Model for Receptor-Controlled Cytosolic Calcium Oscillations and for External Influences on the Signal Pathway

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ABSTRACT The external stimulation of many cells by a hormone, for example, often leads to an oscillating cytosolic calcium concentration. This periodic behavior is now designated the cytosolic calcium oscillator. A theoretical model is presented that describes this behavior on the basis of inositol(1,4,5)trisphosphate-induced calcium oscillations. In contrast to other models only a single positive feedback loop is taken into account to obtain oscillations.

The model includes important innovations compared to other approaches. It includes the contribution of extracellular calcium and its modification after the stimulation of the cell. Furthermore, the signal pathway that leads to cytosolic calcium oscillations is described in more detail than in other models. This enables investigations on the influence of additional parameters like external electromagnetic fields on the signal transduction pathway. The model and the calculations are based on the theory of nonlinear self-sustained oscillators.

1. INTRODUCTION

In recent years it has become increasingly evident that oscillating phenomena play a fundamental role for both the functioning and information processing of biological systems (Berridge and Rapp, 1979; Goldbeter, 1989). Rhythmic phenomena are responsible for the creation, stabilization, and maintenance of a certain kind of biological order. The corresponding frequencies extend over an immense scale reaching from the sub-hertz to the gigahertz region. What all these biological oscillators have in common is that they are essentially nonlinear systems. It is the inherent nonlinearity that makes possible the occurrence of temporal, spatial, and spatio-temporal structures within the system. These states are created via self-organizing processes. The steady and periodic structures are stabilized far away from thermal equilibrium, and transitions from one state to another have to be regarded as nonequilibrium phase transitions (Kaiser, 1987, 1992).

On the cellular level many experiments have revealed that as a response to an external stimulation the intracellular calcium concentration can display an oscillating behavior (Berridge and Galione, 1988; Berridge, 1990; Fewtrell, 1993; Meyer and Stryer, 1991; Tsien and Tsien, 1990). The oscillation periods typically lie in the range of 1 s to several minutes. This periodic behavior is now designated the cytosolic calcium oscillator. The importance of this oscillator is explained by the fact that calcium controls a large variety of subcellular processes; that is, calcium is one of the few second messengers of the cell (Berridge and Galione, 1988).

For a specific cell type the oscillation pattern can be a function of both the kind of agonist applied and its concentration (Meyer and Stryer, 1991). This has led to the interpretation that the calcium messenger system is frequency encoded (Berridge and Galione, 1988). There are comprehensive reviews of cytosolic calcium oscillations (Berridge and Galione, 1988; Berridge, 1990, 1993; Fewtrell, 1993; Goldbeter, 1989; Meyer and Stryer, 1991; Tsien and Tsien, 1990). Therefore we will not stress experimental details but instead concentrate on theoretical considerations.

The cytosolic calcium oscillator is, like all biological oscillators, an essentially nonlinear system. This property is connected with the existence of feedback loops that are created via nonlinear dissipative processes within the cell. In this context it becomes evident that a theoretical description of such phenomena has to be based on the concept of nonlinear dynamics in general and on the theory of self-sustained (i.e., limit-cycle) oscillators in detail (Kaiser, 1977, 1987, 1988; Kaiser and Eichwald, 1991). These systems represent an ideal theoretical basis for a description of intracellular calcium oscillators.

The autonomous limit-cycle oscillation has to be regarded as a singular degree of freedom of the system that is partially decoupled from the rest of the system (Kaiser, 1992). This can be seen by the fact that the intracellular calcium concentration varies in the range of 0.1 μM to at most several μM during one oscillation period. However, the extracellular calcium concentration and the calcium concentration in intracellular calcium stores are 3 orders of magnitude higher. Therefore it is evident that the cytosolic calcium oscillator has to be shielded from these conditions to obtain an effective information processing. In this context an oscillating messenger will be much more effective than an amplitude-encoded one (Berridge and Galione, 1988).

Since the limit-cycle oscillation is created via nonlinear dissipative processes this also accounts for the strong stability and specific resistance of such oscillators against noise, which is of fundamental importance for any information-creating process. On the other hand it is well known from nonlinear dynamics that self-sustained oscillators exhibit a

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certain kind of extreme sensitivity against periodic perturbations even at very weak intensities. Examples are sharp resonances and strong frequency- and intensity-dependent responses. It should be stressed that this kind of behavior is found in theoretical investigations as well as in experiments (Blackman et al., 1989; Grundler and Kaiser, 1992; Grundler et al., 1992; Kaiser and Eichwald, 1991; Kaiser, 1992).

The basic elements of the signal pathway that lead to a change in the intracellular calcium concentration have become widely accepted (Berridge and Galione, 1988; Berridge and Irvine, 1989; Berridge, 1990; Cuthbertson, 1989; Meyer and Stryer, 1990, 1991; Tsien and Tsien, 1990). The hormone-mediated stimulation of a receptor at the cell surface leads to the activation of specific G-proteins at the inner side of the cell membrane. Activated G-proteins facilitate the activation of phospholipase C (PLC). This in turn leads to the hydrolysis of phosphatidylinositol(4,5)bisphosphate (PIP_2) into inositol(1,4,5)trisphosphate (IP3) and diacylglycerol. IP_3 diffuses into the intracellular cell compartment, where it releases calcium from internal stores (like the endoplasmic reticulum) by causing the opening of ion channels (Meyer et al., 1988; Meyer and Stryer, 1990).

Besides these basic features of the signal pathway further processes are required to account for an oscillating cytosolic calcium concentration (Meyer and Stryer, 1991). These processes include mechanisms of positive and negative feedback between several components of the cell system. The detailed nature of these mechanisms is still a matter of speculation and further investigations. One has also to take into account the possibility that in different cell types different mechanisms may be important. These offer an explanation of the variations of the oscillation patterns that have been observed.

There are several mechanisms that may lead to intracellular calcium oscillations which have been proposed so far (Berridge and Galione, 1988; Berridge, 1990; Cuthbertson, 1989; Fewtrell, 1993; Meyer and Stryer, 1991; Tsien and Tsien, 1990). All involve a certain kind of feedback mediated by cytosolic calcium itself. Two mainstreams have evolved. One is based on the process of calcium-induced calcium release (CICR) (Berridge and Galione, 1988; Wakui et al., 1990). Here calcium itself controls its release from intracellular stores. The oscillations are a consequence of the periodic release and pumping back of calcium into the store. On this basis a quantitative model was developed by A. Goldbeter, G. Dupont, and M. J. Berridge (Dupont and Goldbeter, 1989, 1992; Goldbeter et al., 1990). This model fulfils one of the basic requirements mentioned above: it represents a self-sustained nonlinear oscillator. The model was compared with the results of experiments with, for example, pancreatic acinar cells (Wakui et al., 1990) and Jurkat T-lymphocytes (Payet et al., 1991).

A second main approach emphasizes the role of IP_3 more directly. Here the feedback loop that leads to the oscillations is between cytosolic calcium and the IP_3 production pathway (Berridge and Galione, 1988; Harootunian et al., 1991; Meyer and Stryer, 1988, 1991). In this scheme the activation of PLC is catalyzed by cytosolic calcium (positive feedback). The existence of such an interaction has gained experimental support (Cockcraft, 1992). This feedback loop leads to IP_3-induced calcium release. Hints for the occurrence of this process came from, for example, experiments with stimulated fibroblasts (Harootunian et al., 1991) and with fertilized hamster eggs (Miyazaki et al., 1992). But IP_3-induced calcium release was also recognized to be involved in T-lymphocyte activation (Gardner, 1989).

