed in the presence of 10 μM forskolin and 30 μM octopamine, and the AC activity has thus been normalized to the forskolin response in the absence of octopamine (2.54 ± 0.25 times the basal activity).

particular the effector enzyme model, can be lifted out and used for the investigation of, e.g. adenylyl cyclase regulation, adenylyl cyclase being a very important physiological signal integrator.

We have previously discussed some of the results of simulations as such and in relation to the experimental data (Kukkonen et al., 2001). Currently, no absolute conclusions of the nature of receptor–G-protein interaction can be drawn. This is mostly attributable to the lack of experimental data, which in its turn is partly due to the lack of rational experimental design to solve the questions. If the mechanistic basis for the receptor–G-protein interaction is to be solved,
signaling at different receptor and G-protein levels is to be compared, and dominant-negative (i.e. receptor-interacting but effector-dead) G-proteins would give very useful information. It also appears that there might be differences associated with the systems. Thus, in some cases complexing model seems to give the best fit of the data (see Kukkonen et al., 2001), but in other cases, such as the one depicted in Figure 5, some freedom is required. A highly speculative but very interesting possibility would be that different G-proteins might have different properties with respect to diffusion and receptor chelation.

The models presented here are, of course, coarse simplifications of the physiological system. For a realistic model, a complex network of equilibrium and kinetic equations is required. However, at present we cannot extract the information needed to supply the multitude of constants with realistic values. Considering these problems we would suggest that equilibrium models are very useful; however, it is important to understand what assumptions we are making by using these. Equilibrium model requires that the measured (and modeled) receptor responses relatively rapidly reach an equilibrium or pseudo-equilibrium. Translated to the responses modeled in this study, it would mean that (positive and negative) receptors signals to adenylyl cyclase do not decay, but cAMP is produced at a constant rate instead. In such case, mechanistic information can be extracted from the analysis. If not, mechanistic information is of dubious kind but the analysis can still give useful suggestions.

ACKNOWLEDGEMENTS
I would like to thank Dr Lisa Johansson for careful scrutiny of the formula derived. Economical support from the Göran Gustafsson Foundation, the Lars Hierta Foundation, the Åke Wiberg Foundation, the Novo Nordisk Foundation and the European Union (contract no. QLG3-CT-2002-00826) is gratefully acknowledged.

REFERENCES