Metabotropic receptor activation, desensitization and sequestration—I: modelling calcium and inositol 1,4,5-trisphosphate dynamics following receptor activation

G. Lemon, W.G. Gibson, M.R. Bennett

Abstract

A mathematical account is given of the processes governing the time courses of calcium ions ($Ca^{2+}$), inositol 1,4,5-trisphosphate ($IP_3$) and phosphatidylinositol 4,5-bisphosphate ($PIP_2$) in single cells following the application of external agonist to metabotropic receptors. A model is constructed that incorporates the regulation of metabotropic receptor activity, the G-protein cascade and the $Ca^{2+}$ dynamics in the cytosol. It is subsequently used to reproduce observations on the extent of desensitization and sequestration of the P2Y2 receptor following its activation by uridine triphosphate (UTP). The theory predicts the dependence on agonist concentration of the change in the number of receptors in the membrane as well as the time course of disappearance of receptors from the plasmalemma, upon exposure to agonist. In addition, the extent of activation and desensitization of the receptor, using the calcium transients in cells initiated by exposure to agonist, is also predicted. Model predictions show the significance of membrane $PIP_2$ depletion and resupply on the time course of $IP_3$ and $Ca^{2+}$ levels. Results of the modelling also reveal the importance of receptor recycling and $PIP_2$ resupply for maintaining $Ca^{2+}$ and $IP_3$ levels during sustained application of agonist.

Keywords: Receptors; Desensitization; Sequestration; Calcium; $IP_3$; G protein; PLC; $PIP_2$

1. Introduction

Agonist-induced activation of second messenger systems plays an important role in the mobilization of stored $Ca^{2+}$ inside cells (Berridge, 1993; Miyazaki, 1995). A first stage in this process is the binding of a ligand to a G-protein coupled receptor, the metabotropic receptor. This sets off a cascade of events leading to the activation of the enzyme phospholipase C (PLC) which hydrolyses the membrane-bound phospholipid, phosphatidylinositol 4,5-bisphosphate ($PIP_2$) to inositol 1,4,5-trisphosphate ($IP_3$) and diacylglycerol. $IP_3$ then diffuses into the cytosol and interacts with $Ca^{2+}$ channels in the endoplasmic reticulum (ER) causing the release of stored $Ca^{2+}$ (Tsien and Tsien, 1990; Amundson and Clapham, 1993). At present there is no complete and unified model of the processes enumerated above, starting from the binding of ligand to metabotropic receptors and leading, via a G-protein cascade, to the production of $IP_3$ and the release of $Ca^{2+}$ from the endoplasmic reticulum.

Although there is no comprehensive model of the events that occur after ligand binding to metabotropic receptors, theoretical consideration has been given to various elements of the process. A number of models have been proposed for the interactions between receptors, ligands and G-proteins (Zigmond et al., 1982; Linderman and Lauffenburger, 1988; French and Lauffenburger, 1997; Lauffenburger and Linderman, 1993) with the cubic ternary model (Weiss et al., 1996a, b) providing the most general description of the interaction between the three species. Monte Carlo style simulations have also been used to analyse the possible stochastic nature of the interactions (Mahama and Linderman, 1994; Felber et al., 1996; Shea and
The discovery of desensitization of receptors following phosphorylation on ligand binding with subsequent internalization of the receptors has prompted the inclusion of these processes in more recent attempts to build quantitative models (Riccobene et al., 1999; Adams et al., 1998).

Modelling the formation of IP$_3$ by the hydrolysis of PIP$_2$, followed by the dynamics of Ca$^{2+}$ and IP$_3$ in the cytosol, has also been attempted. These models may include PIP$_2$ depletion and replenishment (Haugh et al., 2000) and also show how IP$_3$ can induce Ca$^{2+}$ oscillations (Cuthbertson and Chay, 1991; De Young and Keizer, 1992; Atri et al., 1993) as well as Ca$^{2+}$ waves (Jafri and Keizer, 1994, 1995; Schaff et al., 1997). With the discovery of elementary Ca$^{2+}$ events, such as ‘sparks’ and ‘blips’, that arise from the behaviour of either single-ion channels or clusters of them, concentration has centred on modelling these formations following the opening of IP$_3$-sensitive Ca$^{2+}$ channels (Smith et al., 1998; Swillens et al., 1998).

Although quantitative models of the action of hormones in inducing secretion have recently been presented (Blum et al., 2000) there is as yet no comprehensive model of how activation of metabotropic receptors leads to a response. Construction of such a model involves consideration of ligand–receptor binding and its desensitization through phosphorylation and internalization (Fig. 1, box A), of the G-protein cascade leading to production of IP$_3$ (Fig. 1, box B) and finally of the IP$_3$-induced Ca$^{2+}$ release from the endoplasmic reticulum (Fig. 1, box C). We present such a unified model here and use it to predict observations on the results of P$_2$Y$_2$ receptor stimulation by ligands.

2. Methods

This section contains the basic equations that define the model. In order to streamline the presentation, additional considerations involved in constructing the model are reserved for the Discussion section and detailed mathematical derivations are given in the appendices.

2.1. Regulation of metabotropic receptor activity

The regulation of metabotropic receptor activity has several components: phosphorylation of the receptors and their uncoupling from G-proteins; sequestration or internalization of the receptors; down-regulation of the receptors as a consequence of their destruction in lysosomes or alternatively dephosphorylation and recycling of the receptors to the membrane. The model presented here is an adaptation of that given by Hoffman et al. (1996) for the N-formyl peptide receptor for neutrophils, the difference being that internalized receptors are allowed to recycle to the surface. The elements of the model are depicted in Fig. 1 within the

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Fig. 1. Schematic diagram of the complete model. Box A shows the interaction between ligand and receptors as well as the processes of receptor endocytosis and recycling. Box B shows the G-protein interaction, leading to the formation of IP$_3$ by the hydrolysis of PIP$_2$. Box C outlines the Ca$^{2+}$ dynamics in the cytosol and the endoplasmic reticulum.
box marked A, where reactions involving ligand (L) and receptors (R) are given. Receptors on the cell surface bind extracellular ligand reversibly, with forward and backward rate constants $k_1^+$ and $k_1^-$, respectively. It is assumed that ligand is not depleted by binding with receptors and hence has a predetermined concentration.

Receptors occupied with ligand, LR, are phosphorylated irreversibly to $LR_p$ at a rate $k_p$ but phosphorylated receptors, $R_p$, remain free to interact with the ligand, with possibly different binding kinetics governed by rates $k_2^+$ (Hoffman et al., 1996; Adams et al., 1998; Riccobene et al., 1999). Phosphorylation causes desensitization of the receptors and so G-protein may only be activated by unphosphorylated receptors (R and LR), as indicated by the broken lines joining boxes A and B in Fig. 1. The model presented here has an aspect in common with the cubic ternary complex model (Weiss et al., 1996a, b) in that G-proteins are allowed to bind to receptors which are both bound and unbound with ligand. However, analysis of the model as given in the appendices shows that with certain assumptions the receptor/ligand and G-protein systems largely decouple and only the proportion of activated receptors needs to be specified in the G-protein cascade model.

Phosphorylated receptors are internalized at a rate that is dependent on agonist occupancy and this is incorporated into the model by having the bound phosphorylated receptors, $LR_p$, internalized at rate $k_r$. These internalized receptors, $R_i$, are then dephosphorylated and recycled back to the surface at a rate $k_r$.

The equations describing the processes depicted in box A of Fig. 1 are:

$$\frac{d[R]}{dt} = -k_1^+[L][R] + k_1^-[LR] + k_r[R_i],$$

$$\frac{d[LR]}{dt} = k_1^+[L][R] - (k_1^+ + k_p)[LR],$$

$$\frac{d[LR_p]}{dt} = k_2^+[L][R_p] - (k_2^- + k_p)[LR_p] + k_p[LR],$$

$$\frac{d[R_p]}{dt} = -k_2^-[L][R_p] + k_2^+[LR_p],$$

$$\frac{d[R_i]}{dt} = -k_r[R_i] + k_r[LR_p],$$

where $[L]$ denotes the concentration of ligand L, $[R]$ and $[LR]$ are the numbers of unbound and bound receptors, $[R_p]$ and $[LR_p]$ are the corresponding phosphorylated quantities and $[R_i]$ is the number of internalized receptors. Adding Eqs. (1)–(5) gives zero and so $[R] + [LR] + [LR_p] + [R_p] + [R_i] = [R_T]$, where $[R_T]$, the total number of receptors, is a constant.

The kinetics of ligand binding are considered to be fast relative to the other processes in the model. Eqs. (1)–(5) can be combined to leave only the slow kinetics, giving

$$\frac{d[R^s]}{dt} = k_r[R_i] - k_p[LR],$$

$$\frac{d[R^s_p]}{dt} = -k_r[LR_p] + k_p[LR],$$

where $[R^s] = [R] + [LR]$ is the total number of unphosphorylated surface receptors and $[R^s_p] = [R_p] + [LR_p]$ is the total number of phosphorylated surface receptors.