An argument in favor of a premier involvement of IP_3 in the calcium release process is that IP_3 has a much longer spatial range as a second messenger within the cell because it is not buffered very fast (Allbritton et al., 1992).

The activation of PLC leads to an enhanced PIP_2 hydrolysis, which results in an increase of the IP_3 concentration within the cell. On this basis T. Meyer and L. Stryer developed a model they called the IP_3-Ca^{2+} cross-coupling (ICC) model (Meyer and Stryer, 1988, 1991). The ICC model consists of equations for the temporal development of the intracellular concentrations of calcium and IP_3. These first equations alone did not reproduce oscillations. Therefore the authors included a third equation describing an inhibition parameter that characterizes the response of the calcium channels of the intracellular stores (Meyer and Stryer, 1991).

The purpose of this paper is twofold. First, we want to discuss a different approach for IP_3-induced calcium oscillations that will show that it is not necessary to include additional terms to account for cytosolic calcium oscillations. We found it necessary to develop an alternative model because the ICC model does not explain cytosolic calcium oscillations on the basis of an IP_3-induced mechanism. The oscillations in the ICC model are solely a product of the negative feedback of calcium on the response of the calcium channels. This can be seen if the equation for the time development of IP_3 is eliminated adiabatically. This procedure does not extinguish the oscillations (Eichwald and Kaiser, unpublished results). The ICC model was also recently criticized for other reasons (Kraus and Wolf, 1993).

We present a model where the mechanism that leads to cytosolic calcium oscillations is mediated solely through the positive feedback of intracellular calcium on the PLC activation. The approach is not designed to meet a specific experimental situation but rather to gain some general insights into the theoretical explanation of cytosolic calcium oscillations. This should lead to new concepts for future experiments. Therefore the model should also allow for extensions that describe a specific experimental situation more realistically.

Besides these aspects it is our intention to describe the production pathway of IP_3 in more detail than is done in other models. Because it is more realistic, this approach also offers the possibility of investigating the activation pathway under the influence of additional external perturbations. This kind of interaction becomes more and more interesting because of the increasing literature on the effects of external low-intensity electromagnetic fields on the calcium metabolism of the cell (Adey, 1989; Frey, 1993; Grundler et al., 1992;

Walleczek, 1992). Experiments on T-lymphocytes in particular have obtained a sophisticated level (Cadossi et al., 1992; Lyle et al., 1991; Walleczek and Liburdy, 1990; Walleczek, 1992; Walleczek and Budinger, 1992).

Since some of these experiments are investigations of activated cells that display an oscillating intracellular calcium concentration, a theoretical concept that describes these interactions is in order (Kaiser, 1992; Polk, 1992). In this context the recent use of the Fura 2 technique will lead to new and exciting results (Frey, 1993). This will lead to an increasing understanding of possible interaction mechanisms and interaction sites of low-intensity electromagnetic fields with the calcium metabolism of the cell.

2. MODEL FOR IP_3-INDUCED CALCIUM OSCILLATIONS

The basic concept of the model under consideration is shown in Fig. 1, which includes the activation pathway for the IP_3 production as well as the individual contributions for changes in the intracellular calcium concentration. The following set of differential equations is proposed:

$$\frac{d\Gamma}{dt} = V_T \frac{\beta^l}{K_H^l + \beta^l} \frac{1 - \Gamma}{K_T + 1 - \Gamma} - \kappa_T \Gamma \tag{1}$$

$$\frac{dQ}{dt} = \sigma \Phi(\Gamma, Z) - \kappa_Q Q \tag{2}$$

$$\frac{dY}{dt} = \nu_2(Z) - \nu_3(Q, Y) - k_F Y \tag{3}$$

$$\frac{dZ}{dt} = -\nu_2(Z) + \nu_3(Q, Y) + k_F Y + \nu_0 + \nu_1(Q) - kZ. \tag{4}$$

The variables of Eqs. 2–4 are the intracellular concentrations of IP_3 and calcium and the concentration of calcium in an intracellular calcium store, abbreviated as Q, Z, and Y. All concentrations and rates are defined with respect to the total intracellular volume.

Equation 1 characterizes the fraction of activated G-proteins, that is, $\Gamma = [G]_{activated}/[G]_{total}$. The first term on the right side of Eq. 1 describes the activation process of the G-proteins. Here only the following simple reaction is taken into account (Gilman, 1987):

$$HR + G_0 \rightleftharpoons HRG_0 \rightarrow HR + G,$$

where HR, HRG_0, G_0, and G stand for the concentrations of hormone–receptor complexes, hormone–receptor–G-protein complexes, inactivated G-proteins, and activated G-proteins, respectively. We define β as the fraction of receptors where a hormone is bound (or another kind of stimulus is present), that is, $\beta = (HR)_{total}/R_{total} = (HR + HRG_0)/R_{total}$. With that definition β characterizes the strength of the stimulus ($0 \leq \beta \leq 1$). For simplicity the total amount of G-proteins in the cell membrane is given by $G_{total} = G_0 + G$. This is of course an approximation because G-proteins are also bound in other reactions. One example is the subsequent activation of PLC. A careful inspection of this approximation leads to a modification of the first term of Eq. 1, which will be discussed below. Furthermore, it is assumed that the activation of the G-protein is a fast process.

With the definitions given above the activation ratio of the G-proteins is proportional to $\beta(1 - \Gamma)/(K_T + 1 - \Gamma)$ (assuming Michaelis-Menten kinetics, K_T Michaelis-Menten constant). The linear dependence of this ratio on the parameter β is only a first approximation. A generalization of this term should be considered for the following reasons. First, the interaction of the G-proteins with the activated receptor complexes has to be taken into account. Activated G-proteins will in turn interact with PLC. Finally, it is well known that the hormone-receptor complexes operate catalytically; one receptor can interact with more than one G-protein over a certain period of time (Gilman, 1987). Taken together these points yield a complicated dependence of the activation ratio of the G-proteins on the parameter β, the concentration of PLC, and on the variable $\Gamma(t)$.

A detailed calculation shows that a reasonable approximation is given by the following generalization of the parameter β: $\beta \rightarrow \beta^l/(K_H{}^l + \beta^l)$. Here K_H and the exponent l have to be regarded as effective parameters that characterize the activation of the G-proteins ($l \geq 1$, not necessarily an integer). This changes the dependence of the activation ratio on β to a sigmoidal shape. The procedure described above simplifies the calculations without affecting the general basis of the model. In particular the mechanism that leads to cytosolic calcium oscillations is not altered.

Finally, the second term on the right side of Eq. 1 describes the decay of activated G-proteins by hydrolysis. Only this contribution is considered because in most cases this is the main deactivation process for G-proteins.

