Switching from Simple to Complex Oscillations in Calcium Signaling

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ABSTRACT We present a new model for calcium oscillations based on experiments in hepatocytes. The model considers feedback inhibition on the initial agonist receptor complex by calcium and activated phospholipase C, as well as receptor type-dependent self-enhanced behavior of the activated G_α subunit. It is able to show simple periodic oscillations and periodic bursting, and it is the first model to display chaotic bursting in response to agonist stimulations. Moreover, our model offers a possible explanation for the differences in dynamic behavior observed in response to different agonists in hepatocytes.

INTRODUCTION

Calcium ions are part of an information-processing system in the cells of animals and plants. In such cells cytoplasmic free calcium ions act as second messengers in response to a variety of stimuli. They thereby participate in the control of general functions such as excitability, contraction, metabolism, or exocytosis directly via the modification of enzymatic function or gene expression (Berridge et al., 1998).

The temporal behavior of cytoplasmic free calcium in eukaryotic cells has attracted much attention, especially after it was shown that calcium concentration displays oscillatory behavior in response to agonist stimulation in a variety of cells (Toescu, 1995; Goldbeter, 1996). Moreover, in the last couple of years evidence has been accumulating that altering the frequency of the oscillations affects gene expression (Li et al., 1998; Dolmetsch et al., 1998) and enzymatic activities (De Koninck and Schulman, 1998; Oancea and Meyer, 1998).

Since their discovery by Woods et al. (1986), calcium oscillations have been studied extensively in hepatocytes. Even though this oscillatory behavior changes somewhat when cells are stimulated with different agonists, it is possible to classify it qualitatively into two groups: 1) simple periodic spikes and 2) bursts. If, for example, vasopressin or phenylephrine is supplied at a constant level, simple spiking behavior of calcium ions is observed (Fig. 1). The frequency of these oscillations increases with the concentration of the agonist (Woods et al., 1986), a behavior that is called frequency encoding. On the other hand, stimulation with such agonists as ATP (Dixon et al., 1990) and UTP (Dixon et al., 2000) results in complex oscillations in addition to simple spiking behavior. These complex oscillations consist of "bursts," where one large spike is followed by a series of secondary oscillations (Fig. 2). Two consecutive bursts are separated by a refractory period of constant calcium concentration of less than 100 nM. The bursting behavior is highly irregular (i.e., nonperiodic) in almost all cases.

In addition to the agonist used, various other factors affect the oscillatory behavior of calcium ions in hepatocytes. For example, it was shown that the removal of external calcium stops the oscillations in most cases (Woods et al., 1990; Sanchez-Bueno et al., 1997), i.e., excessive calcium influx from the extracellular medium is crucial for the oscillations to occur.

A number of theoretical models have been proposed to explain the oscillatory behavior of calcium ions. The basis for most of these models is the following general scheme of signal transduction via calcium ions, which is rather well established. After the binding of an agonist to the extracellular side of a membrane-bound receptor molecule, the G_α subunit at the intracellular side of the receptor-coupled G-protein is activated. The activated G-protein in turn stimulates a phospholipase C (PLC), which catalyzes the hydrolysis of the membrane lipid phosphatidyl inositol-4,5-bisphosphate to form inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. IP_3 binds to specific receptors in the membrane of, for example, the endoplasmic reticulum. This binding leads to the opening of calcium channels, which again results in a massive flux of calcium ions from intracellular stores into the cytoplasm (Taylor and Marshall, 1992). The initial rise in concentration of calcium ions in the cytoplasm also stimulates the release of additional calcium ions. This latter mechanism is called calcium-induced calcium release (CICR) (Berridge, 1993), and it plays a central role in the propagation of the calcium signal. It was also shown that at high calcium concentrations the IP_3 receptors can be inhibited (Bezprozvanny et al., 1991; Finch et al., 1991). Even though this general scheme is widely accepted, details concerning the kinetics of the initial G-protein and PLC activation as well as details regarding the interaction of other processes involved have still not been determined.