Applying the rapid ligand kinetics assumption (cf. the rapid buffer approximation: Wagner and Keizer, 1994) gives the following relations:

$$[R] = \frac{K_1[R^s]}{K_1 + [L]},$$

$$[LR] = \frac{[L][R^s]}{K_1 + [L]},$$

$$[LR_p] = \frac{[L][R^s_p]}{K_2 + [L]},$$

$$[R_p] = \frac{K_2[R^s_p]}{K_2 + [L]},$$

where $K_1 = k_1^+ / k_1^-$, $K_2 = k_2^- / k_2^+$. Substituting into Eqs. (7)–(8) and using Eq. (6) gives

$$\frac{d[R^s]}{dt} = k_r[R_i] - \left( k_r + \frac{k_p[L]}{K_1 + [L]} \right) \times [R^s] - k_p[R^s_p],$$

$$\frac{d[R^s_p]}{dt} = [L] \left( \frac{k_p[R^s]}{K_1 + [L]} - \frac{k_r[R^s_p]}{K_2 + [L]} \right).$$

It is likely that some fraction of the surface receptors do not recycle, perhaps through being immobile, and are prevented from being endocytosed (see, for example, Zigmound et al. (1982), where there is a discrepancy between the theoretical and experimental surface receptor numbers at equilibrium). This effect is incorporated into the present model by supposing that a fraction $\zeta$ of receptors are mobile, so that the total number of mobile receptors is now $\zeta[R_T]$ and the remaining receptors, numbering $1 - \zeta[R_T]$, are immobile and are assumed not to participate in second-messenger signalling.

The equilibrium solution of Eqs. (13) and (14), that is, the solution for which the ligand concentration has been held constant for a very long time, is found by setting $d[R^s]/dt = d[R^s_p]/dt = 0$ and solving for $[R^s]$ and $[R^s_p]$. 

This gives the equilibrium number of surface receptors,

$$[R^S_{EF}] = \lim_{t \to \infty} \left( \left[R^S\right] + \left[R^S_{EF}\right] \right),$$

as

$$[R^S_{EF}] = \frac{k_r}{1 + \frac{k_p}{k_e} \left( \frac{K_2 + [L]}{K_1 + [L]} \right)}$$

$$\times \left( 1 - \frac{\xi [R_T]}{[R_T]} \right).$$ (15)

### 2.2. G-protein cascade

The elements of this part of the overall model are shown in box B of Fig. 1, and involve the hydrolysis of membrane bound PIP$_2$, its subsequent replenishment and the degradation of IP$_3$ in the cytosol. A simplified model for the G-protein cascade is used, where the activation rate of G-protein is proportional to both the amount of active receptor and inactive G-protein (G · GDP) and the deactivation rate is proportional to the amount of active G-protein (G · GTP). Whereas the ligand bound receptor (LR) most strongly activates PLC, there is the possibility that the unbound receptor (R) may also contribute to IP$_3$ production, albeit at a lower rate, and this is taken to account for the basal concentration of IP$_3$. The resulting equation for the amount of Gz · GTP, denoted by [G], is

$$\frac{d[G]}{dt} = k_d(\delta + \rho_g)([G_T] - [G]) - k_d[G],$$ (16)

where $k_d$ and $k_d$ are the G-protein activation and deactivation rate parameters, $[G_T]$ is the total number of G-protein molecules, $\delta$ is the ratio of the activities of the ligand unbound and bound receptor species and $\rho_g$ is the ratio of the number of ligand bound receptors to the total number of receptors, $\rho_g = [LR]/([R_T])$, so from Eq. (10),

$$\rho_g = \frac{[LR]}{[R_T]}$$ (17)

The assumptions involved in the derivation of the simplified model are that the binding of subspecies participating in the G-protein cascade (see Fig. 2) is rapid relative to the other time-scales in the model and this binding is well below saturation. Additional requirements are that the dissociation of G · GTP into the subunits Gz · GDP, Gβγ is irreversible, as is also the association of the Gz · GDP and Gβγ subunits into G · GDP. Full details of the modelling of the G-protein cascade, leading to the above simplified version, are given in Appendix A.

PLC (PLC-β) is considered to be fully activated when bound to both Gz · GTP and Ca$^{2+}$. Although, as shown in Appendix A, the model allows for a contribution by unbound PLC towards the hydrolysis of PIP$_2$ (Rhee and Bae, 1997), this has not been included here. The rate of hydrolysis of PIP$_2$, assuming rapid kinetics for the binding of Ca$^{2+}$, is $r_h[PIP_2]$ where $[PIP_2]$ is the number of PIP$_2$ molecules and the rate coefficient is

$$r_h = \alpha \left( \frac{[Ca^{2+}]}{K_c + [Ca^{2+}]} \right)[G],$$ (18)

where $\alpha$ is an effective signal gain parameter, [Ca$^{2+}$] is the cytosolic Ca$^{2+}$ concentration and $K_c$ is the dissociation constant for the Ca$^{2+}$ binding site on the PLC molecule. Details of the derivation of Eq. (18) are given...
in Appendix A. Hydrolysis of PIP2 forms IP3 molecules at a rate $r_6[\text{PIP2}]$ and these are free to diffuse into the cytosol where they are degraded by intracellular kinases. The degradation of IP3 is assumed to occur at a rate $k_{\text{deg}}$ and hence the equation for the total number of IP3 molecules, $[\text{IP3}]$, is

$$\frac{d[\text{IP3}]}{dt} = r_6[\text{PIP2}] - k_{\text{deg}}[\text{IP3}], \quad (19)$$

Replenishment of PIP2 is required for IP3 production to be maintained over sustained periods of agonist stimulation. Although the means by which this regeneration takes place is complex (Batty et al., 1998; Takenawa et al., 1999; Cockcroft and De Matteis, 2001), the essentials of this process are captured by assuming that there exists an intracellular pool of phospholipid, $[\text{H}]$ (see Fig. 1, box B) to which IP3 is degraded. This phospholipid is then phosphorylated and returned to the cell surface at a constant rate $r_s$, so the equations for the numbers of PIP2 and hydrolysed IP3 molecules are thus

$$\frac{d[\text{PIP2}]}{dt} = -r_6[\text{PIP2}] + r_s[H], \quad (20)$$

$$\frac{d[H]}{dt} = k_{\text{deg}}[\text{IP3}] - r_s[H]. \quad (21)$$

Adding Eqs. (19)–(21) and integrating over time shows that $[\text{PIP2}] + [\text{IP3}] + [H] = ([\text{PIP2}]_0 + [\text{IP3}]_0)$ where $([\text{PIP2}]_0)$ is the constant total number of PIP2 molecules and so Eq. (20) can be written as

$$\frac{d[\text{PIP2}]}{dt} = -(r_h + r_r)[\text{PIP2}] - r_s[\text{IP3}] + r_s([\text{PIP2}]_0). \quad (22)$$

Later work will require the molar concentration of IP3, so $[\text{IP3}]$ is now replaced by $N_a[v[\text{IP3}]]$ where $v$ is the volume of the cell, $N_a$ is Avogadro’s constant and $[\text{IP3}]$ is the molar concentration of IP3.

The full equations for the G-protein cascade are thus

$$\frac{d[G]}{dt} = k_a(\delta + \rho_r)[G_T] - [G] - k_{a}[G], \quad (23)$$

$$\frac{d[\text{IP3}]}{dt} = r_hN_a^{-1}v^{-1}[\text{PIP2}] - k_{\text{deg}}[\text{IP3}], \quad (24)$$

$$\frac{d[\text{PIP2}]}{dt} = -(r_h + r_r)[\text{PIP2}] - r_sN_a[v[\text{IP3}]] + r_s([\text{PIP2}]_0), \quad (25)$$

$$r_h = \frac{[\text{Ca}^{2+}]}{K_c + [\text{Ca}^{2+}]} \cdot [G], \quad (26)$$

$$\rho_r = \frac{[L][R_1]}{[G_T][K_{1} + L]} \cdot (27)$$

In principle, these equations enable calculation of the amount $[G]$ of activated G-protein, the amount $[\text{PIP2}]$ of PIP2 and the concentration $[\text{IP3}]$ of IP3 (see below for details of the solution method). The missing ingredient is the free cytosolic $\text{Ca}^{2+}$ concentration, $[\text{Ca}^{2+}]$, and this requires a model for the calcium dynamics in the cytosol and endoplasmic reticulum (box C of Fig. 1), for which the crucial input is the IP3 concentration, $[\text{IP3}]$.

2.3. Cytosolic $\text{Ca}^{2+}$ dynamics

The cytosol contains an endoplasmic reticulum (ER) which behaves as a $\text{Ca}^{2+}$ store and exchanges $\text{Ca}^{2+}$ with the cytosol via IP3 sensitive channels, calcium pumps and leaks. Both the cytosol and the ER are assumed to contain calcium buffers. The following model is an adaptation of the one given by Li and Rinzel (1994) for simplified IP3 channel kinetics, but includes rapid calcium buffering as described by Wagner and Keizer (1994). The equation governing the concentration of free cytosolic calcium, $[\text{Ca}^{2+}]$, is

$$\frac{d[\text{Ca}^{2+}]}{dt} = \beta \left\{ \epsilon_v[\eta_1m_\infty^3h^3 + \eta_2][\text{Ca}^{2+}_{\text{ER}}] - [\text{Ca}^{2+}] \right\} - \eta_3 \left( \frac{([\text{Ca}^{2+}]^2)}{k_3 + ([\text{Ca}^{2+}]^2)} \right), \quad (28)$$

where $[\text{Ca}^{2+}]$ is the concentration of free $\text{Ca}^{2+}$ in the ER, $\beta$ is related to the buffering (see below), $\eta_1$, $\eta_2$ and $\eta_3$ are effective permeability constants for the IP3 channels, membrane leakage and $\text{Ca}^{2+}$ pumps, respectively, and $k_3$ is the pump dissociation constant. The quantity $h$ is the fraction of IP3 channels not yet inactivated by $\text{Ca}^{2+}$ binding, and its time course is governed by the differential equation

$$\frac{dh}{dt} = \frac{h_{\infty} - h}{\tau_h}, \quad (29)$$

where

$$\tau_h = \frac{1}{a_2(\zeta + [\text{Ca}^{2+}])}, \quad (30)$$

$$h_{\infty} = \frac{\zeta}{\zeta + [\text{Ca}^{2+}]}, \quad (31)$$

$$\zeta = d_2^2[\text{IP3}] + d_2 \frac{d_1}{d_3}[\text{IP3}]^2 + d_3 \quad (32)$$

where $d_1$, $d_2$, $d_3$ and $a_2$ are channel kinetic parameters and $[\text{IP3}]$ is the concentration of IP3 in the cytosol. The remaining quantity is

$$m_{\infty} = \left( \frac{[\text{IP3}]}{d_1 + [\text{IP3}]} \right) \left( \frac{[\text{Ca}^{2+}]_3}{d_5 + [\text{Ca}^{2+}]_3} \right). \quad (33)$$