So far Eq. 1 is not coupled to the other model equations. However, the existence of feedback processes on the activation status of the G-proteins will provide a coupling of Eq. 1 with the other model equations. Mechanisms of negative feedback on the activated G-proteins can exist. One example is a negative feedback loop created by activated PLC that would enhance the GTPase activity of the

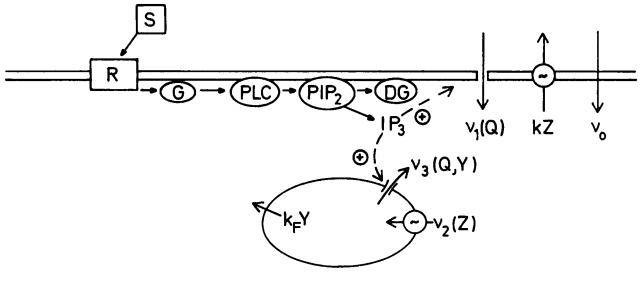

FIGURE 1 Basic concept of the model under consideration. Included is the activation pathway that leads to the production of IP_3 and the individual contributions for changes in the intracellular calcium concentration. *S*, external biochemical stimulus; *R*, receptor; *G*, G-protein. The intracellular concentrations of IP_3 and calcium and the calcium concentration of an intracellular calcium store are denoted as Q, Z, and Y. The terms ν_i ($i = 0, 1, 2, 3$), $k_F Y$ and kZ represent different calcium fluxes (see text for details).

G-protein bound to it (Cuthbertson, 1989). Another possibility is based on a negative feedback loop exerted by protein kinase C (PKC), which is activated by diacylglycerol and intracellular calcium (Nishizuka, 1992) onto the activated G-proteins (Cobbold et al., 1988; Cuthbertson, 1989). In these schemes the second term on the right side of Eq. 1 must be supplemented. This discussion will be continued in section 3.3.

The first term on the right side of Eq. 2 describes the formation of IP_3 due to the activation of PLC, where the latter process is assumed to be fast. The following set of reaction equations forms the basis for the activation of PLC and the subsequent formation of IP_3:

$$jG + P \overset{Ca^{2+}}{\rightleftharpoons} P'$$

$$P + Q_2 \rightleftharpoons PQ_2 \overset{V_Q}{\rightarrow} P + Q$$

$$P' + Q_2 \rightleftharpoons P'Q_2 \overset{V_{Q'}}{\rightarrow} P' + Q,$$

where $P(P')$ is the concentration of unactivated (activated) PLC and Q_2 is the concentration of PIP_2 (other definitions as before). The parameter j accounts for the possibility that the activation of PLC could require multiple G-proteins (Cuthbertson, 1989). The production ratio of IP_3 will then be proportional to $V_Q PQ_2/P_{total} + V'_Q P'Q_2/P_{total}$, where $V'_Q \gg V_Q$, and $P_{total} = P + P' + PQ_2 + P'Q_2$. Finally, it is assumed that the PLC activation is catalyzed by cytosolic calcium (positive feedback) (Cockcraft, 1992; Meyer and Stryer, 1988, 1991). Therefore the forward reaction rate of the first reaction equation (which describes the activation of PLC) is modified by a Hill-type expression proportional to $Z^p/(K_C^p + Z^p)$.

With all the definitions given above the production ratio of IP_3 (first term on the right side of Eq. 2 reads as follows (assuming Michaelis-Menten kinetics again, K_G, K_Q, K'_Q Michaelis-Menten constants):

$$\sigma\Phi(\Gamma, Z) = \sigma \frac{\mu_1 K_C^p + (\mu_1 + \Gamma^j)Z^p}{\mu_2 K_C^p + (\mu_2 + \Gamma^j)Z^p}. \tag{5}$$

The parameters σ, μ_1, and μ_2 include all constants:

$$\sigma = V_Q' \frac{Q_2}{K_Q' + Q_2},$$

$$\mu_1 = \frac{V_Q K_Q'}{V_Q' K_Q} \frac{K_G}{G_{total}},$$

$$\mu_2 = \frac{1 + Q_2/K_Q}{1 + Q_2/K_Q'} \frac{K_G}{G_{total}}.$$

The second term on the right side of Eq. 2 is a first-order decay term for IP_3.

Equation 3 describes the time variation of the calcium content of the intracellular store. The term $\nu_2(Z)$ characterizes the influx of calcium into the store through the action of a calcium pump and $\nu_3(Q, Y)$ the IP_3-induced efflux of calcium out of the store. The ATP-driven calcium pump is described by (Goldbeter et al., 1990)

$$\nu_2(Z) = V_{M2} \frac{Z^n}{K_P^n + Z^n}. \tag{6}$$

The IP_3-induced efflux of calcium out of the store is taken as (Meyer and Stryer, 1991)

$$\nu_3(Q, Y) = V_{M3} \frac{Q^m}{(K_R + Q)^m} Y, \tag{7}$$

where the exponent m characterizes the degree of cooperativity in the opening of calcium channels by IP_3. Early results indicated that $m \geq 3$ (Meyer et al., 1988; Meyer and Stryer, 1990). However, recent experiments revealed that $m = 1$ is also possible for certain cell types (Finch et al., 1991).

There are investigations that demonstrate the existence of additional feedback mechanisms of intracellular calcium on IP_3-induced calcium release (Bezprozvanny et al., 1991; Iino and Endo, 1992). For example, it was found that elevated levels of intracellular calcium inhibit IP_3-induced calcium release while lower levels may stimulate it (Bezprozvanny et al., 1991; Finch et al., 1991; Iino and Endo, 1992). In this scheme the term ν_3 (Eq. 7) must be supplemented by a functional dependence on the intracellular calcium concentration Z. We do not consider such a possibility here because it is not clear whether such a feedback mechanism is the prime cause for cytosolic calcium oscillations. Instead a model is considered where the oscillations result solely from the feedback loop between intracellular calcium and the activation of PLC.

The last term on the right side of Eq. 3 is a passive leakage flux of calcium out of the store (rate constant k_F).

Finally, Eq. 4 is a differential equation for the time evolution of the intracellular calcium concentration. It includes the interchange of calcium with the store leading to the same terms as on the right side of Eq. 3 but with opposite signs. In addition to these contributions the flux of calcium through the plasma membrane must also be included (Nemeth and Carafoli, 1990). The terms ν_0 and kZ describe the passive leakage of extracellular calcium into the cytosol and, respectively, the activity of the calcium pumps of the plasma membrane. For simplicity the latter one assumes just a linear dependence on Z (rate constant k).

In many cases the stimulation of the cell leads to an altered influx of extracellular calcium (Lewis and Cahalan, 1989; Nemeth and Carafoli, 1990). This behavior can be attributed to two different origins (Berridge and Irvine, 1989). First, the emptying of the intracellular calcium stores can lead to an increased influx of extracellular calcium (Fewtrell, 1993; Hoth and Penner, 1992; Mason et al., 1991). Recent experiments with T-lymphocytes indicate that for this cell type the degree of filling of the intracellular calcium stores is correlated to the plasma membrane permeability for calcium ions (Alvarez et al., 1992; Haverstick and Gray, 1993; Mason

et al., 1991). In T-lymphocytes the emptying of the stores may be signaled to the plasma membrane by a calmodulin-dependent process (Haverstick and Gray, 1993), possibly through the action of a cytochrome P-450 (Alvarez et al., 1992). Similar results were obtained in experiments with stimulated hepatocytes, where it was shown that intracellular calcium oscillations depend on the activity of calmodulin (Somogyi and Stucki, 1991).