Basically, there are two different kinds of models: those in which the instability responsible for the oscillations is confined to the receptors in the membrane surrounding the intracellular calcium pool (e.g., ER) and thus does not

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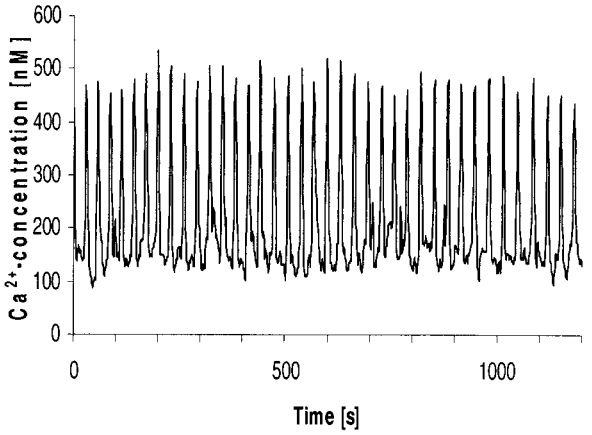


FIGURE 1 Simple oscillations (spiking) of the concentration of free calcium ions in rat hepatocytes stimulated with 2 μM phenylephrine.

depend on oscillations in the concentration of IP_3 (for a review see Sneyd et al., 1995), and those in which important dynamic aspects are found in the respective receptor-complexes of the plasma membrane (Cuthbertson and Chay, 1991; Meyer and Stryer, 1988). The latter models suggest oscillations in IP_3 preceding the oscillations of the calcium ions. Most models of both kinds can exhibit only simple periodic oscillations. A few models can also exhibit chaotic oscillations (Borghans et al., 1997; Shen and Larter, 1995; Houart et al., 1999), but chaotic bursting has not been published so far.

To be able to explain the difference in the experimentally observed behavior in response to different stimuli and at the same time be able to display the complexity of the observed oscillations in response to ATP/UTP, we present a new model for calcium oscillations. Our model proposes that depending on the type of receptor, the activation of the G_α subunit of the receptor complex can be self-enhanced. Changing the receptor-specific terms and parameters of the model results in a receptor-dependent switching from simple to complex oscillations. The complex oscillations fit the experimental data very well. Moreover, our model is able to show under- and overstimulation, frequency encoding, and simple oscillations with constant IP_3. Current models of calcium oscillations in mammalian cells display one or more of these properties. However, none of these models are able to show the same richness in dynamics as the present model. We discuss the implications of the proposed model.

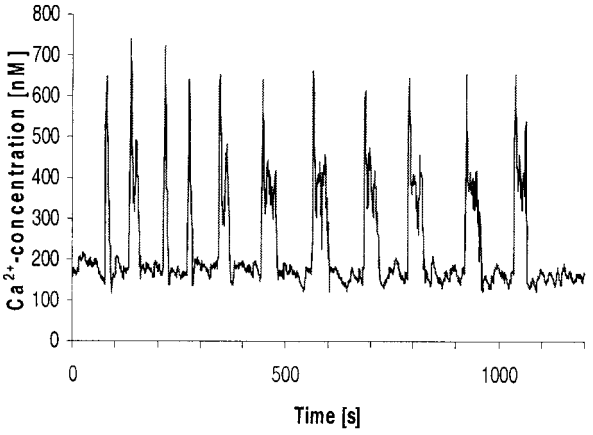


FIGURE 2 Time series with complex oscillations (bursting) of the concentration of calcium in hepatocytes stimulated with 1.2 μM ATP.

MATERIALS AND METHODS

Experimental

Aequorin was provided by Prof. O. Shimomura (Marine Biological Laboratory, Woods Hole, MA). Collagenase was obtained from Roche Diagnostics (Lewes, UK), and William's medium E (WME) was from GIBCO (Paisley, UK). Agarose and agonists were purchased from Sigma-Aldrich (Poole, UK).

Single hepatocytes were isolated from fed male Wistar-strain rats (150–250 g) by collagenase perfusion as described previously (Dixon et al., 1995). Briefly, the hepatic portal vein was cannulated, and an initial Ca^{2+}-free perfusion was followed by perfusion with collagenase (0.04% w/v) and Ca^{2+} (3.8 mM) for 15 min. The perfusion rate was 30 ml/min throughout. The cells were harvested and incubated at 37°C at low density (1000 cells/ml) in 2% type IX agarose in WME. Single hepatocytes were prepared for microinjection with the bioluminescent Ca^{2+} indicator aequorin as described previously (Cobbold and Lee, 1991). The injected cell was transferred to a perfusable cup held at 37°C, positioned under a cooled, low-noise photomultiplier, and continuously superfused with WME, to which agonists were added. Photon counts were sampled every 50 ms by computer. At the end of an experiment, the total aequorin content of each cell was determined by discharging the aequorin by lysing the cell. The data were normalized retrospectively by computer, by calculating the photon counts per second divided by the total counts remaining. The computed fractional rate of aequorin consumption could then be plotted as $[Ca^{2+}]_i$, using in vitro calibration data and exponential smoothing with time constants: for resting $[Ca^{2+}]_i$, 12 s; for transients, 1 s.