The $\text{Ca}^{2+}$ buffers in the cytosol are assumed to comprise an endogenous stationary buffer and an exogenous mobile buffer, in this case Fura-2. The buffering function $\beta$ is defined as

$$\beta = \left( 1 + \frac{K_a[B_s]}{(K_a + [\text{Ca}^{2+}]^2) + \frac{K_a[B_s]}{(K_a + [\text{Ca}^{2+}]^2)} \right)^{-1} \quad (34)$$
where \([B_r]\) and \(K_r\) are the total concentration and dissociation constant, respectively, of the endogenous buffer and \([B_c]\), \(K_c\) are the corresponding parameters for the exogenous buffer.

The \(Ca^{2+}\) buffer in the ER is assumed to be of high concentration and low affinity, implying that the total concentration of \(Ca^{2+}\) in the ER, \([Ca^{2+}_{ER}]\), is approximately equal to that bound to the buffer and thus approximately equal to \(([ER]/K_{ER})[Ca^{2+}_{ER}]\) where \([ER]\) is the total concentration of ER buffer and \(K_{ER}\) is the ER buffer dissociation constant.

The conservation condition for total \(Ca^{2+}\), both bound and unbound in cytosol and ER, is

\[
e_r([Ca^{2+}_{ER}]_T) + ([Ca^{2+}_{cyt}]_T) = ([Ca^{2+}]_T),
\]

(35)

where \(e_r\) is the ratio of the ER volume to the cytosol volume and \([Ca^{2+}_T]\) is the total concentration of \(Ca^{2+}\) in terms of the cytosolic volume. Substituting the above approximations and also the ratio \(\gamma\) of free to total \(Ca^{2+}\) in the cytosol,

\[
\gamma = \left(1 + \frac{[B_c]}{K_c + [Ca^{2+}_c]} + \frac{[B_r]}{K_r + [Ca^{2+}_r]}\right)^{-1}, \tag{36}
\]

into Eq. (35), gives the relation between free ER \(Ca^{2+}\) and free cytosolic \(Ca^{2+}\):

\[
[Ca^{2+}_{ER}] = \frac{K_{ER}([Ca^{2+}]_T)}{[ER]r} ([Ca^{2+}_c] - [Ca^{2+}_r]/\gamma). \tag{37}
\]

The free \(Ca^{2+}\) concentration, \([Ca^{2+}_c]\), is determined by solving Eqs. (28) and (29), with definitions (30)–(34), (36), (37) and with initial values of \([Ca^{2+}_c]\) and \(h\).

2.4. Initial conditions and methods of solution

The stimulus applied to the cells is taken to be a step application of agonist,

\[
[L](t) = \begin{cases} 
0 & \text{if } t < 0, \\
[L] & \text{if } t \geq 0.
\end{cases}
\]

Eqs. (13), (14), (23)–(27) and (28)–(29), with the appropriate initial conditions, suffice to determine the transient in the modelled quantities for this stimulus.

The basal levels of \([PIP2]\), \([Ca^{2+}]_b\), \([IP3]\) and \(h\), respectively \([PIP2]_b\), \([Ca^{2+}]_b\), \([IP3]_b\) and \(h_b\) were determined by integrating the equations for a sufficiently long time prior to agonist stimulation. The basal level of \(Gx\cdot GTP\), \([G]_b\), can be determined exactly by setting \(d[G]/dt = 0\) in Eq. (23) and noting that \([L] = 0\) implies \(\rho = 0\). Solving for \(G\) gives

\[
[G]_b = \frac{k_p \delta(G_T)}{k_m \delta + k_d}. \tag{39}
\]

The appropriate initial conditions for the model equations are thus

\[
[R^+_0](0) = \gamma[R_T], \quad [R^-_0](0) = 0, \quad [G](0) = [G]_b,
\]

\[
[PIP2](0) = [PIP2]_b, \quad [IP3](0) = [IP3]_b,
\]

\[
[Ca^{2+}]_b(0) = [Ca^{2+}]_b,
\]

\[
h(0) = h_b. \tag{40}
\]

Approximate expressions for \([PIP2]_b\), \([Ca^{2+}]_b\), \([IP3]_b\) and \(h_b\), can be obtained as follows. The parameter values governing the \(Ca^{2+}\) dynamics are such that in the absence of ligand the contribution of the IP3 channels to the \(Ca^{2+}\) current across the ER membrane is small compared to that of the leak and pumps. Also, both the free and total cytosolic \(Ca^{2+}\) is small compared to the free and total ER \(Ca^{2+}\). Using these approximations, Eq. (37) becomes

\[
[Ca^{2+}_{ER}] = \frac{K_{ER}([Ca^{2+}]_T)}{[ER]_r} \quad \text{and at equilibrium the free \(Ca^{2+}\) concentration as given by Eq. (28) reduces to}
\]

\[
\eta_2K_{ER}([Ca^{2+}]_T) - \eta_2[Ca^{2+}_c]^2 \frac{K_{ER}}{K_c + [Ca^{2+}_c]} = 0. \tag{41}
\]

Solving Eq. (41) for \([Ca^{2+}_c]\) gives an approximation for \([Ca^{2+}]_b\):

\[
[Ca^{2+}]_b = \frac{k_3}{\sqrt{\eta_2 [ER]/\eta_2 K_{ER}([Ca^{2+}]_T) - 1}}. \tag{42}
\]

Next, approximate expressions for \([IP3]_b\) and \([PIP2]_b\) can be found by setting \(d[IP3]/dt = d[PIP2]/dt = 0\) in Eqs. (24) and (25) with \(r_b = (r_b)_b = \delta([Ca^{2+}]_b/(K_c + [Ca^{2+}]_b))[G]_b\). Solving for \([IP3]_b\) and \([PIP2]_b\) gives

\[
[IP3]_b = \frac{\nu N_d(k_p((r_b)_b + r) + (r_b)_b)(r_b)}{k_p((r_b)_b + r) + (r_b)_b}. \tag{43}
\]

\[
[PIP2]_b = \frac{k_p \rho r ([PIP2]_T)}{k_p((r_b)_b + r) + (r_b)_b}. \tag{44}
\]

and the parameter \(\delta\) in Eq. (39) may be chosen to give the desired value of \([IP3]_b\). Finally, Eq. (29) implies that in equilibrium \(h = h_b\) and substituting \([Ca^{2+}]_b\) and \([IP3]_b\) into Eqs. (31) and (32) gives the approximation for \(h_b\):

\[
h_b = \frac{d_2(d_1 + [IP3]_b)}{d_1 d_2 + [IP3]_b ([Ca^{2+}]_b + d_2) + d_3([Ca^{2+}]_b)}. \tag{45}
\]

The nonlinear nature of the equations for the \(Ca^{2+}\) dynamics precludes an analytic solution to the full equations and solutions were instead computed numerically using the MATLAB computer package. However, the equations for \([R^+_0]\) and \([R^-_0]\), Eqs. (13) and (14), are linear and an explicit solution can be determined. The solution for the total number of surface receptors, \([R^+_0]\),
given stimulus (38) and initial conditions of Eq. (40) is

\[
[R_T^2] = \begin{cases} 
\{[R_T]\} & \text{if } t < 0, \\
\{[R_T]\} + \frac{\{[R_T] - [R_T^2]\}}{(\lambda_2 - \lambda_1)}([\lambda_2 e^{\lambda_1 t} - \lambda_1 e^{\lambda_2 t}]) & \text{if } t \geq 0,
\end{cases}
\]

where the eigenvalues \(\lambda_1, \lambda_2\) are the roots of the quadratic equation

\[
\lambda^2 + \left(\frac{k_r + \frac{k_p[L]}{K_1 + [L]} + \frac{k_s[L]}{K_2 + [L]}}{K_1 + [L]}\right) \lambda + \left(\frac{k_r + \frac{k_p[L]}{K_1 + [L]} - \frac{k_s[L]}{K_2 + [L]} + \frac{k_s k_p[L]}{K_1 + [L]}\right) = 0. 
\]

(47)

It is of interest to note that for sufficiently high ligand concentration one eigenvalue depends only on the desensitization parameter \(k_p\) and the other depends only on the internalization and recycling parameters \(k_r\) and \(k_s\). This dependence occurs when \([L]\) is sufficiently large that the term \(k_s k_p[L]/(K_2 + [L])\) in the constant term of Eq. (47) is negligible compared to the other two terms. In this case, Eq. (47) reduces to an equation that has solutions

\[
\hat{\lambda}_1 = -\frac{k_p[L]}{K_1 + [L]},
\]

(48)

\[
\hat{\lambda}_2 = -k_r - \frac{k_s[L]}{K_2 + [L]}.
\]

(49)

In the limit \([L] \to \infty\) these solutions are \(\hat{\lambda}_1, \infty = -k_p\) and \(\hat{\lambda}_2, \infty = -(k_r + k_s).

