A model of inositol 1,4,5-trisphosphate and calcium dynamics in single cells following metabotropic receptor activation

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Abstract

The application of external agonist to purinergic metabotropic receptors on single cells leads to transient inositol 1,4,5-trisphosphate (IP_3) and calcium (Ca^{2+}) responses. A mathematical model is formulated for the processes governing these responses, including the regulation of the receptor activity, the G-protein cascade and the cytosolic Ca^{2+} and IP_3 dynamics. This model is used to reproduce experimental observations on the extent of desensitization and sequestration of the P_2Y_2 receptor following its activation by uridine triphosphate and also the IP_3 and Ca^{2+} responses following this activation.

Keywords: Calcium; Inositol 1,4,5-trisphosphate; Metabotropic receptor; Endoplasmic reticulum; G-protein cascade

Introduction

Ca^{2+} is a fundamental and universal signalling agent in living organisms, transmitting information both inside cells and between them. It is literally present at conception, when a wave of Ca^{2+} sweeps across the fertilized egg and initiates cell division; at the other extreme, excess Ca^{2+} released inside a cell can lead to its death (apoptosis). In between, it regulates many cellular processes, including secretion, contraction, proliferation, neural signalling and learning and even gene transcription [1]. Cells have internal stores containing a high concentration of Ca^{2+} and an important signalling mechanism involves the activated release of Ca^{2+} from these stores. This release can be initiated by the binding of an agonist to a G-protein coupled receptor, leading to a chain of events that results in the release of Ca^{2+} from the endoplasmic reticulum (Fig. 1).

We present a unified model for these processes, starting from the binding of agonist to metabotropic receptors and leading, via a G-protein cascade, to the production of IP_3

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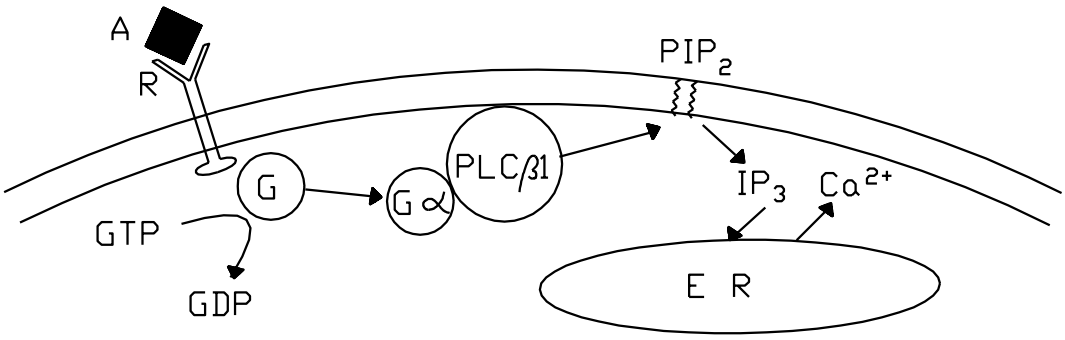

Figure 1: Schematic diagram of the principal steps leading to Ca^{2+} release from internal stores in a cell. A transmitter molecule (A) binds to a receptor (R) in the cell membrane and thereby activates a G-protein (G) inside the cell. This sets off a cascade of events that includes the activation of the phospholipase PLCβ1 and the hydrolysis of the membrane phospholipid PIP_2, giving the second-messenger molecule IP_3. This latter molecule diffuses into the cytosol and opens Ca^{2+} channels in the endoplasmic reticulum (ER) leading to the release of Ca^{2+} into the cytosol.

and the release of Ca^{2+} from the ER. The modelling falls into three linked modules: the first concerns the agonist-receptor binding and involves consideration of desensitization through phosphorylation and internalization (sequestration) of the receptors; the second concerns the G-protein cascade, leading to the production of IP_3 and the final section treats the IP_3-induced Ca^{2+} release from the ER.