Computational

Simulations were performed using the Rosenbrock, LSODE, and Adams routine for the numerical solution of stiff differential equations. The software used was MADONNA (University of Berkeley, Berkeley, CA) and Dynamical Software (Dynamical Software, Tucson, AZ). Bifurcation diagrams were calculated using Dynamical Software.

RESULTS

We have developed a new qualitative model of calcium signaling in hepatocytes on the basis of previous experimental observations and data like those shown in Figs. 1 and 2. The model uses the following four variables: 1) the concentration of the active G_α subunit, 2) the concentration of active PLC, 3) the concentration of free calcium in the cytosol, and 4) the concentration of calcium in the intracellular stores such as the ER. In contrast to most other models, the concentration of IP_3 is not considered here as a separate variable. For the sake of simplicity, we assumed that IP_3 is

in a quasistationary state, i.e., the concentration of IP_3 simply follows the dynamics of active PLC. In the equations shown below a denotes the concentration of active G_α subunit, b denotes the concentration of active PLC, c denotes the concentration of free calcium in the cytosol, and d denotes the concentration of calcium in the intracellular stores.

The first variable to be considered in our model is the concentration of active G_α subunits of the G-protein, which are responsible for the activation of PLC. Only a few studies have attempted to elucidate the kinetics of the initial receptor–G-protein complexes. Experiments on reconstituted vesicles with muscarinic cholinergic receptors (Biddlecome et al., 1996) showed that the kinetics upon agonist binding is rather complex, and an acceleration in the rate of GTP/GDP exchange and hence in the formation of active G_α subunits was observed. The latter observation is possible evidence that the formation of active G_α is autocatalytic. We modeled the possible occurrence of such a behavior with a constant term (k_1) representing the spontaneous activation of the G_α subunit and a term ($k_2 * a$) that represents the accelerated formation of active G_α after binding of agonist to the membrane receptor. Constant k_2 represents the product of the second-order association constant and the concentration of agonist. Because the inactivation of the G_α subunits (due to GTP hydrolysis) was shown to be accelerated by active PLC (Bourne and Stryer, 1992), we have included a term ($k_3 * a * b/(a + K_4)$) to represent this fact. Furthermore, some authors have proposed a negative feedback of calcium-dependent kinase on the active receptor complex (Berridge et al., 1988; Woods et al., 1987; Sanchez-Bueno et al., 1990). We have modeled this by the term $k_5 * a * c/(a + K_6)$, which assumes a linear dependency of the activity of this kinase on calcium concentration. The change in the concentration of the active G_α subunit is then represented by the differential equation

$$\frac{da}{dt} = k_1 + k_2 * a - k_3 * a * b/(a + K_4) - k_5 * a * c/(a + K_6) \quad (1)$$

Of course this equation is a simplification of the events responsible for the activation of G_α, because there are probably additional interactions involved in reality. For instance, it has been shown that the $G_{\beta\gamma}$ subunit of the G-protein is also involved in the formation of active PLC (Sternweis, 1994). Furthermore, the kinetic terms used in the equation represent the minimum required to yield the experimentally observed dynamics. For example, our simulations showed that it is possible to obtain the same qualitative dynamics using either a Michaelis-Menten or a Hill term ($k_2 * a^n/(K_2^n + a^n)$) corresponding to cooperative binding instead of the term $k_2 * a$. However, because we would gain very little in terms of the qualitative overall dynamics in replacing the simple autocatalytic term with the more complex Michaelis-Menten or Hill expressions, we did not include such terms, to keep the model as simple and clear as possible.

The second variable in our model is the concentration of active PLC (Eq. 2). The activation of PLC depends on the concentration of the active G_α subunits, which is modeled by a first-order term, $k_7 * a$. Active PLC is inactivated enzymatically, and this is modeled by the expression $k_8 * b/(b + K_9)$:

$$\frac{db}{dt} = k_7 * a - k_8 * b/(b + K_9) \quad (2)$$

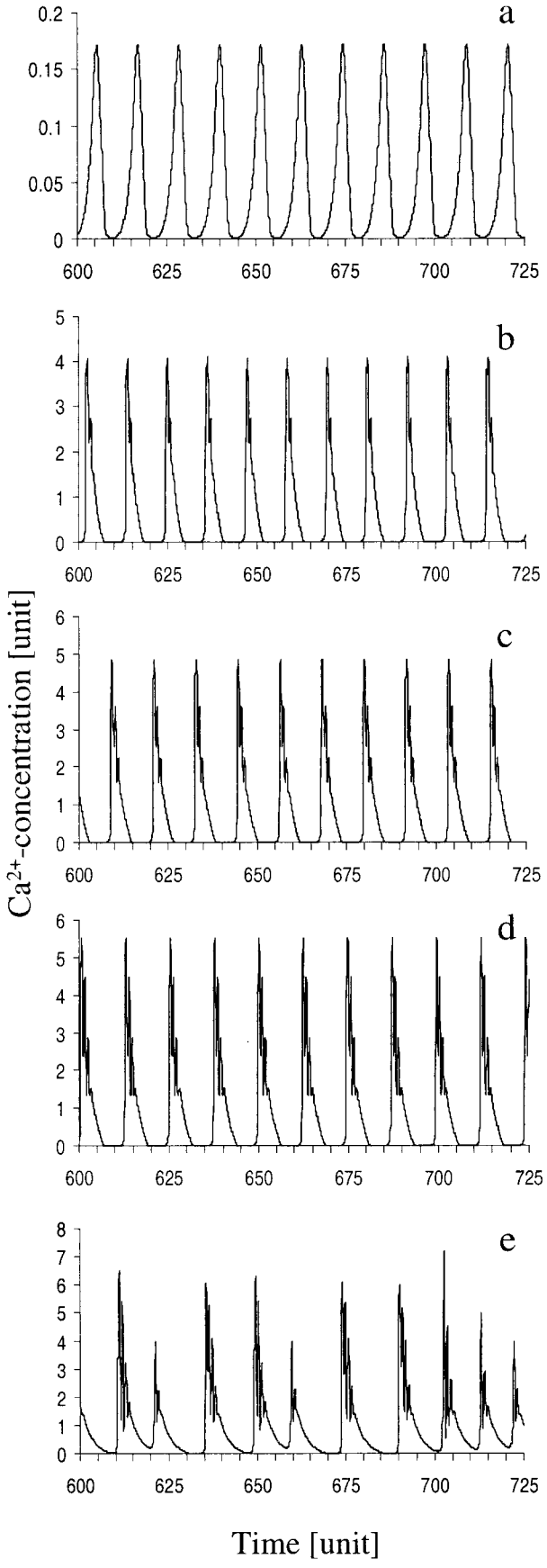
The third variable in our model is the concentration of free calcium in the cytosol (Eq. 3). After influx of calcium from internal stores to the cytoplasm, the concentration of free calcium increases. This influx is stimulated by IP_3 and by calcium itself (Tsien and Tsien, 1990; Sanchez-Bueno and Cobbold, 1993). Because IP_3 is generated by a reaction catalyzed by active PLC and we assumed that IP_3 is in a quasistationary state with respect to the concentration of active PLC, the calcium inflow from the internal stores will depend on PLC. We model this behavior with a term $k_{10} * c * b * d/(d + K_{11})$. In addition, IP_3 has been shown to stimulate calcium influx from the extracellular space (Hansen et al., 1991; Striggow and Bohnensack, 1994), which leads to a term $k_{12} * b$. Because a receptor-operated calcium influx has been shown in a vast number of cases (Tsien and Tsien, 1990; Benham and Tsien, 1987, Dubyak and El-Moatassim, 1993), we include an influx term, $k_{13} * a$. The extracellular concentration of calcium is assumed to be constant. Two ATP-dependent ion pumps pump calcium ions back into the ER and into the extracellular space (Pietrobon et al., 1990). We simulate these pumps by using simple Michaelis-Menten terms with respect to calcium concentration in the cytosol ($k_{14} * c/(c + K_{15})$ and $k_{16} * c/(c + K_{17})$):

$$\frac{dc}{dt} = k_{10} * c * b * d/(d + K_{11}) + k_{12} * b + k_{13} * a - k_{14} * c/(c + K_{15}) - k_{16} * c/(c + K_{17}) \quad (3)$$

The fourth variable in our model is the concentration of calcium in the ER (Eq. 4). The respective terms are the same as those terms in Eq. 3 that simulate the calcium exchange between the cytoplasm and the ER, but with opposite signs:

$$\frac{dd}{dt} = -k_{10} * c * b * d/(d + K_{11}) + k_{16} * c/(c + K_{17}) \quad (4)$$

For certain sets of parameter values this model displays simple periodic (spiking) oscillations, periodic bursting, and chaotic (irregular) bursting (Fig. 3). Using k_2, i.e., the concentration of agonist, as a bifurcation parameter, we find that above a certain threshold the calcium response changes from a low steady state (not shown) to simple periodic