The full set of parameters used in the model together with their numerical values are listed in Table 1. References are given to those parameters whose values were obtained from the literature. Other parameters were determined by fitting the solutions of the equations to the experimental data of Garrad et al. (1998) in this paper and Hirose et al. (1999) in a following paper (Lemon et al., 2003). This procedure is discussed in detail below.

3. Results

3.1. Model predictions for desensitization and sequestration

The theory developed above is now used to calculate the effects of applying UTP to P2Y2 receptors, where comparison can be made with the experimental results of Garrad et al. (1998). Fig. 3(a) shows the equilibrium number of surface receptors \([R_T]\), computed using Eq. (15), expressed as a percentage of \([R_T]\), as a function of the concentration of UTP. Also shown is the corresponding experimental data taken from Fig. 5A of Garrad et al. (1998). The transient behaviour of the number of surface receptors, following a 1 mM application of UTP at \(t = 0\), is shown in Fig. 3(b), where the solid line comes from the solution of Eq. (46) and the points with error bars are experimental values from Fig. 4A of Garrad et al. (1998).

Eq. (46) shows that the theoretical transient in Fig. 3(b) can be expressed as the linear combination of the two solutions \(e^{\lambda_1 t}\) and \(e^{\lambda_2 t}\), where the eigenvalues \(\lambda_1\) and \(\lambda_2\) are solutions of Eq. (47). Fig. 4 shows the variations in the real parts of these two eigenvalues with respect to ligand concentration. The large relative difference in magnitude between the eigenvalues is evident for ligand concentrations above about 1 \(\mu\text{M}\). Here, the eigenvalues are real and so any solution is the sum of two decaying exponential terms. The transient in \([R_T]\) shown in Fig. 3(b) comprises two such terms plus a constant term due to the immobile receptors, as seen from Eq. (46). The asymptotic values of the two curves labelled \(\hat{\lambda}_1\) and \(\hat{\lambda}_2\) in Fig. 4 are \(-0.0300\) and \(-0.00622\) s\(^{-1}\) which are in good agreement with the theoretical limiting values of \(\lambda_1\) and \(\lambda_2\): \(\lambda_1, \infty = -0.03\) and \(\lambda_2, \infty = -6.175 \times 10^{-3}\) s\(^{-1}\). The approximations for \(\hat{\lambda}_1\) and \(\hat{\lambda}_2\) given by Eqs. (48) and (49) are good also for \([L] = 1\) mM. Because of the large relative difference between the eigenvalues, for a sufficiently long time after agonist application (\(\approx 2\) min) the contribution of the \(e^{\hat{\lambda}_1 t}\) term can be ignored and the transient in \([R_T]\) shown in Fig. 3(b) can thus be approximated by a single exponential term proportional to \(\exp(-0.00618t)\). In the vicinity of 0.1 \(\mu\text{M}\) there is a range of ligand concentrations for which the eigenvalues have imaginary parts, meaning that \([R_T]\) exhibits damped oscillatory behaviour; however, the imaginary parts turn out to be too small for these oscillations to be readily observed over the time scale used.

Figs. 5(a)–(d) show the theoretical transients in IP3 concentration, Ca\(^{2+}\) concentration, amount of PIP2 and amount of activated G-protein (Gx·GTP), respectively, for a 1 mM application of UTP at \(t = 0\). These transients were obtained from Eqs. (13), (14) and (23)–(29), using initial conditions given by Eq. (40). In Figs. 5(a)–(c), solutions are plotted for three different values of the PIP2 replenishment rate parameter: \(r_r = 10, 0.1\) and 0.015 s\(^{-1}\). The smaller the value of \(r_r\) the greater the maximum depletion in the amount of PIP2 (Fig. 5(e)) and the faster the rate of decay of the IP3 and Ca\(^{2+}\) concentrations (Figs. 5(a) and (b)). Lowering the value of \(r_r\) lowers the peak IP3 concentration and to a lesser extent the peak Ca\(^{2+}\) concentration. The peak in activated G-protein (7.1\%) is low due to the low ratio of \(k_p\) to \(k_d\); also, the amount of activated G-protein does not depend on \(r_r\).

Binding of ligand to the receptors causes an initial period of increase of IP3 concentration, with it reaching a maximum at approximately 14.1, 8.0 and 7.2 s after application of agonist for \(r_r = 10, 0.1\) and 0.015 s\(^{-1}\),
respectively. Similarly, the Ca\(^{2+}\) concentration peaks after 16.5, 11.5 and 10.1 s. The initial increase of IP\(_3\) and Ca\(^{2+}\) concentration follows the initial rise of activated G-protein levels but the time to peak is decreased by depletion of PIP\(_2\) levels. The time for activated G-protein to peak is 13.1 s and this is determined mainly by the GTPase rate parameter. Thereafter, desensitization of the agonist bound receptors causes a decrease in [IP\(_3\)], [Ca\(^{2+}\)] and [Gz⋅GTP] on a time-scale of minutes.

In the steady state, the IP\(_3\) and Ca\(^{2+}\) concentrations remain slightly above the basal level due to the slow recycling to the surface of internalized, dephosphorylated receptors. The large difference between the peak and steady-state concentrations of IP\(_3\), for the three different values of \(r_r\), is evident in Figs. 6(a) and (b). These curves were produced by solving Eqs. (13), (14) and (23)–(29) with initial conditions given by Eq. (40) and ligand stimulus by Eq. (38). In each figure, both the maximum and steady-state curves are monotonic.

Table 1
Model parameter values

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Value</th>
<th>Cell type</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>([R_2])</td>
<td>Total no. of P(_2)Y(_2) receptors</td>
<td>(2 \times 10^4)</td>
<td>1321N1</td>
<td>Garrad et al. (1998)</td>
</tr>
<tr>
<td>(K_1)</td>
<td>Unphosphorylated receptor dissociation constant</td>
<td>5 (\mu)M</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(K_2)</td>
<td>Phosphorylated receptor dissociation constant</td>
<td>100 (\mu)M</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(k_r)</td>
<td>Receptor recycling rate</td>
<td>(1.75 \times 10^{-4}) s(^{-1})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(k_d)</td>
<td>Receptor phosphorylation rate</td>
<td>0.03 s(^{-1})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(k_e)</td>
<td>Receptor endocytosis rate</td>
<td>(6 \times 10^{-3}) s(^{-1})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(\xi)</td>
<td>Fraction of mobile receptors</td>
<td>0.85</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>([G_x])</td>
<td>Total number of G-protein molecules</td>
<td>(1 \times 10^5)</td>
<td>Various</td>
<td>Mahama and Linderman (1994)</td>
</tr>
<tr>
<td>(k_{d_0})</td>
<td>IP(_3) degradation rate</td>
<td>1.25 s(^{-1})</td>
<td>Smooth muscle</td>
<td>Fink et al. (1999)</td>
</tr>
<tr>
<td>(k_a)</td>
<td>G-protein activation rate</td>
<td>0.017 s(^{-1})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(k_d)</td>
<td>G-protein deactivation rate</td>
<td>0.15 s(^{-1})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>([\text{PIP}_2]_2)</td>
<td>Total number of PIP(_2) molecules</td>
<td>(5.0 \times 10^4)</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(r_e)</td>
<td>PIP(_2) replenishment rate</td>
<td>10, 0.1, 0.015 s(^{-1})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(\delta)</td>
<td>G-protein intrinsic activity parameter</td>
<td>1.234, 1.235, 1.238 (\times 10^{-3})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(K_e)</td>
<td>Dissociation constant for Ca(^{2+}) binding to PLC</td>
<td>0.4 (\mu)M</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(\alpha)</td>
<td>Effective signal gain parameter</td>
<td>2.781 (\times 10^{-5}) s(^{-1})</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(v)</td>
<td>Cell volume</td>
<td>(5 \times 10^{-16}) m(^3)</td>
<td>See text</td>
<td></td>
</tr>
<tr>
<td>(N_o)</td>
<td>Avogadro’s constant</td>
<td>6.0225 (\times 10^{23})</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
increasing and show characteristic saturation at large concentrations of agonist. The peak IP₃ concentration decreases for decreasing values of \( r \), because there is greater depletion of PIP₂ and hence the maximum rate of IP₃ production will be lower (see also Fig. 5(a)). The equilibrium IP₃ concentration is less sensitive to changes in \( r \), because in the steady state the level of receptor activity does not significantly perturb the amount of PIP₂ from the basal level, \([\text{PIP}_2]_{\text{basal}}\). The peak IP₃ concentration actually begins at the basal level of IP₃ (10 nM) at low agonist concentrations but this is difficult to distinguish from zero concentration of IP₃ in Fig. 6(a).

