Intracellular signalling

Receptor-specific messenger oscillations

The cytosolic molecule inositol-1,4,5-trisphosphate (InsP₃) acts as a messenger to link receptors at the cell surface with alterations in calcium concentration inside the cell, but it is not clear how production of InsP₃ is related to the often-complex calcium response. Here we use a fluorescent biosensor to visualize InsP₃ synthesis in individual cells in real time and show that this is periodically switched on and off in a receptor-specific manner. Our findings are consistent with intracellular calcium oscillations being generated by either fluctuating or sustained concentrations of InsP₃, which may allow diversity of signalling through the same signal-transduction pathway.

Oscillations in intracellular calcium concentration ([Ca²⁺]ᵢ) induced by G-protein-coupled receptors in the membrane provide a versatile encoding mechanism that uses variations in the amplitude, frequency and duration of signals to control cellular processes. Models to explain these oscillations are broadly based on dynamic uncoupling of the phospholipase C (PLC)/InsP₃ signalling pathway, or on the self-propagating regulatory properties of Ca²⁺ on the InsP₃ receptor (known as Ca²⁺-induced Ca²⁺ release). Distinction between the two schemes relies on whether InsP₃ oscillations, from repetitively switching phospholipase C on and off, are the driving force, or whether Ca²⁺ alone controls this process by enhancing or inhibiting its own release from internal stores at low and high concentrations, respectively.

The pleckstrin-homology (PH) domain of the PLC-δ1 enzyme allows these alternatives to be tested, as it binds to the precursor of InsP₃, phosphatidylinositol-4,5-bisphosphate, which is associated with the cell membrane, but translocates to the cytosol after activation of phospholipase C and InsP₃ synthesis. Tagging the PH domain with green fluorescent protein (GFP) allows this process to be visualized (Fig. 1a) and the fractional increase in cytosolic fluorescence can be used as an index of InsP₃ production (Fig. 1b–d).

We studied two types of PLC-linked G-protein-coupled receptor that induce oscillatory Ca²⁺ responses: the metabotropic glutamate receptor, mGluR5a, and the M₂-muscarinic receptor. Stimulation through mGluR5a produced oscillatory patterns of InsP₃ production, which were consistently paralleled by changes in [Ca²⁺]ᵢ (Fig. 1a–c). We found that oscillations were controlled by protein kinase C, as prolonged treatment with phorbol ester to downregulate this enzyme resulted in sustained InsP₃ production, which failed to oscillate even at low agonist concentrations (Fig. 1b, inset).

These results reveal, to our knowledge for the first time, [Ca²⁺]ᵢ changes that are induced by InsP₃ oscillations through dynamic and rapid uncoupling. This probably occurs through phosphorylation by protein kinase C of a single residue in mGluR5a (ref. 11). In contrast, although oscillatory and sustained Ca²⁺ signals induced by M₂-muscarinic receptors are dependent on agonist concentration (Fig. 1d), we detected no InsP₃ oscillation. This suggests that [Ca²⁺]ᵢ alone does not significantly stimulate InsP₃ production and that mGluR5a-induced InsP₃ oscillations are not secondary to increases in [Ca²⁺]ᵢ.

We propose that at least two mechanisms — Ca²⁺-induced Ca²⁺ release and dynamic uncoupling determined by the intrinsic properties of the receptor — can drive [Ca²⁺]ᵢ oscillations in the same cell background. For the latter scheme, which is typified by mGluR5a, cyclical desensitization and resensitization dynamically controls the activity of phospholipase C. In contrast, mGluR5a-expressing cells treated with 10 μM quisqualate, intracellular Ca²⁺ concentration was determined using Fura-2 dye and is expressed as the ratio of fluorescence at 340 nm and 380 nm. Traces showing the changes in InsP₃ and [Ca²⁺]ᵢ in a single M₂-expressing cell treated with 0.1 μM methacholine, washed and treated again with 1 μM methacholine.
the bifurcating phospholipase C pathway. In contrast, Ca^{2+}-induced Ca^{2+} oscillations at low agonist concentration may partially isolate the activities of Ca^{2+} release from those of protein kinase C activation. The full range of oscillatory and sustained-plateau Ca^{2+} signals can thus be elicited from a single receptor. This complex Ca^{2+} signalling not only increases the flexibility with which cellular processes can be controlled, but also allows different G-protein-coupled receptors, using the same transmembrane signalling in the same cell, to achieve specificity.

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COMMUNICATION ARISING

Hospital waiting-lists

Do power laws imply self-regulation?

Negative feedback leading to self-regulatory behaviour is an important phenomenon that affects time-series fluctuations in a range of systems and is critical in forecasting and management, particularly when complex dynamics are possible. Smethurst and Williams argue that the lengths of waiting-lists are self-regulating on the grounds that the relative changes in the size of waiting-lists follow a power law, with large changes being relatively rare compared with small ones. Here we show that similar power laws can also be obtained from unregulated, random time series. The existence of a power law that governs fluctuations in time series is not sufficient to prove the existence of self-regulatory behaviour, and we argue that a more sophisticated analysis is required.

Figure 1a shows a power law derived from the data of Smethurst and Williams. In their analysis, the rarity of large changes in the length of waiting-lists is used as evidence for negative feedback: people are more likely to join short rather than long queues, thereby buffering changes. Figure 1b reveals a similar relationship in the number of people waiting at the bar of a public house. The similarity of the plots for the two systems could be taken as evidence of a general power law governing the behaviour of queues. However, the data in Fig. 1a, b have not been tested against a null model, and it is not clear what pattern would be expected in a system with no self-regulatory behaviour.

The appropriate null model for a system with no regulation is a random walk (brownian motion). If data are following a random walk (that is, if the time series is unregulated), the frequency distribution of relative changes will be expected to show power-law-like behaviour. This is because even in unregulated time series, relatively large fluctuations would be expected to be less common than relatively small ones.

To illustrate this point, Fig. 1c shows the results of applying Smethurst and Williams’ analysis to randomly generated data from brownian-motion time series. These time series yield frequency distributions of relative changes that are strikingly similar to the plots in Fig. 1a, b, and which have a power-law slope that is indistinguishable from —2. This indicates that Smethurst and Williams’ data are entirely consistent with models in which self-regulation does not occur. The existence of this power-law relationship cannot therefore be used to infer the operation of self-regulatory mechanisms.

Providing evidence for such negative feedback is difficult, as shown, for example, by the problems of detecting density-dependence in ecology. We suggest three approaches to the problem of detecting negative feedback in hospital waiting-lists, on the basis of approaches taken by population ecologists. One is to adjust queue lengths experimentally and examine the responses. The second is to plot the rate of change in queue length against the length of the queue, although this is subject to statistical problems. The third is to use behaviour-based population modelling to determine the range of trade-offs made by individuals — for example, specific trade-offs can be quantified between the expected waiting time for treatment and the probability of choosing private treatment. Knowledge of such responses could then be applied to reducing waiting times. By contrast, relationships of the type shown in Fig. 1 constitute a poor method of characterizing and forecasting such systems.

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