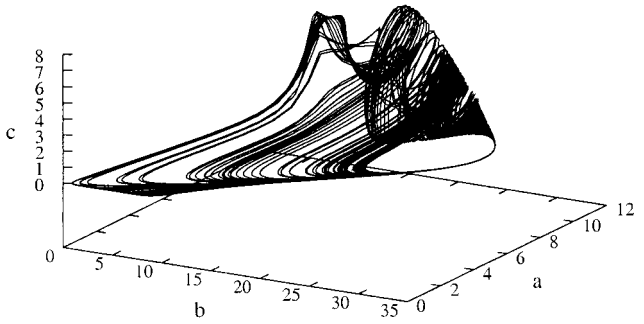


FIGURE 4 Phase plot corresponding to Fig. 3 *e*.

oscillations (Fig. 3 *a*) and then switches to complex periodic oscillations as k_2 is increased. These complex periodic oscillations display two different maxima (a main spike followed by one secondary oscillation; Fig. 3 *b*). A further increase in k_2 results in complex oscillations with first three maxima (Fig. 3 *c*), then four (Fig. 3 *d*) and five (not shown) maxima, and finally yields chaotic oscillations (Fig. 3 *e*). Finally, the system responds with an overstimulated elevated steady state from which the calcium concentration will no longer return to small values (not shown). This behavior is called overstimulation and is observed experimentally (Rooney et al., 1991). The elevated steady state in our simulations has an ~50-fold higher calcium concentration compared to the steady state at low k_2. However, in accordance with many experimental observations (e.g., Green et al., 1995), the steady-state concentration is lower than the maximum amplitude during complex bursting. The parameter range of k_2 in which simple periodic oscillations are observed is rather small compared to the parameter range where periodic and complex bursting occurs. On the other hand, with this set of parameters the range of k_2 values where chaotic bursting is observed is also rather small. This is undoubtedly a result of our choice of the simple autocatalytic term k_2 in preference to the more complex Michaelis-Menten and Hill expressions. However, the resulting time series, which exhibit not only irregular numbers of secondary oscillations but also irregular spacing between the main spikes, are in close correspondence to the experimental results. The parameter range of other constants in the model leading to chaotic dynamics is rather broad.

The time series shown in Fig. 3 *e* exhibits chaotic bursting. The phase plot (Fig. 4) represents the corresponding attractor. Similar irregular or chaotic oscillations in bio-

FIGURE 3 Periodic and complex oscillations (bursting) of the concentration of free calcium in response to increasing degrees of agonist stimulation in Eqs. 1–4. Parameters: $k_1 = 0.09$, $k_3 = 0.64$, $K_4 = 0.19$, $k_5 = 4.88$, $K_6 = 1.18$, $k_7 = 2.08$, $k_8 = 32.24$, $K_9 = 29.09$, $k_{10} = 5.0$, $K_{11} = 2.67$, $k_{12} = 0.7$, $k_{13} = 13.58$, $k_{14} = 153$, $K_{15} = 0.16$, $k_{16} = 4.85$, $K_{17} = 0.05$. Initial conditions: $a = 0.01$, $b = 0.01$, $c = 0.01$, $d = 20$. k_2 varies from 1.3 (*a*), to 2.35 (*b*), to 2.5 (*c*), to 2.6 (*d*), to 2.7738 (*e*).

chemical systems were proposed many years ago (Rössler, 1976). As in the experiments, an oscillation starts from a small calcium concentration with a large, steep spike followed by an irregular number of secondary smaller oscillations around an elevated mean value. The spike maxima vary irregularly, and between spikes the free calcium decreases to a low concentration. In the experimental system, an increase in frequency was observed (Green et al., 1997) for the main spikes, when the concentration of agonist was increased. This behavior is also found in our model with a slightly different set of parameters (Fig. 5).

During the chaotic bursting of c in our model, variables a (concentration of active G_α subunits) and b (concentration of active PLC) display oscillatory behavior around a higher elevated level, whereas variable d (concentration of calcium in the ER) shows oscillations that are antiparallel to the oscillations of variable c.

Because the assumed self-enhancement in the initial receptor complex is not necessarily present in every receptor type, we removed the receptor-specific autocatalytic term in Eq. 1:

$$\frac{da}{dt} = k_1 - k_3 * a * b/(a + K_4) - k_5 * a * c/(a + K_6) \qquad (5)$$

The resulting model system, which uses Eq. 5 instead of Eq. 1, is able to display simple oscillations with the same receptor independent parameters ($k_8 - K_{12}$ and $k_{14} - K_{17}$) as used in the above calculations for the complex oscillations. The frequency of these simple oscillations is higher compared to the bursting shown before and increases with increasing agonist concentration (Fig. 6), as is observed, for example, in the experiments using phenylephrine as a stimulus (Woods et al., 1986). Moreover, the frequency of the oscillations has been shown to increase when the pumps that pump calcium out of the cell are inhibited (Green et al., 1997). In our model the frequency of the oscillations rises, if the velocity constant k_{14} is lowered. However, our simulations show a rise in amplitude when the agonist concentration is increased, whereas experimental data show a rather constant amplitude. Thus additional factors seem to play a role here, and in fact it has been postulated that the constant amplitude results from an interplay with the calcium-buffering capacity of the mitochondria (Marhl et al., 1998). During the periodic oscillations, variable a also displays periodic oscillations between a very low and an elevated level, b oscillates around a high elevated level, and d is again antiparallel to c. This model also reproduces the understimulation by the agonist, in the sense that at low concentrations of agonist neither oscillations nor elevations in the intracellular concentration of calcium are observed.