The model can also be used to reproduce the results of experiments performed by Garrad et al. (1998) (see their Fig. 3A) designed to measure the agonist concentration dependence of receptor activation and desensitization. In their work, the activation curve was constructed by measuring the peak Ca²⁺ concentration produced after application of agonist. The desensitization curve was constructed by incubating the cells with agonist of the specified amount for 5 min, followed by a wash period and then by the reapplication of the EC₅₀ amount of agonist derived from the activation curve (the amount of agonist required to illicit half-maximal Ca²⁺ response). This experimental procedure can be simulated by extending Eq. (38) in a piecewise fashion and solving Eqs. (13), (14) and (23)–(29) with initial conditions given by Eq. (40). A wash period of 100 s was used in the simulations so as to allow the return of the model variables \([\text{IP}_3], [G]\) and \([\text{Ca}^{2+}]\) to their equilibrium values. The results of the simulations are shown in Fig. 7(a) along with the experimental data from Garrad et al. (1998, Fig. 3A). The shape of the desensitization curve is a result of there being a low desensitization rate at low agonist concentrations, thus leaving an abundance of unphosphorylated receptors when the agonist is re-applied. At high agonist concentrations, most of the receptors have been phosphorylated by the time agonist is applied a second time, hence leading to a smaller response. The activation and desensitization curves have been plotted for both \( \lambda_1 \) and \( \lambda_2 \), because in the steady state the level of receptor activity does not significantly perturb the amount of PIP₂ from the basal level, \([\text{PIP}_2]_{\text{basal}}\). The peak IP₃ concentration actually begins at the basal level of IP₃ (10 nM) at low agonist concentrations but this is difficult to distinguish from zero concentration of IP₃ in Fig. 6(a).

Fig. 4. Variations in the real parts of the eigenvalues of Eqs. (13) and (14) with respect to ligand concentration. The eigenvalues, \( \lambda_1 \) and \( \lambda_2 \), are the roots of Eq. (47) and this equation was solved for different values of \( [L] \) over the range of ligand concentrations shown. The time rate of decay of the number of surface receptors (see Fig. 3(b)) is governed by \( e^{l_1} \). For ligand concentration above about 1 μM, \( |\lambda_1|>|\lambda_2| \) so the term \( e^{l_1} \) vanishes rapidly with increasing time and the decay is governed almost entirely by \( e^{l_2} \).
experimental data. For \( r_r = 10 \text{ s}^{-1} \), the theoretically determined EC50 and IC50 levels (the latter being the amount of agonist required to produce half-maximal desensitization) values were 180 and 489 nM, respectively. For comparison, the experimental values for EC50 and IC50 determined by Garrad et al. (1998) are 250 ± 30 and 430 ± 100 nM (for the wild-type receptor).

Fig. 7(b) shows theoretical calcium transients resulting from the simulated experimental procedure used to construct the theoretical activation–desensitization curves in Fig. 7(a). Curves for an application of a small (50 nM) and a large (5 μM) concentration of ligand are shown, for both values of \( r_r \). For each of the curves in Fig. 7(b), the magnitude of the first peak corresponds to the height of the activation curve in Fig. 7(a) and the magnitude of the second peak corresponds to the height of the desensitization curve.

### 3.2. Parameter value selection

In this section, some explanation of how the unknown parameter values (indicated by the ‘See text’ column in Table 1) were chosen. In some cases, recourse was made to the parameter values determined in the second paper (Lemon et al., 2003) where the model was used to fit other experimental data for purinergic stimulation of cells.

In the absence of a definite value for \( K_1 \) for the P2Y2 receptor, a value of 5 μM has been used. This value was found to be optimal in fitting the model to experimental data. Once \( K_1 \) is set, the horizontal positioning of the theoretical curve in Fig. 3(a) is determined by the value \( K_2 \) (and to a less extent by the values of \( k_r \) and \( k_e \), see below) and this was chosen according to the experimental data in that figure.

The parameter \( \zeta \) was adjusted to set the concentration of surface receptors at saturating agonist levels evident in the experimental data in Fig. 3(a). Without an immobile fraction of receptors, the ratio \( k_r/k_e \) would have to be made larger to give a higher rate of recycling of receptors. This however would mean a higher fraction of dephosphorylated receptors at large agonist concentrations and would not allow a good fit of the theoretical desensitization curve to the data in Fig. 7(a). Hence, the
ratio of the values of $k_r$ and $k_c$ was kept small and adjusted to obtain a best fit in Fig. 3(a). The value of $k_c$ itself was chosen to fit the decay rate of the experimental transient in the number of surface receptors in Fig. 3(b). The value for $k_a$ used in this paper was adapted from the results of Mahama and Linderman (1994) for stochastic simulations of G-protein dynamics. In their paper, a simplified equation is given where the rate of G-protein activation is proportional to the numbers of inactive G-proteins and ligand bound receptors (see their Eq. (6)). The simplified theory is shown to be applicable for the case of low diffusivity and no ligand switching, for which the cell averaged encounter rate constant $k_c = 1 \times 10^{-6} \text{ s}^{-1}$ (see their Fig. 8). The value for $k_a$ is assigned the value of this rate in the presence of the maximum number of signalling receptors in this paper, hence $k_a = k_c 4[R_f]$. Estimates for the GTPase rate parameters vary widely between cell types; for example, a range of $0.02$–$2 \text{ s}^{-1}$ for the GTPase rate, is quoted by Mahama and Linderman (1994). A suitable value for the G-protein deactivation rate parameter, $k_d$, determined in Lemon et al. (2003) from experimental rise time data, lies in this range, and the same value was used in this paper. Also determined in Lemon et al. (2003) was the value of the dissociation constant for the binding of $\text{Ca}^{2+}$ to PLC, $K_c = 0.4 \text{ mM}$, and this value was also used in the present paper.

In this paper the 1321N1 cells will be assumed to have a volume, $v = 5 \times 10^{-16} \text{ m}^3$. This value is based on a spherically shaped cell of diameter approximately 10 $\mu$m.

The value of $r_l$ determines the rate of recovery of PIP$_2$ levels after most of the P$_2$Y$_2$ receptors have been...
choices for concentrations were attained by making appropriate
of d
Balla (1998). In practice, the levels and rate of PIP 2
tion, similar to that shown in Fig. 4B of V
van and Balla (1998). In practice, the levels of PIP 2
replenishment are likely to vary widely between different
cells. Hence the other two values of r, were chosen to
give a range of lower depletion levels of PIP 2 during
agonist stimulation.

The desired basal level of Ca 2+ was to be approximately 100 nM (a typical value widely used in theoret-
ical studies and the same as that used in Lemon et al., 2003). The desired maximum possible change in Ca 2+
concentration was chosen to be approximately 310 nM
which lies within the experimentally determined range
282±80 nM given in Garrad et al. (1998). These
concentrations were attained by making appropriate
choices for n1, n2, n3 and [(Ca 2+ )], which are listed in
Table 1. The numerically determined values of [Ca 2+ ]bas
and hbas are 96.1 nM and 0.6158 which are close to the values of [Ca 2+ ]bas = 115.6 nM and hbas = 0.5737,
computed using Eq. (42).

Based on the observation by Luzzi et al. (1998) that in
an unstimulated cell the concentration of IP 3 is not more
than a few tens of nano molar, the basal concentration
of IP 3 has been chosen to be 10 nM. The three values
of δ corresponding to the three values of r, chosen to
to obtain [IP 3 ]bas = 10 nM, are given in Table 1. Also
shown, for the case of r, = 10 s −1 , are the correspon-
ding values of [G]bas, [PIP 2 ]bas, [Ca 2+ ]bas and hbas. These
values do not differ significantly from those of the cases
r, = 0.1 and 0.015 s −1.

The value of k p was chosen, first roughly, so as to make the Ca 2+
and IP 3 transients reach equilibrium
levels, at saturating ligand concentrations, within
approximately 5 min. The values of z, k p and K 1 all
affect the horizontal positioning of the theoretical
activation and desensitization curves (and hence the
EC 50 values of these curves). These parameters were
adjusted to match the experimental data in Fig. 7(a).

4. Discussion

Mathematical modelling has been carried out of the
processes leading from agonist stimulation of cells
containing membrane metabotropic receptors, to the
subsequent Ca 2+ IP 3 and PIP 2 response. A simplified
and yet realistic model has been produced that captures
the essential elements of each part of the signal
transduction process. A quantitative account has suc-
cessfully been given of the second messenger
response of single cells upon application of external
ligand to metabotropic receptors.

The precise details of the mechanisms involved in
metabotropic receptor activation and desensitization
depend on the receptor type (reviewed in Ferguson,
2001). For example, the β 2 -adrenergic receptor (β 2 -AR)
is phosphorylated by G-protein coupled receptor
kinases (GRKs) which preferentially bind to the
agonist-bound receptor. Subsequent binding of β-arrest-
ins precludes interaction of the receptor with G-proteins
hence causing desensitization. β-arrestin also acts as an
endocytotic adaptor protein targeting the β 2 -AR for
internalization via clathrin coated pits. Other proteins
such as the Rab4 and Rab5 GTPases, both located in
early endosomal membranes, are involved in internal-
ization, sorting and recycling of the β 2 -AR (Seachrist
et al., 2000).

Less is known about the corresponding mechanisms
for the P 2Y 2 receptor. This receptor has sites for
phosphorylation by protein kinase C (PKC) and
possible sites for GRK phosphorylation (Garrad et al.,
1998). Indeed, PKC has been shown to have a role in
P 2Y 2 receptor desensitization (Chen and Lin, 1999;
Otero et al., 2000) but phosphorylation by other protein
kinases may be involved (Otero et al., 2000). In the
absence of definite information regarding the biochem-
ic mechanisms for the regulation of the P 2Y 2 receptor,
the mechanisms given above for the β 2 -AR have been
used to construct the mathematical model in this paper.
This is justified by the fact that the regulation of the
P 2Y 2 receptor involves the same processes given above.