Methods

The first stage involves modelling the interaction of agonist A with the receptors, R. This reversible reaction leads to the production of bound receptor AR which can then be phosphorylated to AR_P and this reversibly dissociates into A and R_P:

$$A + R \underset{k_1^-}{\overset{k_1^+}{\rightleftharpoons}} AR \overset{k_p}{\longrightarrow} AR_P \underset{k_2^-}{\overset{k_2^+}{\rightleftharpoons}} A + R_P \tag{1}$$

The other process involves the internalization (at rate k_e) of the phosphorylated bound receptor AR_P and its subsequent recycling (at rate k_r) to the surface as unbound receptor R. This scheme is a subset of the full cubic ternary model [6] and can be formulated as a set of differential equations involving the appropriate rate constants. The steps are described by differential equations; for example, the equation for the rate of production of bound receptor is

$$d[AR]/dt = k_1^+[A][R] - k_1^-[AR] - k_p[AR] \tag{2}$$

Let $[R^S] = [R] + [AR]$ be the total number of unphosphorylated receptors and $[R_P^S] =$

$[R_P] + [LR_P]$ be the total number of phosphorylated receptors. Then the above scheme reduces to

$$\frac{d[R^S]}{dt} = k_r[R_T] - \left(k_r + \frac{k_p[A]}{K_1 + [A]}\right)[R^S] - k_r[R_P^S], \tag{3}$$

$$\frac{d[R_P^S]}{dt} = [A]\left(\frac{k_p[R^S]}{K_1 + [A]} - \frac{k_e[R_P^S]}{K_2 + [A]}\right), \tag{4}$$

where $[R_T]$ is the total number of receptors, $K_1 = k_1^-/k_1^+$ and $K_2 = k_2^-/k_2^+$. In making this reduction, it is assumed that agonist binding is fast relative to the other processes and use has been made of a rapid binding approximation [5].

The next stage is the interaction of the bound receptor AR with the G-protein G.GDP leading to the replacement of GDP with GTP and its subsequent dissociation into the subunits $G_\alpha \cdot$ GTP and $G_{\beta\gamma}$. $G_\alpha \cdot$ GTP then binds to a site on PLC and this activated unit binds to PIP_2 to form the complex G_α·GTP·PLC·PIP_2. The final step in this stage is the binding of Ca^{2+} to a catalytic site on this complex leading to the hydrolysis of PIP_2 and the production of IP_3. Schematically,

$$\begin{array}{ccc} AR \cdot G & \rightleftharpoons & G \cdot PLC \cdot PIP_2 \longrightarrow IP_3 \\ & & \uparrow\downarrow \\ & & \mathrm{Ca}^{2+} \end{array} \tag{5}$$

A simplified model for the G-protein cascade is used, where the activation rate of G-protein is proportional to both the concentration of active receptor and inactive G-protein (G·GDP) and the deactivation rate is proportional to the concentration of active G-protein (Gα·GTP). Whereas the agonist bound receptor (AR) 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 concentration of $G\alpha \cdot$ GTP, denoted by $[G]$, is

$$\frac{d[G]}{dt} = k_a(\delta + \rho_r)([G_T] - [G]) - k_d[G], \tag{6}$$

where k_a and k_d are the G-protein activation and deactivation rate parameters, $[G_T]$ is the total number of G-protein molecules, δ is the ratio of the activities of the agonist unbound and bound receptor species and ρ_r is the ratio of the number of agonist bound receptors to the total number of receptors, $\rho_r = [AR]/([R_T])$

The rate of hydrolysis of PIP_2, assuming rapid kinetics for the binding of Ca^{2+}, is $r_h[\mathrm{PIP}_2]$ where $[\mathrm{PIP}_2]$ is the number of PIP_2 molecules and the rate coefficient is

$$r_h = \alpha\left(\frac{[\mathrm{Ca}^{2+}]}{K_c + [\mathrm{Ca}^{2+}]}\right)[G], \tag{7}$$

where α 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. Replenishment of PIP_2 is required for IP_3 production to be maintained over extended periods of agonist stimulation, so we have assumed that there is a recycling of IP_3 via an intracellular pool of phospholipid.

The final step to be modelled is the release of Ca^{2+} from the endoplasmic reticulum via IP_3 acting on IP_3-sensitive channels in the ER wall, and here we have used a modified version of the theory of Li and Rinzel [4].

The overall scheme is a set of equations relating agonist application to the production of IP_3 and Ca^{2+}. A number of simplifications are incorporated (in particular, the rapid binding approximation [5]) in order to concentrate on the most significant steps and reduce the number of parameters required. Those parameter whose values were not available directly from experiment were chosen in order to fit the experimental data of Garrad et al. [2] on receptor desensitization and sequestration.