One proposal for the regulation of extracellular calcium influx is the capacitative model of J.W. Putney, Jr. (Putney, 1986). However, newer experiments revealed that there is no direct link between the store and the plasma membrane calcium permeability (Haverstick and Gray, 1993). This means that calcium enters the cytosol before it is sequestrated. This behavior necessitates the existence of a second messenger, which might include calmodulin-dependent processes as described above.

A second possibility for an altered influx of extracellular calcium is the existence of second-messenger-operated calcium channels in the plasma membrane. These kinds of channels are found in several cell types (Berridge, 1989, 1993; Berridge and Irvine, 1989; Tsien and Tsien, 1990). Examples are T-lymphocytes again (Gardner et al., 1989; Gardner, 1989, 1990; Khan et al., 1992), olfactory cells (Restrepo et al., 1990) and *Xenopus* oocytes (Snyder et al., 1988; Girard and Clapham, 1993). The opening of the plasma membrane calcium channels may be caused by IP_3 directly or by another inositol phosphate derivate (Lückhoff and Clapham, 1992).

Taken together the data reveal the necessity to include an additional influx term into the model. This is done by adding a contribution $\nu_1(Q)$ to Eq. 4:

$$\nu_1 = V_{M1} \frac{Q^q}{(K_M + Q)^q}. \tag{8}$$

This term is not responsible for the onset of oscillations in the model, and oscillations are possible without this contribution. However, it will change the basal level of the intracellular calcium concentration. This also accounts for the observation that the rate of rise and the amplitude of the oscillations remain constant for different values of the strength of the external stimulus (Berridge and Irvine, 1989). Together with an appropriate choice of the parameters V_{M1}, K_M, and the exponent q this yields a suitable description of an altered influx of extracellular calcium.

The system of Eqs. 2–4 in combination with Eqs. 5–8 is sufficient to reproduce cytosolic calcium oscillations. In other words, Eqs. 2–8 with Γ as an input parameter in the form of a solution of Eq. 1 represent a self-sustained oscillator in the sense of nonlinear dynamics if the strength of the external stimulus β exceeds a critical value. The oscillations are a product of the complicated balance between influx and efflux terms. The expressions ν_0, $\nu_1(Q)$, $\nu_3(Q, Y)$, and $k_F Y$ have to be regarded as influx terms that increase the intracellular calcium concentration, whereas the pump terms $\nu_2(Z)$ and kZ have the opposite behavior. An increasing strength of the external stimulus that leads to an increasing IP_3 formation will change this balance. This kind of behavior causes the nonoscillating steady-state solution of Eqs. 1–8 to become unstable via a Hopf bifurcation. An oscillating intracellular calcium concentration results.

3. DISCUSSION

A detailed investigation of the system of Eqs. 1–8 with the methods of nonlinear dynamics reveals that the model represents a self-sustained oscillator. This can be seen, for example, by evaluating the eigenvalue equation of the linearized matrix of the steady states of Eqs. 1–8. An investigation of this relation yields two important points. First, in contrast to the ICC model the oscillations are exclusively a consequence of the positive feedback loop between cytosolic calcium and PLC activation. This means that after an adiabatic elimination of the dynamics of IP_3 (Eq. 2) the model no longer predicts intracellular calcium oscillations. The interpretation that follows from this point is that the dynamics of the IP_3 concentration is a necessary prerequisite for IP_3-induced calcium oscillations. This is an important condition for any model that explains intracellular calcium oscillations on the basis of an IP_3-calcium cross-coupling mechanism.

The second remark is that the limit-cycle behavior results exclusively from the stimulation of the cell; that is, a certain threshold for the onset of oscillations exists. The steady state for $\beta = 0$ is stable and nonoscillatory (real negative eigenvalues). It is given by the following relations:

$$Q_S^{\beta=0} = \frac{\sigma\mu_1}{\kappa_Q\mu_2}$$

$$Y_S^{\beta=0} = \left(V_{M2}\frac{Z_S^n}{K_P^n + Z_S^n}\right) \Big/ \left(V_{M3}\frac{Q_S^m}{(K_R + Q_S)^m} + k_F\right)$$

$$Z_S^{\beta=0} = \left(\nu_0 + V_{M1}\frac{Q_S^q}{(K_M + Q_S)^q}\right) \Big/ k.$$

One can see that the parameters σ, μ_1, μ_2, κ_Q, ν_0, V_{M1}, K_M, and k determine the levels of intracellular IP_3 and calcium of the quiescent cell. The latter is typically in the range of 0.1 μM. This value has been used in the following investigations, and the IP_3 concentration without stimulation of the cell has been fixed to 0.05 μM (Meyer and Stryer, 1991). This choice causes constraints on the parameters mentioned above.

The parameter κ_Q, which is a rate constant for the time scale of IP_3, is fixed to $\kappa_Q = 1$ s. This value was estimated in, for example, measurements of the diffusion constant of IP_3 in the cytosol in *Xenopus* oocytes (Allbritton et al., 1992).

The investigations revealed that the self-sustained behavior of the system under consideration is largely determined by the parameters V_{M2}, K_P, V_{M3}, K_R, and k_F. This is easy to understand since these parameters characterize the detailed balance between the internal calcium fluxes within the cell. Cytosolic calcium oscillations are found in large ranges of these parameters. In most calculations presented here the value of the Michaelis-Menten constant of the calcium pump of the intracellular calcium store is fixed to $K_P = 0.1$ μM,

which is a reasonable value, because the basal calcium concentration of the cell is about 0.1 μM. The other parameters mentioned above can vary from cell to cell since they can depend on the detailed intracellular structure (e.g., location of the stores, cell volume) (Fewtrell, 1993). Furthermore, in Eqs. 6–7 the exponents were set to $n = 2$ and $m = 4$, assuming that the calcium pump transports two ions, and assuming cooperativity in the opening of calcium channels by IP_3 (Meyer et al., 1988; Meyer and Stryer, 1990). On the other hand the parameters that characterize the feedback of intracellular calcium on the activation of PLC cannot be attributed to experimental findings so far.

In order to demonstrate the variability of the model different sets of parameters are discussed. For each combination of these parameters considered here the model exhibits a Hopf bifurcation for a threshold value of the strength of the external stimulus β (hard-mode excitation). For a certain range of β oscillations exist. If β exceeds a critical value an inverse Hopf bifurcation occurs. This leads to a sustained rise in the intracellular calcium concentration for high levels of the strength of the external stimulus (nonoscillating state).

In Fig. 2 some representative oscillation diagrams of the intracellular calcium concentration are shown. In each case the stimulus is applied after $t = 10$ s. It is seen that very different kinds of calcium oscillations can be reproduced with the model. Depending on the specific choice of the parameters that determine the internal calcium fluxes, the following types of oscillations are observed: (*a*) oscillations that are fairly symmetric (Fig. 2 *a*), (*b*) very brief pulses with a long period in between (Fig. 2 *b*), (*c*) asymmetric oscillations (Fig. 2 *c*), or (*d*) a single pulse followed by a sustained rise in calcium (Fig. 2 *d*).

All of these different types of cytosolic calcium oscillations have been found in experiments. For example, asymmetric oscillations similar to those of Fig. 2 *c* were observed in recent investigations on rat gonadotropes stimulated with GnRH (Tse et al., 1993). The other types can be related to, for example, calcium oscillations in hepatocytes (Rooney et al., 1989; Somogyi and Stucki, 1991; Woods et al., 1986).