The effects of internalization of ligand (for example, ligand-dependent sorting in endosomes) have been
neglected, as have also receptor degradation and
insertion at the cell surface, since these processes
generally become significant only after hours of con-
tinual application of agonist whereas the present model
is only concerned with times of not more than several
tens of minutes.

Recent studies suggest the importance of the spatial
localization of membrane and cytosolic components of
second messenger signalling (Haugh and Lauffenburger
1998; Golovina and Blaustein, 1997). The model
presented here, while neglecting this consideration, was
found to be adequate in describing the experimental
data. It is straightforward, however, to generalize the
model to include diffusion of subspecies, but this is
beyond the scope of the present paper.

The processes linking active metabotropic receptors
to production of IP 3 may comprise more than one
pathway depending on the cell type. Each pathway may
comprise different subtypes of G-protein subunits and
PLC. In smooth muscle the P 2Y 2 receptor activates both
PLC-β1 via Gzq/11 and PLC-β3 via Gβ13 (Murthy and Makhlouf, 1998). In Chinese hamster ovary cells, however, P2Y2 functionally couples only to PLC-β3 (Strassheim and Williams, 2000). In the model presented here we have assumed that there is only one pathway with one type of G-protein (Gzq/11) and PLC (PLC-β1).

All types of phospholipase C (PLC-β, PLC-γ, PLC-δ) require the presence of Ca^{2+} for activation (Rebecchi and Pentyala, 2000). Strong activation of PIP₂ hydrolysis by Ca^{2+} is evident in many cell systems (Harootunian et al., 1991; Taylor et al., 1991; Capozzi et al., 1999) but there is evidence that this is only true when PLC-δ is involved (Allen et al., 1997). This activation is also evident in the results of Hirose et al. (1999), to which the present model will also be applied (Lemon et al., 2003), and so Ca^{2+} activation of PLC is included in the model. However, the model G-protein cascade involves only PLC-β and so the catalytic effect of Ca^{2+} on PIP₂ hydrolysis is assumed to be due to activation of PLC-β by Ca^{2+}.

Degradation of IP₃ may involve a 3-kinase (Takazawa et al., 1990) or a 5-phosphatase (Verjans et al., 1994). The 3-kinase has shown to be stimulated by Ca^{2+} resulting in a Ca^{2+} dependent IP₃ degradation rate. For the purposes of this study, however, it is assumed that IP₃ is metabolized by the Ca^{2+} independent 5-phosphatase which justifies using a constant value for kdeg.

A simplified yet realistic model has been chosen for the IP₃ channel, a model that has the potential to allow Ca^{2+} oscillations and waves (see for example De Young and Keizer, 1992; Wagner and Keizer, 1994). The parameter values chosen here were such that no oscillations occur for any concentration of IP₃, this being consistent with the experimental evidence. Simulations show that both sustained and damped Ca^{2+} oscillations are ubiquitous when the lower value for the Ca^{2+} pump dissociation constant, k₃ = 0.1 µM representing the SERCA2 isoform, is used. That the higher value used here, k₃ = 0.4 µM corresponding to the SERCA1 isoform, suppresses the occurrence of oscillations may indicate this is the dominant isoform in these cells.

Many of the rate parameter values for the chemical reactions in the detailed signal transduction model are unknown. It has been the approach here to use, without evidence to the contrary, the assumptions of rapid kinetics and subsaturation binding to simplify the equations for the reactions (see Appendix A). These assumptions do not affect the qualitative behaviour of the system as a whole but do allow each submodule in the model to be characterized by a minimal number of free parameters. This can allow unambiguous determination of parameter values for fitting the model to experimental data.

The relative difference in magnitudes between the desensitization rate parameter kₚ and the receptor endocytosis and recycling parameters, kₑ and kᵣ, respectively, has ensured that desensitization occurs over a faster time scale than internalization. As seen in Fig. 5(b), for a step application of 1 nM UTP, the theoretical IP₃ concentration transient has a decay half time of approximately 30 s whereas, as shown in Fig. 3(a), the decay in the number of surface receptors has a half-time of approximately 2.5 min. That desensitization occurs more rapidly than internalization appears to be typical of the P₂Y₂ receptor, although the absolute rates may vary between preparations. For example, in another experiment of the stimulation of 1321N1 cells with UTP (Stromek and Harden, 1998) maximal accumulation of inositol phosphates occurred with a half-time of approximately 2.5 min, whereas the half-time of the decay of surface receptors was approximately 15 min. The faster rate of desensitization relative to internalization is typical of other types of metabotropic receptors; for a review see Ferguson (2001) and Bünemann et al. (1999).

In Figs. 6(a) and (b) both the maximum and steady-state IP₃ concentration curves are monotonic increasing and plateau at large concentrations of agonist due to receptor saturation. For raised levels of Ca^{2+} and IP₃ to be maintained over extendend periods, recycling of dephosphorylated receptors and resupply of membrane PIP₂ are essential. Typically in experiments cells are treated with LiCl and measurements of total inositol phosphate (including species of phosphorylated IP₃) accumulation is measured after some fixed time. Treatment with LiCl alters the pathway of resupply of PIP₂ to the membrane and for this reason such data is not directly applicable to this model. There is however some data available of measurements of equilibrium IP₃ concentration with respect to ligand (Waugh et al., 1999) which confirms the qualitative form of the curves in Fig. 6(b).

The theoretical time taken for IP₃ concentration to peak after application of agonist is in agreement with the 5–20 s range observed by Strøbæk et al. (1996) for purinergic stimulation of human coronary smooth muscle cells. Experimental studies suggest that the range of concentrations of IP₃ attainable in cells is from tens of nM to tens of µM (Luzzi et al., 1998; Khodakah and Ogden, 1993; Horstman et al., 1988). The modelling in this paper has predicted a concentration range for IP₃ of 10 nM (basal) to approximately 12 µM at saturating levels of agonist.

An important result of this study comes from the modelling of mechanisms for PIP₂ depletion and resupply. Using reasonable estimates for the replenishment rate parameter, rₑ, and level of total PIP₂, [PIP₂]₀, the results show that the level of PIP₂ depletion has a significant effect on the Ca^{2+} and IP₃ response. For the lowest rate of PIP₂ resupply, rₑ = 0.015 s⁻¹, the IP₃ and Ca^{2+} concentrations were
decreased by up to 80% and 30%, respectively, compared to the concentrations at the highest rate of resupply, \( r_r = 10 \, \text{s}^{-1} \) (see Figs. 5(a) and (b)). Depletion of PIP\(_2\) may therefore contribute significantly to the deactivation of the signal transduction processes in cells.

Estimates of the density of PIP\(_2\) molecules in the plasma membrane are not widely available from the literature. However, using the method explained above to determine a likely value for \([\text{PIP}_2]_T\), the number of PIP\(_2\) molecules turns out to be comparable to the number of receptors and G-protein molecules, \([R_T]\) and \([G_T]\), respectively.

### Appendix A. Detailed mathematical model of the G-protein cascade

In this section, a detailed mathematical model is formulated for the G-protein cascade occurring due to a homogeneous distribution of P\(_2\)Y\(_2\) receptors in the cell membrane. By making various assumptions a reduced and simplified set of model equations are then derived. The schematic diagram for the G-protein cascade is shown in Fig. 2.

#### A.1. P\(_2\)Y\(_2\) receptor model

The following process by which any G-protein coupled receptor activates G-protein molecules is well established, see for example Lamb and Pugh (1992), Lauffenburger and Linderman (1993, pp. 185–187). A P\(_2\)Y\(_2\) receptor binds a molecule of G\(-\)GDP from the membrane and brings about the exchange of a GTP nucleotide (assumed to be immediately available from the cytosol) for the GDP nucleotide bound to the receptor. These processes can be modelled with the scheme

\[
\begin{align*}
G \cdot \text{GDP} + P_2Y_2 & \xrightarrow[k_1^+]{k_1^-} P_2Y_2 \cdot G \cdot \text{GDP} \\
& \xrightarrow[k_2^-]{k_2^+} P_2Y_2 \cdot G \cdot \text{GTP}
\end{align*}
\]

for forward and reverse rate constants \(k_1^+, k_1^-\). In Appendix B it is shown that

\[
k_2 = \hat{k}_2 + (k_2^* - \hat{k}_2)\rho_r,
\]

where \(k_2^*, \hat{k}_2\) are the exchange rates for the ligand bound and unbound receptor. \(\rho_r\) is the ratio of the amount of ligand bound receptor to total receptor and is determined independently of the G-protein kinetics.

#### A.2. G-protein subunit kinetics

The kinetics for the interaction of the G-protein subunits and the trimer is assumed to depend on whether the nucleotide bound to it is GTP or GDP. Hence

\[
G \cdot \text{GTP} \xrightarrow[k_1^-]{k_1^+} G \cdot \text{GTP} + G\beta y
\]

and

\[
G \cdot \text{GDP} + G\beta y \xrightarrow[k_3^-]{k_3^+} G \cdot \text{GDP},
\]

for forward rate constants \(k_3^+, k_3^-\) and reverse rate constants \(k_1^-, k_1^-\).

#### A.3. PLC effector model

The activity of the PLC molecule is mediated by several different binding sites (Rebecchi and Pentyala, 2000). Of relevance to the present study are the three sites each of which bind to the G\(_\alpha\) subunit, Ca\(^{2+}\) ion and PIP\(_2\) molecule. It is assumed that the probability of binding to any of these sites is not dependent on the state of occupancy of the other sites.