Because of the presence of CICR, our model is also able to produce simple periodic oscillations while the concentra-

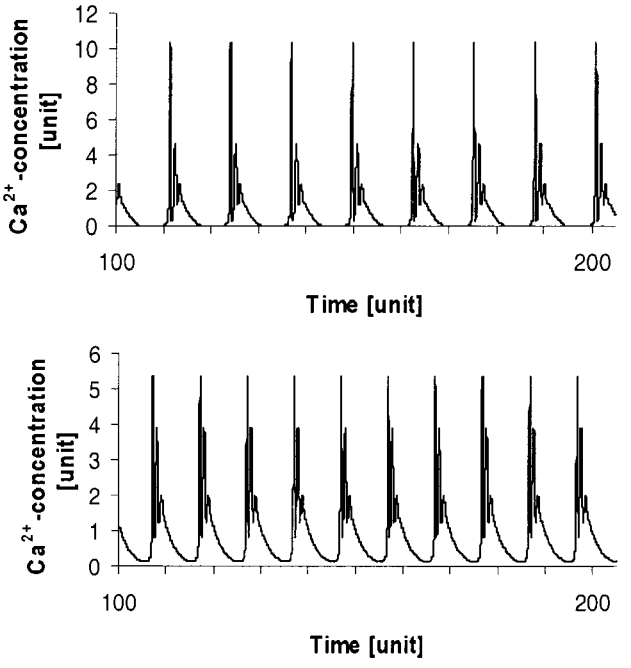


FIGURE 5 Simulated frequency coding in the frequency of the main spikes in bursting of the concentration of calcium in response to agonist stimulation produced by our model (Eqs. 1–4). Parameters: $k_1 = 0.09$, $k_3 = 1.27$, $K_4 = 0.19$, $k_5 = 3.73$, $K_6 = 0.73$, $k_7 = 1.24$, $k_8 = 32.24$, $K_9 = 29.09$, $k_{10} = 2.0$, $K_{11} = 2.67$, $k_{12} = 0.05$, $k_{13} = 13.58$, $k_{14} = 153$, $K_{15} = 0.16$, $k_{16} = 4.85$, $K_{17} = 0.05$. Initial conditions: $a = 0.01$, $b = 0.01$, $c = 0.01$, $d = 20$. $k_2 = 2.33$ (*top*) and $k_2 = 2.42$ (*bottom*).

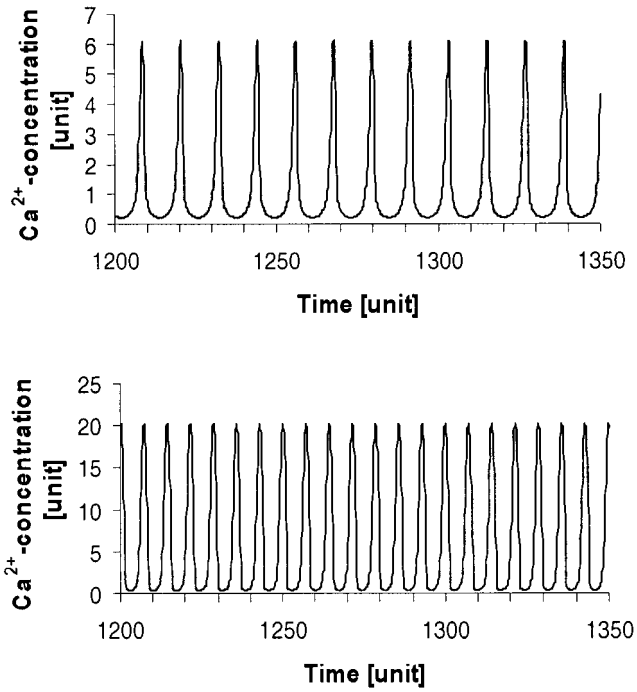


FIGURE 6 Frequency encoding in Eqs. 2–5. Parameters: $k_3 = 0.0001$, $K_4 = 0.788$, $k_5 = 1.45$, $K_6 = 0.18$, $k_7 = 5.82$, $k_8 = 32.24$, $K_9 = 29.09$, $k_{10} = 5.0$, $K_{11} = 2.67$, $k_{12} = 0.7$, $k_{13} = 0.12$, $k_{14} = 153$, $K_{15} = 0.16$, $k_{16} = 4.85$, $K_{17} = 0.05$. Initial conditions: $a = 0.01$, $b = 0.01$, $c = 0.01$, $d = 20$. $k_1 = 1.5$ (*top*) and $k_1 = 3.82$ (*bottom*).

tion of PLC is kept constant (data not shown). However, parameters (concentrations of pumps and enzymes) have to be changed substantially to observe such oscillations.