The different types of cytosolic calcium oscillations can be observed in single cell types even under identical chemical stimulation since the response to a given agonist is a function of the individual cell (Fewtrell, 1993). Examples are experiments with hepatocytes stimulated with phenylephrine or vasopressin (Rooney et al., 1989; Somogyi and Stucki, 1991; Woods et al., 1986) and with cultured smooth muscle cells in response to histamine (Himpens et al., 1992). In the modeling approach the different types of calcium oscillations are obtained by varying some internal, single-cell-specific parameters (V_{M2}, K_R, k_F, K_C). Therefore the results indicate that the variability in the response of a single cell type to a given stimulation as observed in experiments can be explained in this way.

In addition to these basic features the model also accounts for specific experimental findings. As a general result it was observed that the latency between the time where the stimulus is applied and the appearance of the first calcium spike is shortened when the strength of the stimulus is higher (in the present situation this means that β has a larger value). Furthermore, a correlation exists between the calcium oscillation period and the latency of the first calcium spike: a longer latency is connected with a longer oscillation period. This behavior was found, for example, in experiments with hepatocytes stimulated by phenylephrine (Rooney et al., 1989).

Another important experimental result is that for many cell types the frequency of the cytosolic calcium oscillations depends on the strength of the external stimulus (Berridge and

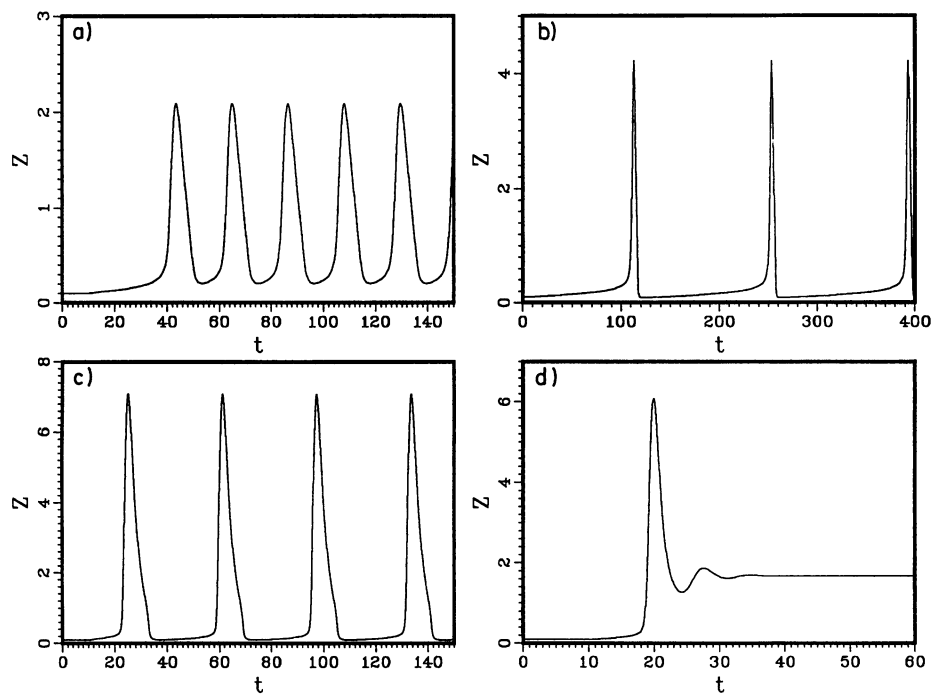

FIGURE 2 Time evolution of the intracellular calcium concentration. Parameters: $V_T = 3.2\ s^{-1}$, $K_H = 1.1$, $K_T = 0.1$, $\kappa_T = 1\ s^{-1}$, $\sigma = 5\ s^{-1}$, $\mu_1 = 0.01$, $\mu_2 = 1$, $\kappa_Q = 1\ s^{-1}$, $\nu_0 = 0.8\ \mu M/s$, $V_{M1} = 25\ \mu M/s$, $K_M = 0.2\ \mu M$, $k = 10\ s^{-1}$, $K_P = 0.1\ \mu M$, $V_{M3} = 10\ \mu M/s$, $l = 2$, $j = 1$, $p = 1$, $n = 2$, $m = 4$, $q = 3$, (*a*) $V_{M2} = 50\ \mu M/s$, $K_R = 1\ \mu M$, $k = 0.2\ s^{-1}$, $K_C = 0.5\ \mu M$ (*b*) $V_{M2} = 20\ \mu M/s$, $K_R = 1\ \mu M$, $k_F = 0.1\ s^{-1}$, $K_C = 3\ \mu M$ (*c*) $V_{M2} = 50\ \mu M/s$, $K_R = 2.2\ \mu M$, $k = 0.1\ s^{-1}$, $K_C = 1\ \mu M$ (d) $V_{M2} = 50\ \mu M/s$, $K_R = 1\ \mu M$, $k = 0.3\ s^{-1}$, $K_C = 1.5\ \mu M$ The cell is activated after $t = 10$ s. (*a*) $\beta = 0.25$; (*b*) $\beta = 0.4$; (*c* and *d*) $\beta = 0.6$.

Galione, 1988). The model reproduces this behavior if the parameters mentioned above (V_{M2}, K_P, V_{M3}, K_R, k_F) are chosen appropriately. Fig. 3 is an example of the calcium oscillations of Fig. 2 *b*. A rise in the strength of the external stimulus results in a substantial decrease in the oscillation period. This specific example can be related qualitatively (spike form) and quantitatively (oscillation frequency) to experiments with hepatocytes stimulated by vasopressin (Woods et al., 1986).

On the other hand the symmetric oscillation type (Fig. 2 *a*) does not exhibit a correlation between the frequency of the calcium oscillations and the strength of the external stimulus. This result conforms with experimental findings. So far there is no experimental evidence that symmetric (sinusoidal) calcium oscillations show such a correlation (Fewtrell, 1993).

In many cell types the cytosolic calcium oscillations can be influenced by extracellular calcium. This leads to a slowing down of the oscillation period or even to the abolishment of oscillations. The former case was observed, for example, in hepatocytes stimulated with phenylephrine (Rooney et al., 1989), and the latter accounts for the situation in, for example, T-lymphocytes (Lewis and Cahalan, 1989; Payet et al., 1991). Fig. 3 shows an example of the calcium oscillation type of Fig. 2 *b* ($\beta = 0.6$). The influx of extracellular calcium is modified by varying the parameter ν_0 of Eq. 4. One finds that the oscillation period is strongly affected by extracellular calcium influx. A complete abolishment necessitates that all parameters that represent external calcium sources are set at zero, that is, $\nu_0 = 0$ and $V_{M1} = 0$.