##### A.3.1. Binding of the G\(_\alpha\) subunit

The \(G\alpha \cdot \text{GTP} (G\alpha_{q/11} \cdot \text{GTP})\) molecules are deactivated by binding to GTPase activating proteins (GAPs; see Iyengar, 1997). PLC-\(\beta 1\) (hereafter PLC) itself acts as a GAP for G\(_q\) (Morris and Scarlata, 1997; Mukhopadhyay and Ross, 1999) hence the interaction of the G-protein with PLC is modelled with the following kinetics:

\[
G\alpha \cdot \text{GTP} + \text{PLC} \xrightarrow[k_1^-]{k_1^+} G\alpha \cdot \text{GTP} \cdot \text{PLC}
\]

\[
\xrightarrow[k_3^-]{k_3^+} G\alpha \cdot \text{GDP} \cdot \text{PLC} \xrightarrow[k_3^-]{k_3^+} G\alpha \cdot \text{GDP} + \text{PLC},
\]

for forward rate constants \(k_1^+, k_1^-\) and reverse rate constant \(k_3^-\).

##### A.3.2. Binding of Ca\(^{2+}\)

The kinetics for the binding of Ca\(^{2+}\) from the cytosol to PLC are

\[
\text{PLC} + \text{Ca}^{2+} \xrightarrow[k_4^-]{k_4^+} \text{PLC} \cdot \text{Ca}^{2+}.
\]

##### A.3.3. Binding of PIP\(_2\)

PLC binds to the membrane at the pleckstrin homology domain of PIP\(_2\). PLC which is also bound to Ca\(^{2+}\) and G\(_\alpha\) \cdot GTP is considered to be most effective in hydrolysing PIP\(_2\) molecules to give IP\(_3\) and diacylglycerol. The kinetics for the interaction of PLC and
A.4. Receptor–effector equations

The quantity of that species, for forward and reverse rate constants \( k_i^+ \), \( k_i^- \). In a similar calculation to that in Appendix B it can be shown that

\[
k_i = k_i^+ + (k_i^+ - k_i^-)p_b, \tag{A.1}
\]

with \( p_b \) being the ratio of the amount of \( \mathrm{Ca}^{2+} \) and \( \zeta \) GTP bound PLC to total PLC.

A.4. Mathematical formulation

Using the law of mass action, the kinetic schemes presented above can be formulated in terms of a set of coupled ordinary differential equations. It suffices to give these equations in the shorthand form

\[
\frac{du}{dt} = Au + Bu_u, \tag{A.2}
\]

\[
u = u_0 \quad \text{at} \quad t = 0, \tag{A.3}
\]

where \( u \) is the vector of species concentrations, \( A \) is a matrix specifying the forward reaction steps and \( B \) is a rank 3 tensor specifying the reverse reaction steps. In what follows, a bracketed species name is used to denote the quantity of that species.

A.4.1. Receptor–effector equations

It can be shown using Eq. (A.2) that (\( d/dt \) \([P_2 Y_2] + [P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}] + [P_2 Y_2 \cdot \zeta \cdot \mathrm{GTP}] = 0 \) and \( d/dt \) \([\mathrm{PLC}] + [\zeta \cdot \mathrm{GTP} \cdot \mathrm{PLC}] + [\zeta \cdot \mathrm{GDP} \cdot \mathrm{PLC}] = 0 \), from which follows the conservation conditions for the total number of receptors, \([P_2 Y_2]_T\), and PLC, \([\mathrm{PLC}]_T\),

\[
\begin{align*}
[P_2 Y_2] + [P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}] + [P_2 Y_2 \cdot \zeta \cdot \mathrm{GTP}] &= [(P_2 Y_2)_T], \\
[\mathrm{PLC}] + [\zeta \cdot \mathrm{GTP} \cdot \mathrm{PLC}] + [\zeta \cdot \mathrm{GDP} \cdot \mathrm{PLC}] &= [(\mathrm{PLC})_T].
\end{align*} \tag{A.4}
\]

To simplify the initial value problem (A.2)–(A.3), it is assumed that all processes act over a fast time scale except the GTP-GDP exchange and GTP hydrolysis. The fast processes are treated as being in dynamic equilibrium hence

\[
\begin{align*}
[G \cdot \mathrm{GDP}] [P_2 Y_2] &= K_1 [P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}], \\
[\mathrm{PLC}] [\zeta \cdot \mathrm{GTP}] &= K_5 [\zeta \cdot \mathrm{GTP} \cdot \mathrm{PLC}], \\
[P_2 Y_2] [\zeta \cdot \mathrm{GTP}] &= K_5 [P_2 Y_2 \cdot \zeta \cdot \mathrm{GTP}], \\
[\mathrm{PLC}] [\zeta \cdot \mathrm{GDP}] &= K_5 [\zeta \cdot \mathrm{GDP} \cdot \mathrm{PLC}], \\
[\zeta \cdot \mathrm{GTP}] &= K_5 [\zeta \cdot \mathrm{GTP}], \\
[\zeta \cdot \mathrm{GDP}] [\zeta \cdot \beta \gamma] &= \hat{K}_3 [\zeta \cdot \mathrm{GDP}], \tag{A.5}
\end{align*}
\]

where \( K_1 = \hat{k}_i^- / k_i^+ \) except that \( K_3 = k_i^+ / k_i^- \).

By taking sums and differences of combinations of the equations constituting (A.2), new equations are obtained where the rate constants for the rapid kinetics do not appear. There are at most three such equations that are linearly independent there being 11 variables but 8 constraints (A.4)–(A.5). Alternatively, these three equations can be reasoned by applying the law of mass action to groups of species as follows. Firstly, the rate of change of species bound with the GDP nucleotide is the difference between the rate of deactivation of \( \zeta \cdot \mathrm{GTP} \) and the rate of activation of \( \zeta \cdot \mathrm{GDP} \) hence

\[
\begin{align*}
\frac{d}{dt}([\zeta \cdot \mathrm{GDP}] + [P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}] &+ [\zeta \cdot \mathrm{GTP} + [\zeta \cdot \mathrm{GDP} \cdot \mathrm{PLC}]) \\
&= \hat{k}_2 [\zeta \cdot \mathrm{GTP} \cdot \mathrm{PLC}] - k_2 [P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}]. \tag{A.6}
\end{align*}
\]

Similarly for molecules bound with \( \zeta \cdot \mathrm{GTP} \) the equation

\[
\begin{align*}
\frac{d}{dt}([\zeta \cdot \mathrm{GTP}] + [P_2 Y_2 \cdot \zeta \cdot \mathrm{GTP}] &+ [\zeta \cdot \mathrm{GTP} + [\zeta \cdot \mathrm{GDP} \cdot \mathrm{PLC}]) \\
&= k_2 [P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}] - \hat{k}_2 [\zeta \cdot \mathrm{GTP} \cdot \mathrm{PLC}]. \tag{A.7}
\end{align*}
\]

is obtained. Next, since the GTPase does not alter the total number of \( \zeta \) subunits, it follows that the difference between the rate of change of the numbers of \( \zeta \beta \gamma \) and \( \zeta \) subunits is zero hence

\[
\begin{align*}
\frac{d}{dt}([\zeta \beta \gamma] - \frac{d}{dt}([\zeta \cdot \mathrm{GTP}] &+ [P_2 Y_2 \cdot \zeta \cdot \mathrm{GTP}] + [\zeta \cdot \mathrm{GTP} + [\zeta \cdot \mathrm{GDP} \cdot \mathrm{PLC}] + [\zeta \cdot \mathrm{GDP}] = 0. \tag{A.8}
\end{align*}
\]

A more appropriate third equation is derived by taking the sum of Eqs. (A.7) and (A.8) which is

\[
\begin{align*}
\frac{d}{dt}([\zeta \beta \gamma] &+ [\zeta \cdot \mathrm{GTP}] + [P_2 Y_2 \cdot \zeta \cdot \mathrm{GTP}] - [\zeta \cdot \mathrm{GTP} + [\zeta \cdot \mathrm{GDP} \cdot \mathrm{PLC}] + [\zeta \cdot \mathrm{GDP}] = k_2 [P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}] - \hat{k}_2 [\zeta \cdot \mathrm{GTP} \cdot \mathrm{PLC}]. \tag{A.9}
\end{align*}
\]

It is possible to eliminate all but the three dependent variables \([\zeta \cdot \mathrm{GDP}]\), \([\zeta \beta \gamma]\) and \([\zeta \cdot \mathrm{GTP}]\) from Eqs. (A.6), (A.7) and (A.9). The remaining variables are expressed in terms of these three using Eqs. (A.4) and (A.5) thus

\[
\begin{align*}
[P_2 Y_2 \cdot \zeta \cdot \mathrm{GDP}] &= \frac{[P_2 Y_2]_T [\zeta \cdot \mathrm{GDP}]}{K_1 + [\zeta \cdot \mathrm{GDP}] + K_5 [\zeta \beta \gamma] [\zeta \cdot \mathrm{GTP}]}
\end{align*}
\]
\[
\frac{d[G\beta_I]}{dt} = \frac{\dot{k}_2([PLC]_T)[Gz \cdot GTP]}{K_1 + [G \cdot GDP]} + \frac{k_2([P_2Y_2]_T)[G \cdot GDP]}{K_1 + [G \cdot GDP]}. \tag{A.13}
\]

### A.4.2. \(Ca^{2+}\) binding equations

A similar development is carried out to determine the relation between the numbers of \(Ca^{2+}\) bound PLC and total PLC. Only two equations are required for this namely the conservation equation, \([PLC] + [PLC \cdot Ca^{2+}] = ([PLC]_T)\), and the fast kinetics assumption, \([PLC][Ca^{2+}] = K_c[PLC \cdot Ca^{2+}]\), where \(K_c = k_{-4}^+ / k_4^+\). Together these equations imply \([PLC \cdot Ca^{2+}] = ([PLC]_T)[Ca^{2+}]/(K_c + [Ca^{2+}])\).