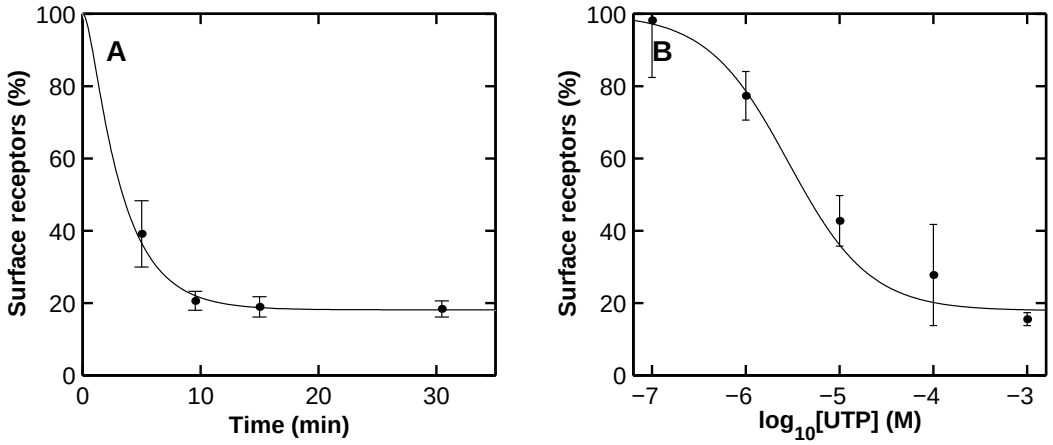

Figure 2: Experimental and theoretical number of surface receptors as a function of time following the step application of 1 mM of UTP at time $t = 0$ (panel A) and as a function of UTP concentration under equilibrium conditions (panel B). Points with error bars give the experimental data taken from Figures 4A and 5A of [2]. In the calculations, it is assumed that 15% of the receptors are immobilized in the surface and do not recycle.

Results

The first set of results concern surface receptor density following the application of agonist. Calculations were performed for both the transient and equilibrium surface receptor densities following the step application of uridine triphosphate (UTP) and good agreement was obtained with the experimental results [2] for both the time-dependence of the

response and for the equilibrium receptor density as a function of UTP concentration (Fig. 2).

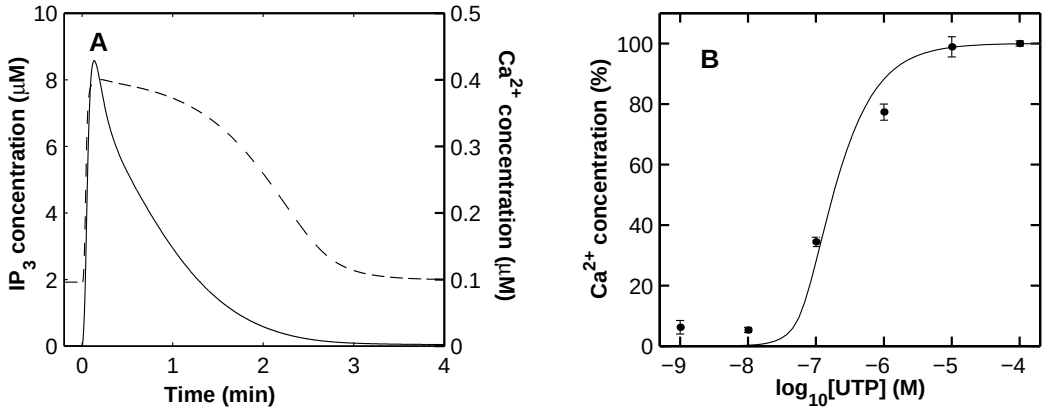

Figure 3: Panel A shown the theoretical concentrations of IP_3 (solid line, scale on left axis) and Ca^{2+} (broken line, scale on right axis) as a function of time following the step application of 1 mM UTP at $t = 0$. Panel B shown the Ca^{2+} response, as a percentage of maximum response, as a function of the applied concentration of UTP. The points with error bars show the experimental data from [2], Figure 3A.

Next, IP_3 and Ca^{2+} concentrations resulting from the step application of 1 mM UTP were calculated and their time courses are shown in Fig. 3A. Also the Ca^{2+} response as a function of agonist concentration was calculated and shown to reproduce the results of [2] (Fig. 3B).

Conclusion

This work provides a unified mathematical model of the steps leading from the activation of metabotropic receptors to the production of IP_3 and Ca^{2+}. The model has been specifically applied to systems involving the application of UTP to astrocytoma cells [2] and the application of ATP to MDCK cells [3] (results not given here) and successfully reproduces the main experimental results. However, the model is generally applicable to other systems that involve the production of IP_3 following metabotropic receptor activation, such as norandrenergic transmission in the autonomic nervous system.

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Biosketches

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