In the model the cytosolic calcium oscillations are accompanied by oscillations of IP_3. Fig. 4 shows the temporal

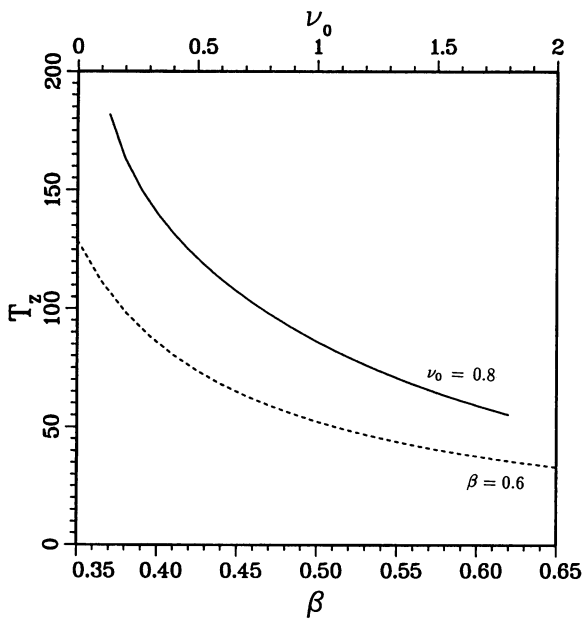

FIGURE 3 Oscillation period T_Z of cytosolic calcium oscillations of the type of Fig. 2 *b*. *Solid line,* oscillation period as a function of the strength of the external stimulus measured by the parameter $\beta(\nu_0 = 0.8)$. *Dashed line,* oscillation period as a function of the influx of extracellular calcium measured by the parameter ν_0 ($\beta = 0.6$). Other parameters as in Fig. 2 *b*.

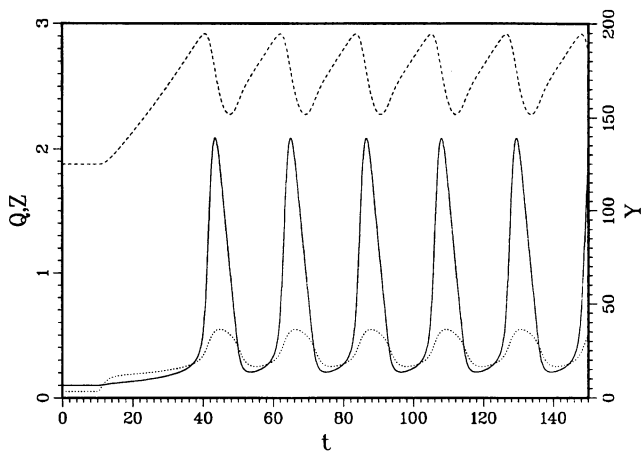

FIGURE 4 Time dependence of the intracellular concentrations of inositol(1,4,5)trisphosphate (Q, ·····) and calcium (Z, ——), and of the calcium concentration in the calcium store (Y, - - - -). The stimulus is applied after $t = 10$ s; parameters as in Fig. 2 *a*.

evolution of these variables and of the calcium concentration in the intracellular store in more detail. After the external stimulus is applied ($t \geq 10$ s) the IP_3 concentration rises rapidly. This behavior is exclusively due to the activation of the G-proteins. The cytosolic calcium concentration rises much more slowly, and no spikes occur in this initial phase. The influx of calcium into the cell mainly leads to an increase of calcium in the store (compare Fig. 4).

After this initial phase the mutual interaction between the cytosolic calcium and IP_3 pathways causes a further increase in both variables. This continues until the threshold for the onset of oscillations is reached. The threshold is characterized by two supplementary conditions: the concentration of IP_3 and of calcium in the internal store must obtain a certain value. Beyond this value the calcium pressure within the store and the strength of the IP_3 signal lead to a rapid emptying of the store. As soon as the cytosolic calcium concentration rises the different calcium pumps are activated. In this way the intracellular calcium concentration is lowered again. This leads to the formation of cytosolic calcium oscillations.

The behavior described above was observed in recent experiments with permeabilized hepatocytes (Missiaen et al., 1991). Here it was found that internal IP_3-sensitive stores release calcium spontaneously when overloaded with calcium. As discussed above this mechanism was dependent on a threshold level of IP_3. These results strongly support the modeling approach. The inclusion of the term $\nu_1(Q)$ in Eq. 4, which accounts for this behavior, is particularly justified.

3.1 Bistability

So far the conventional behavior of the cytosolic calcium oscillator has been discussed. This means that a rise in the strength of the external stimulus (measured by the parameter β) leads to a Hopf bifurcation that is connected with the onset of self-sustained oscillations. In the system presented here another possibility arises when the exponent p in Eq. 5, which

characterizes the catalyzing behavior of cytosolic calcium, is changed from $p = 1$ to $p = 2$. We do not know any details about the feedback loop on the PLC activation. Therefore different values of p have to be considered.

Fig. 5 is a diagram of the steady-state solutions of Eqs. 1–8 ($p = 2$). In this case the model exhibits a different kind of behavior, which is termed *bistability*. This means that over a finite range of the bifurcation parameter β two stable steady states exist. Systems showing bistability are common in nonlinear optics (Lugiato, 1984). They are also considered in some special enzyme reactions (Li and Goldbeter, 1989). In Fig. 5 it is seen that the intracellular calcium concentration rises only slightly when the strength of the external stimulus β is increased from zero. However, when β exceeds a critical value ($\beta \approx 0.38$) two new states are born via a saddle-node bifurcation. This means that besides the low-concentration, nonoscillating branch a second stable state corresponding to a high intracellular calcium concentration now exists. These two states are seperated by an unstable saddle (compare Fig. 5). A further increase of β yields an inverse saddle-node bifurcation ($\beta \approx 0.68$). Here the low-concentration steady state vanishes together with the saddle. Above this β value only one state exits that is connected with a high intracellular calcium concentration.

An interesting feature of the upper-branch steady state is that it becomes unstable via a Hopf bifurcation at $\beta \approx 0.39$. This is only slightly above the value of the saddle-node bifurcation where the state is born ($\beta \approx 0.38$). In this way an oscillating limit-cycle solution that corresponds to an oscillating intracellular calcium concentration is created. Bistable systems where an oscillating solution exists on the upper stable branch are again found in nonlinear optics (Merkle and Kaiser, 1991).

The behavior described above has consequences for the biological cell system. In the present situation the stimulation of the cell does not yield an oscillating behavior immediately. Instead of this the intracellular calcium concentration rises slowly, thus causing a long transient. If the strength of the external stimulus exceeds a critical value ($\beta \approx 0.68$) the system has to leave the lower branch and jump to the upper branch. This marks the onset of oscillations. An experimental situation where intracellular calcium oscillations are preceded by a long period where calcium stays nearly at its basal level was observed, for example, in pancreatic acinar cells stimulated by acetylcholine (Yule and Gallacher, 1988). These findings might be connected with the situation described by the model.

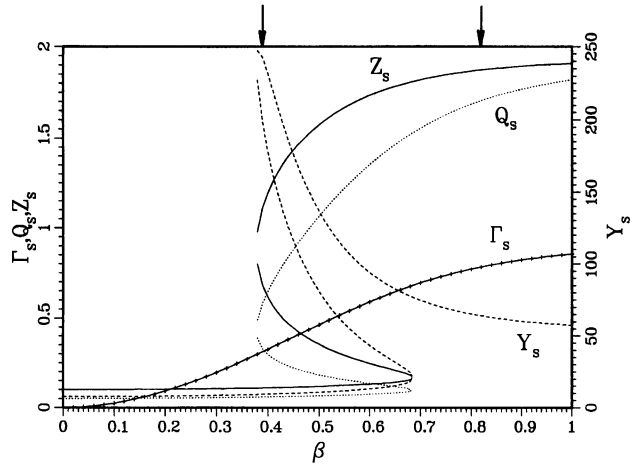

FIGURE 5 Steady-state solutions of Eqs. 1–8 as a function of the strength of the external stimulus β. ++++, Γ_S; ·····, Q_S; - - - -, Y_S; ——, Z_S. The arrows at the curve for the upper branch steady state of Z mark the β range where an oscillating intracellular calcium concentration exists. Parameters: $V_{M2} = 100$ μM/s, $K_P = 0.8$ μM, $V_{M3} = 20$ μM/s, $K_R = 1.8$ μM, $k_F = 0.2$ s^{-1}, $K_C = 1.4$ μM, $p = 2$; others as in Fig. 2.