### A.4.3. \(PIP_2\) binding equations

It is assumed that the binding kinetics are fast whilst the hydrolysis and replenishment rates are slow. From the equations for the reaction kinetics there is obtained the conservation equation

\[
[PLC] + [PLC \cdot PIP_2] = ([PLC]_T), \tag{A.14}
\]

the fast kinetics condition

\[
[PLC][PIP_2]_M = K_4[PLC \cdot PIP_2], \tag{A.15}
\]

where \(K_4 = k_4^- / k_{-4}^+\) and \([PIP_2]_M\) is used to denote the quantity of free \(PIP_2\) in the membrane. From Eqs. (A.14) and (A.15) follows the relation

\[
[PLC \cdot PIP_2] = \frac{([PLC]_T)[PIP_2]_M}{K_4 + [PIP_2]_M}. \tag{A.16}
\]

Taking appropriate combinations of the kinetic equations gives

\[
\frac{d}{dt}([PIP_2]_M + [PLC \cdot PIP_2]) = -k_3[PLC \cdot PIP_2] + r_f[H], \tag{A.17}
\]

where replenishment of \(PIP_2\) to the membrane has been included as described in the text. The total number of \(PIP_2\) molecules in the membrane is

\[
[PIP_2] = [PLC \cdot PIP_2] + [PIP_2]_M, \tag{A.18}
\]

and solving Eqs. (A.14), (A.15) and (A.18) gives a quadratic equation relating \([PLC \cdot PIP_2]\) and \([PIP_2]\),

\[
[PLC \cdot PIP_2]^2 - ([PLC]_T + [PIP_2] + K_4)[PLC \cdot PIP_2] + ([PLC]_T)[PIP_2] = 0, \tag{A.19}
\]

which has solution

\[
[PLC \cdot PIP_2] = \frac{1}{2} \left\{ ([PLC]_T) + [PIP_2] + K_4 - \sqrt{([PIP_2] + [PIP_2] + K_4)^2 - 4([PLC]_T)[PIP_2]} \right\}. \tag{A.20}
\]
Because binding at the sites on the PLC molecule is mutually exclusive, the fraction $\rho_b$ of PLC bound to both Gz \cdot GDP and Ca$^{2+}$ is

$$\rho_b = \frac{[Gz \cdot GDP \cdot PLC] \cdot [PLC \cdot Ca^{2+}] }{[PLC]_f} = \frac{[Gz \cdot GDP]}{K_i + [Gz \cdot GDP]} \cdot \frac{[Ca^{2+}]}{K_c + [Ca^{2+}]}.$$

(A.21)

Combining (A.17), (A.18), (A.21) and (A.1) gives

$$\frac{d[PIP_2]}{dt} = -I + r_i[H],$$

(A.22)

where the IP$_3$ production rate is

$$I = \left( k_{h} + (k^*_a - \bar{k}_a) \right) \frac{[Ca^{2+}]}{K_c + [Ca^{2+}]},$$

$$\times \frac{[Gz \cdot GDP]}{K_i + [Gz \cdot GDP]} \cdot [PLC \cdot PIP_2].$$

(A.23)

A.5. Linearized theory

Eqs. (A.11)–(A.13) are linear when $[G \cdot GDP], [(P_2Y_2)_f] \ll K_i$ and $[Gz \cdot GDP], [(PLC)_f] \ll \bar{K}_1$, in which case

$$\frac{d[G \cdot GDP]}{dt} = k_a[Gz \cdot GDP] - k_a(\delta + \rho_r)$$

$$\times [G \cdot GDP],$$

(A.24)

$$\frac{d[Gz \cdot GDP]}{dt} = -k_d[Gz \cdot GDP] + k_a(\delta + \rho_r)$$

$$\times [G \cdot GDP],$$

(A.25)

where $k_a = (k^*_a - \bar{k}_a)((P_2Y_2)_f)/K_i, k_d = \bar{k}_d[(PLC)_f]/\bar{K}_1$, and the parameter $\delta = K_c/(k^*_a - \bar{k}_a)$ measures the ratio of the activity of ligand unbound and bound receptor. Adding Eqs. (A.24) and (A.25) gives $(d/d\delta)([G \cdot GDP] + [Gz \cdot GDP]) = 0$, so $[G \cdot GDP] + [Gz \cdot GDP] = [G_T]$ and then Eq. (A.25) becomes

$$\frac{d[Gz \cdot GDP]}{dt} = k_a(\delta + \rho_r)([G_T] - [Gz \cdot GDP])$$

$$- k_d[Gz \cdot GDP].$$

Similarly Eqs. (A.22), (A.23) and (A.20) are linearized by assuming $[PIP_2], [(PLC)_f] \ll K_4$. In Eq. (A.20), $2[PLC \cdot PIP_2]/K_4$ is expanded in a double Taylor series in the variables $[(PLC)_f]/K_4$ and $[PIP_2]/K_4$. Retaining the highest order term gives $[PLC \cdot PIP_2] = [(PLC)_f][PIP_2]/K_4$. Eqs. (A.22), (A.23) and (A.20) become

$$\frac{d[PIP_2]}{dt} = -I + r_i[H],$$

$$I = \left( k_{h} + (k^*_a - \bar{k}_a) \right) \frac{[Ca^{2+}]}{K_c + [Ca^{2+}]},$$

$$\times \frac{[(PLC)_f][PIP_2]}{K_4}.$$  

(A.26)

The rate of hydrolysis of PIP$_2$ due to PLC not bound to both Ca$^{2+}$ and Gz \cdot GDP is ignored hence $\bar{k}_h = 0$. It is convenient to re-write (A.26) in the form

$$I = x \left( \frac{[Ca^{2+}]}{K_c + [Ca^{2+}]} \right) [Gz \cdot GDP][PIP_2].$$

Appendix B. Proof of modification of GDP–GTP exchange rate

Consider separately the kinetics for the ligand bound receptor, $P_2Y_2^*$,

$$G \cdot GDP + P_2Y_2^* \xrightarrow{k^*_i} P_2Y_2^* \cdot G \cdot GDP$$

$$\xrightarrow{k_i} P_2Y_2^* \cdot G \cdot GDP,$$

and unbound receptor $P_2\bar{Y}_2$,

$$G \cdot GDP + P_2\bar{Y}_2 \xrightarrow{k^*_i} P_2\bar{Y}_2 \cdot G \cdot GDP$$

$$\xrightarrow{k_i} P_2\bar{Y}_2 \cdot G \cdot GDP,$$

(A.27)

In what follows, a bracketed species name is used to denote the quantity of that species. Using the rapid binding assumption, the ratio $[P_2Y_2^* \cdot G \cdot GDP]/([P_2Y_2^* \cdot G \cdot GDP] + [P_2\bar{Y}_2 \cdot G \cdot GDP])$ is the same as the ratio of bound receptor to total receptor, $\rho_r$, since

$$\rho_r = ([P_2Y_2^*] + [P_2\bar{Y}_2] \cdot G \cdot GDP]$$

$$+ [P_2Y_2^* \cdot G \cdot GDP]/([P_2Y_2^*] + [P_2\bar{Y}_2]$$

$$+ [P_2Y_2^* \cdot G \cdot GDP] + [P_2\bar{Y}_2] \cdot G \cdot GDP]$$

$$+ [P_2Y_2^* \cdot G \cdot GDP] + [P_2\bar{Y}_2] \cdot G \cdot GDP],$$

and substituting $K_1[P_2Y_2^* \cdot G \cdot GDP] = [G \cdot GDP] [P_2Y_2^*]$, $K_1[P_2\bar{Y}_2 \cdot G \cdot GDP] = [G \cdot GDP][P_2\bar{Y}_2]$ and $K_1[P_2Y_2^* \cdot G \cdot GDP] = [G \cdot GDP][P_2\bar{Y}_2]$
Similarly the ratio of unbound receptor to total receptor, is $\rho_r = [P_2Y_2 \cdot G \cdot GDP]/([P_2Y^\bullet 2 \cdot G \cdot GDP] + [P_2Y_2 \cdot G \cdot GDP])$. These results can be arrived at intuitively because the rapid binding assumption means that bound and unbound receptors become rapidly distributed among all G-protein subspecies in the same proportion.

Replacing $k^{\bullet 2}_{2}([P_2Y^\bullet 2 \cdot G \cdot GDP] + [P_2Y_2 \cdot G \cdot GDP])$ by $\rho_r k^{\bullet 2}_{2}([P_2Y^\bullet 2 \cdot G \cdot GDP] + [P_2Y_2 \cdot G \cdot GDP])$ and $k^{\bullet 2}_{2}([P_2Y^\bullet 2 \cdot G \cdot GDP] + [P_2Y_2 \cdot G \cdot GDP])$ in the equations for the kinetic schemes above gives the equations describing the modified kinetic scheme. The effective rate constant is $k_2 = \rho_r k^{\bullet 2}_{2} + \rho_r k^{\bullet 2}_{2}$ but since $\rho_r + \rho_r = 1$ it follows that $k_2 = k^{\bullet 2}_{2} + (k^{\bullet 2}_{2} - k^{\bullet 2}_{2})\rho_r$.

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