Our model system shows a surprising robustness toward the inclusion of additional terms in the equations. Thus it is possible to include feedback activation and/or inhibition of calcium on PLC (which has been proposed in the literature; Hirose et al., 1999) without changing the resulting complex dynamics considerably. We have also studied a variant of the present model, where we included IP_3 as a separate variable. This model showed essentially the same type of complex behavior, even if we included the activation of IP_3 degradation by a calcium-dependent kinase. Moreover, the complex behavior does not crucially depend on the term that represents the calcium influx from internal stores, $k_{10} * c * b * d/(d + K_{11})$, or on the explicit form of this term. This means that differently regulated calcium channels can be incorporated here. This is especially important for the inhibition of the calcium channel by high calcium concentrations. The same holds for the term $k_{12} * b$, which is not crucial for complex oscillations to occur.

Excluding those two terms and variable d (which is not essential for complex behavior) from the model leads to a core model for the chaotic bursting of calcium in hepatocytes in response to stimulation by ATP/UTP:

$$\frac{da}{dt} = k_1 + k_2 * a - k_3 * a * b/(a + K_4) - k_5 * a * c/(a + K_6) \quad (6)$$

$$\frac{db}{dt} = k_7 * a - k_8 * b/(K_9 + b) \quad (7)$$

$$\frac{dc}{dt} = k_{10} * a - k_{11} * c/(c + K_{12}) \quad (8)$$

This core model shows qualitatively the same complex periodic and chaotic behavior (Fig. 7) as described above for Eqs. 1–4. In fact, the model shows a change in behavior very similar to that in Fig. 3, with simple periodic oscillations at low k_2 followed by complex and chaotic oscillations with increasing k_2. From this model we can infer that the key term for elevated small oscillations after a spike is $k_2 * a$. CICR is not required to generate complex behavior.

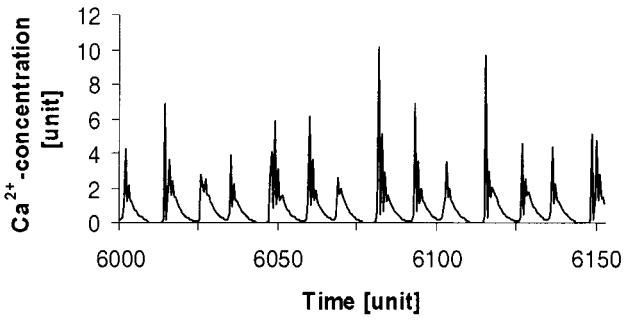


FIGURE 7 Simulated complex oscillations (bursting) of the concentration of calcium in response to agonist stimulation produced by the core model (Eqs. 6–8). Parameters: $k_1 = 0.212$, $k_2 = 2.9259$, $k_3 = 1.52$, $K_4 = 0.19$, $k_5 = 4.88$, $K_6 = 1.18$, $k_7 = 1.24$, $k_8 = 32.24$, $K_9 = 29.09$, $k_{10} = 13.58$, $k_{11} = 153$, $K_{12} = 0.16$. Initial conditions: $a = 0.01$, $b = 0.01$, $c = 0.01$.

DISCUSSION

We developed a new model for the calcium oscillations in hepatocytes on the basis of qualitative experimental results regarding the mechanism of the production and degradation of the involved species.

Our model shows qualitative behavior that matches experimental data very well. Including a receptor-specific self-acceleration ($k_2 * a$) for the active G_α subunit of the G-protein, we observe a transition from simple to complex periodic and chaotic behavior as the concentration of agonist is increased. The analysis of the experimental data in conjunction with the model simulations strongly supports the hypothesis that the observed irregular bursts of cytosolic calcium are of a deterministic nature. The size of the parameter region in which chaotic bursting is observed in our model varies, as do some details that are displayed during bursting if different sets of parameters are used. For the parameter set used for the time series shown in Figs. 3 and 4, the range of k_2 values resulting in chaotic dynamics and frequency coding are rather small. This is most likely a result of our use of a simple autocatalytic term rather than a more complex expression such as Michaelis-Menten or Hill expressions. However, because our simple model gives a much better understanding of the dynamics, we have chosen the simple approach over the more realistic approach. Furthermore, if we relax the condition that all of the different behaviors, such as frequency encoding, chaotic bursting, etc., should be observed with essentially the same parameter set, it is possible to observe chaotic bursting or frequency encoding for much larger ranges of k_2 values, using different sets of parameters.