There are several ways by which the transient behavior may be shortened. One is connected with the existence of fluctuations. It is intuitively clear that fluctuations of a certain strength can cause the system to jump to the upper branch for $\beta < 0.68$. Biological systems like cells are open systems. This means that additional fluctuations do exist (Weaver and Astumian, 1990). The transition from the lower branch to the upper one is connected with the organization of an oscillating state as discussed above. However, this limit-cycle oscillation is much more resistant against the influence of noise compared to the lower-branch steady state.

3.2 The influence of a periodic modulation of the signal pathway

Fluctuations will shorten the transient evolution of the system described in the preceding section. One may also ask whether it is possible to reach the opposite behavior, that is, whether it is possible to cause a considerable delay in the transition from the lower- to the upper-branch steady state. In the present situation this means that the onset of calcium oscillations is partially suppressed.

We want to investigate a case where the signal pathway between the activated hormone–receptor complex and the G-protein is modulated by an external oscillating field. The strength of the signal transduction is characterized by the parameter V_T in Eq. 1. The following substitution is considered:

$$V_T \rightarrow V_T(1 + f(t)) = V_T(1 + F_1 \cos(\omega t)),$$

where ω is the frequency of the field and F_1 is the modulation amplitude ($F_1 < 1$).

The specific choice of a modulation of the parameter V_T is not essential. If another parameter of the first term on the right side of Eq. 1 is modulated the results discussed below are qualitatively the same.

The procedure described here allows for investigations where instead of a shift of chemical equilibria the rate of the signal transduction process is modified (Markin et al., 1992). In the specific example of calcium oscillations this means that one investigates a modulation of the activation of the G-proteins. This will have consequences for subsequent processes.

In Fig. 6 the temporal evolution of the intracellular calcium concentration in the absence ($F_1 = 0$) and in the pres-

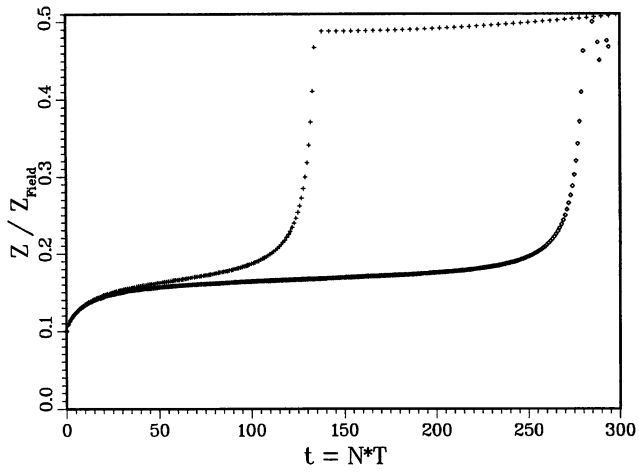

FIGURE 6 Time evolution of the intracellular calcium concentration Z in the absence (+) or in the presence (◇) of an external modulation $F_1 \cos(\omega t)$. The cell is activated after $t = 0$, $\beta = 0.7$. The intracellular calcium concentration is shown for times $t = NT$, $T = 2\pi/\omega$. Parameters: $F_1 = 0.3$, $\omega = 1\ s^{-1}$; others as in Fig. 4. The sharp rise of the curves indicates the onset of oscillations.

ence ($F_1 = 0.3$) of the external field is shown. The sharp rise of the curves indicates the onset of oscillations. One can see that in the presence of the modulation there is a considerable delay. The rise of the cytosolic calcium concentration is slowed down. This behavior results even though the mean value of the modulation vanishes. However, a nonlinear differential equation is considered. This means that the delay in the onset of oscillations is a result of the interaction term with the nonlinear term, which describes the activation of the G-proteins (first term on the right side of Eq. 1). The consequence of this behavior is that the correlation $\langle \Gamma(t)\, f(t) \rangle_t$ does not vanish.

Two conclusions can be drawn from the behavior described above. First, it is clearly seen that a nonlinear interaction of the modulation with the biological system (in this case the signal transduction from the hormone–receptor complex to the G-protein) causes a completely different behavior compared to a linear interaction. This is a well-known feature (Kaiser, 1988). For example, in this context nonlinear interactions of an oscillating electric field on membrane proteins were discussed by R. D. Astumian and B. Robertson (Astumian and Robertson, 1989).

Another point concerns the importance of intracellular calcium as a second messenger. The delay in the onset of cytosolic calcium oscillations automatically requires that the cell's response to the external (first) messenger also be delayed. It is interesting to note that experiments exist where the behavior described in this section has been found. One example is the observation that the activity of cAMP-independent protein kinase (like protein kinase, for example) is reduced in the presence of an external field (Byus et al., 1984). While these experimental results are not yet understood the model presented here offers a hint about a possible explanation.

3.3 Negative feedback on activated G-proteins

The previous discussion was based on the existence of a single feedback loop where cytosolic calcium catalyzes the activation of PLC (positive feedback). In this case Eq. 1, which describes the time evolution of the fraction of activated G-proteins, is not coupled to the other model equations. There are experiments that indicate the existence of negative feedback loops (Berridge and Galione, 1988; Cuthbertson, 1989). Negative feedback on the activated G-proteins could come from activated PLC or from PKC (or from other sources).

In order to investigate the effects of a negative feedback loop on the status of the activation process of the G-proteins, Eq. 1 of the system considered so far has to be modified. We will restrict ourselves to the possibility that activated PLC enhances the deactivation of the G-proteins. However, the procedure to be discussed below is by no means limited to this case.

The negative feedback exerted by activated PLC is taken into account by modifying the last term on the right side of Eq. 1 in the following manner: $\kappa_T \rightarrow \kappa_T V_{MP} P'/(K_{PLC} + P')$, where P' denotes the concentration of activated PLC. As in the derivation of Eq. 5, it is possible to express P' through the fraction of activated G-proteins (Γ) and the intracellular calcium concentration (Z) (one has to use the reaction equations given in section 3 again). This yields the following modified Eq. 1:

$$\frac{d\Gamma}{dt} = V_T \frac{\beta^l}{K_H^l + \beta^l} \frac{1 - \Gamma}{K_T + 1 - \Gamma} - \kappa_T \frac{\Gamma^{j+1} Z}{K_C + (1 + \theta\Gamma^j) Z}, \tag{9}$$

where

$$\theta = \left(1 + \frac{P_{total}}{K_{PLC}(1 + Q_2/K_Q')}\right) \Big/ \mu_2.$$

Fig. 7 shows a typical example for the time evolution of the fraction of activated G-proteins and the intracellular calcium concentration in the presence of an external stimulus.

