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

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1 Abstract

The application of external agonist to purinergic (P_2Y_2) receptors on single cells leads to transient inositol 1,4,5-triphosphate (IP_3) and calcium (Ca^{2+}) responses. A mathematical model is formulated for the processes governing these responses, including the regulation of the P_2Y_2 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. The model is further extended to include the process of translocation of green fluorescent protein-pleckstrin homology domain ($GFP-PHD$) from the cell membrane in the presence of IP_3, thus enabling comparison with experimental measurements of $GFP-PHD$ fluorescence.

2 Introduction

Agonist-induced activation of second messenger systems plays an important role in the mobilization of stored Ca^{2+} in neurons and in smooth muscle cells; in the latter case, this is important for the control of muscle contraction. A first stage in this process is the binding of a ligand (for example, adenosine triphosphate, ATP) to a G-protein coupled 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-biphosphate (PIP_2) to inositol 1,4,5-triphosphate (IP_3) and diacylglycerol. This IP_3 diffuses into the cytosol and interacts with Ca^{2+} channels in the endoplasmic reticulum (ER) causing the release of stored Ca^{2+} (see, for example, Zimmermann 1993). There is also a feed-back loop, in that Ca^{2+} is an activator for the hydrolysis of PIP_2.

We present a unified model for these processes, 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 ER. The modelling falls into three linked modules: the first concerns the ligand-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.

A further extension of this work includes the IP_3-induced translocation from the cell membrane of green fluorescent protein-tagged pleckstrin homology domain, thus allowing comparison with the experimental results of Hirose et al. (1999) and Yang et al. (1997).

3 Methods

The first stage involved modelling the interaction of ligand L (ATP) with the P_2Y_2 receptors, R. This reversible reaction leads to the production of bound receptor LR which can then be phosphorylated to LR_{ds} and this reversibly dissociates into L and R_{ds}. The other process involves the internalization and subsequent recycling of the phosphorylated receptors. The above scheme is a subset of the full cubic ternary model of Weiss et al. (1996a,b) and can be formulated as a set of differential equations involving the appropriate rate constants.

The next stage is the interaction of the bound receptor LR with the G-protein G.GDP leading to the replacement of GDP with GT and its subsequent dissociation into the subunits G_α.GTP and $G_{\beta\gamma}$. G_α.GTP next binds to a site on PLC and this activated unit then binds to PIP_2 to form the complx 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. Again, these steps have been formulated as a set of diffential equations, with the work of Li and Rinzel (1994) being adapted for describing the cytosolic Ca^{2+} dynamics. The final stage models the IP_3-induced translocation of the pleckstrin homology domain (PHD) of PLC from the cell membrane into the cytosol. PIP_2 binds the PHD so that an *in vivo* fluorescent indicator can be obtained by tagging the PHD with green fluorescent protein (GFP). The attachment of IP_3 displaces PIP_2 and causes the translocation of the GFP-labelled complex in the cytosol; thus the fluorescence gives both the spatial and temporal distribution of IP_3 (Hirose et al., 1999). Again, these processes have been formulated a a set of diffential equations.

The overall scheme is a set of equations relating ligand application to the production IP_3 and Ca^{2+} and then to the observed fluorescence changes. A number of simplifications are incorporated (in particular, the rapid binding approximation - Wagner and Keizer, 1994) 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 (1998) on receptor desensitization and sequestration and of Hirose et al (1999) on measurements of GFP-PHD fluorescence.

4 Results

The first set of results concern surface receptor density following the application of agonist. Calculations were performed for both the equilibrium and transient surface receptor densities following the step application of uridine triphosphate (UTP) and good agreement was obtained with the experimental results of Garrad et al. (1998) for both the time-dependence of the response and for the equilibrium receptor density as a function of UTP concentration. The model was also able to reproduce the results of experiments performed by Garrad et al. (1998) designed to measure the agonist concentration dependence of receptor activation and desensitization.

The next set of calculations were concerned with fluorescence measurements and results were obtained for fluorescence as a function of time following receptor activation and also as a function of agonist concentration. Good agreement was obtained with the experimental results of Hirose et al. (1999) for the application of ATP to purinergic P_2Y_2 receptors in MDCK cells.

5 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 (Garrad et al., 1998) and the application of ATP to MDCK cells (Hirose et al., 1999) and sucessfully reproduces the main experimental results. However, the model is more 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.

6 References

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