Apart from the complex oscillations, including chaotic bursting, we were also able to reproduce qualitative experiments performed on hepatocytes.

If the concentration of external calcium is decreased to zero, our model stops to display oscillatory behavior. This observation fits the experimental results where oscillations cease when external calcium is removed from the hepatocytes (Woods et al., 1990; Sanchez-Bueno et al., 1997).

Without the receptor-specific self-enhancement, which we assume to be responsible for the complex behavior observed with ATP as a stimulus, we obtain a model that shows simple oscillations (spiking) and frequency encoding. The frequency of these oscillations rises if the concentration of the stimulus increases or if the pumps that remove

calcium from the cell are inhibited, which corresponds to experimental results.

Our model implies oscillations of IP_3 preceding those of calcium, because the concentration of IP_3 is locked to the concentration of active PLC, which oscillates in our model. The observation that calcium oscillations could be generated in the absence of oscillatory behavior of IP_3 (Wakui et al., 1989) strongly favored those models that were based solely on nonlinearities of the receptor-coupled calcium channels of the ER. However, the identity of the oscillations in the presence of constant IP_3 levels with those being produced by receptor-mediated events was questioned, especially for the case of mammalian cells (Thomas et al., 1996). Moreover, in hepatocytes, experiments showed that very few cells were able to display oscillatory behavior in the absence of receptor stimulation and infusion of IP_3 (Chatton et al., 1998). In addition, recent experiments show an oscillatory behavior of IP_3 in the course of calcium oscillations (Hirose et al., 1999), providing support for those models in which oscillatory behavior of IP_3 is a prerequisite for the oscillations of calcium.

In our model, the switching from simple spiking to complex bursting of calcium concentration in response to different stimuli in hepatocytes is due to the existence or nonexistence of self-accelerated formation of active G_α subunit in the initial stage of stimulation. Such a receptor-specific kinetic property can ensure that different information coming from the outside is transferred into qualitatively different types of dynamics and therefore qualitatively different responses inside the cell. Recently, it was observed experimentally that hepatocytes in the intact organ do not synchronize their calcium oscillations if they are perfused with ATP, whereas they do synchronize if they are perfused with vasopressin. This is strong evidence that a nondiffusible species, which could be a component of the receptor complex, plays an important role for calcium oscillations in response to ATP (Motoyama et al., 1999).

Even though we also show a very simplified and qualitative picture of the real situation, the fact that our model is very robust toward the addition of other terms suggests that it indeed comprises a core mechanism that is responsible for the calcium oscillations in hepatocytes. The explicit kinetics of the calcium channels of the ER is not crucial for the oscillatory and complex behavior to occur. In fact, experimentally it has been verified that there are different channel types with different kinetic properties, especially regarding the inhibition of the channel by high calcium concentrations (Hagar et al., 1998). However, for a quantitative simulation, experimentally determined parameters would be necessary, and probably more terms have to be added to reflect quantitatively the behavior of the system.

In the presence of noisy perturbations, which are unavoidable in biological cells, the distinction between complex periodic and chaotic behavior in the model might become less relevant. It is of relevance to the cell, however, that in the case of bursts a comparatively high concentration of free calcium is maintained for a longer period than during spiking. In addition, we note that a complex burst sequence of deterministic origin preserves more features under repetition of the stimulation and is thus more reliable than a stochastic response. Thus with the proposed mechanism the cell has the capability to reliably encode different external stimulations into qualitatively different pieces of metabolic information by means of the complexity of its response. This aspect has not yet been considered in cellular information processing.

To summarize, our new model for calcium oscillations in hepatocytes is able to display simple oscillatory and chaotic bursting. It is the first model to show chaotic bursting. Moreover, no other model has so far offered an explanation for the receptor-specific qualitatively different behavior in nonexcitable cells in response to different stimuli. This is due mainly to the fact that most models do not include any receptor-specific details and therefore cannot account for the fact that the experimental observations are receptor dependent. The models that do consider receptor-specific details have not been shown to exhibit anything but periodic oscillations in nonexcitable cells. Our model combines receptor-specific properties with the ability to show complex dynamic behavior. Future experimental studies should be undertaken to study the kinetics of the initial receptor complex in detail.

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