The results can be summarized as follows. An additional feedback mechanism (especially a negative feedback loop) may act as a control for the specific shape of the oscillation pattern of intracellular calcium oscillations. For example, the absolute level of cytosolic calcium may be controlled in this way. The interaction of intracellular calcium with the IP_3-induced calcium release of the store may be interpreted in a similar manner.

4. SUMMARY AND OUTLOOK

We have presented a model for IP_3-induced calcium oscillations. The oscillations are a consequence of a single positive feedback loop between intracellular calcium and the activation of PLC. This property makes possible a comparison with the CICR model of Goldbeter et al. In both approaches cytosolic calcium oscillations are connected with a single feedback mechanism. This is either the CICR process or the

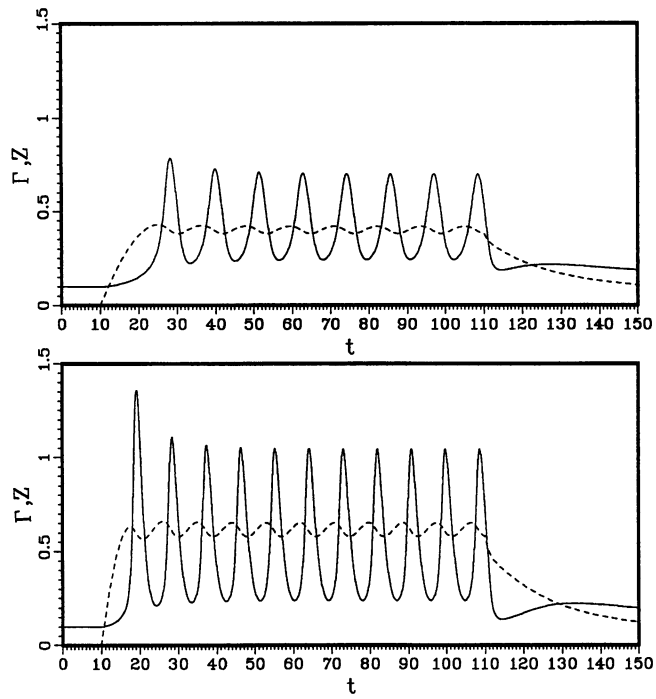

FIGURE 7 Time evolution of the fraction of activated G-proteins (Γ, - - - -) and of the intracellular calcium concentration (Z, ——). Parameters: $V_T = 1\ s^{-1}$, $K_H = 1$, $K_T = 1$, $\kappa_T = 1\ s^{-1}$, $\theta = 2$, $\sigma = 5\ s^{-1}$, $\mu_1 = 0.01$, $\mu_2 = 1$, $\kappa_Q = 1\ s^{-1}$, $K_C = 1\ \mu M$, $\nu_0 = 0.5\ \mu M/s$, $V_{M1} = 10\ \mu M/s$, $K_M = 0.95\ \mu M$, $k = 10\ s^{-1}$, $V_{M2} = 20\ \mu M/s$, $K_P = 0.1\ \mu M$, $V_{M3} = 10\ \mu M/s$, $K_R = 1\ \mu M$, $k_F = 0.4\ s^{-1}$ $l = 2$, $j = 1$, $p = 1$, $n = 2$, $m = 4$, $q = 1$. The stimulus is applied from $t = 10$ s to $t = 110$ s. *Top*, $\beta = 0.33$; *bottom*, $\beta = 0.66$.

positive feedback on the activation of PLC by cytosolic calcium. In both cases the feedback loop is under the control of calcium itself.

It is possible to reproduce a number of experimental findings with the model presented here. These concern the temporal evolution of the cytosolic calcium concentration after the stimulation of the cell as well as the different types of spikes. The latter are closely connected with internal (single-cell-specific) parameters.

The model incorporates two important innovations compared to other approaches: the inclusion of a modified influx of external calcium after the stimulation of the cell and a detailed description of the signal pathway from the receptor activation to an increase in IP_3 production.

The influx of extracellular calcium is not responsible for the onset of oscillations since these are a consequence of changes in the internal calcium balance connected with intracellular calcium stores. However, the stimulation of the cell also leads to an altered influx of extracellular calcium. This can be a consequence of the emptying of the intracellular calcium stores, which may be signaled to the plasma membrane through calmodulin-dependent processes (Haverstick and Gray, 1993). Further experimental evidence will provide information on this point. It will also facilitate the decision about how to modify or extend the model with respect to this additional signal pathway.

Other modifications or extensions of the model include the consideration of additional feedback mechanisms. These may concern the modulation of the IP_3 receptor by calcium (Bezprozvanny et al., 1991). We discussed another example: a negative feedback loop on the activated G-proteins exerted by activated PLC (Cobbold et al., 1988). Such processes can function as additional controls of the calcium signaling process. However, they are not necessary to account for cytosolic calcium oscillations in principle.

Another example that has gained much attention is the interaction of low-frequency electromagnetic fields with cellular systems. There are now reliable experiments that demonstrate that even very weak external fields can influence the calcium metabolism of the cell (Blackman et al., 1989; Lyle et al., 1991; Walleczek and Liburdy, 1990; Walleczek and Budinger, 1992). In particular, experiments on T-lymphocytes revealed a strong dependence on the activation status of the cells. Only activated cells were sensitive to an interaction with the external field (Walleczek and Liburdy, 1990; Walleczek and Budinger, 1992). So far there is no satisfying theoretical explanation for this behavior. However, the dependence of the results on the activation status of the cells points to the involvement of the calcium signal pathway in the interaction (Kaiser, 1992; Walleczek, 1992).

These results strongly suggest that these findings should be included in the model. A modulation of the signal pathway at an early stage by nonlinear interactions can lead to macroscopic changes in the calcium metabolism of the cell. Of course, detailed experiments have to be performed in the future to reveal more information on this point. Of particular importance is the location of the interaction of the external electromagnetic field with the cell, which remains to be determined. The detailed structure of the model presented here makes it possible to include such information. In this way a concept that enables a comprehensive description of such interactions is put forward.

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REFERENCES

Adey, W. R. 1989. Biological effects of radio frequency electromagnetic radiation. *In* Electromagnetic Interaction with Biological Systems. J. C. Lin, editor. Plenum Publishing Corporation, New York. 109–140.

Allbritton, N. L., T. Meyer, and L. Stryer. 1992. Range of messenger action of calcium ion and inositol 1,4,5-trisphosphate. *Science (Washington DC).* 258:1812–1815.

Alvarez, J., M. Montero, and J. Garcia-Sancho. 1992. Cytochrome P450 may regulate plasma membrane Ca^{2+} permeability according to the filling state of the intracellular Ca^{2+} stores. *FASEB J.* 6:786–792.

Astumian, R. D., and B. Robertson. 1989. Nonlinear effect of an oscillating electric field on membrane proteins. *J. Chem. Phys.* 91:4891–4901.

Berridge, M. J., and P. E. Rapp. 1979. A comparative survey of the function, mechanism and control of cellular oscillators. *J. Exp. Biol.* 81:217–279.

Berridge, M. J., and A. Galione. 1988. Cytosolic calcium oscillators. *FASEB J.* 2:3074–3082.

Berridge, M. J., and R. F. Irvine. 1989. Inositol phosphates and cell signalling. 1989. *Nature (Lond.).* 341:197–204.

Berridge, M. J. 1990. Calcium oscillations. *J. Biol. Chem.* 265:9583–9586.

Berridge, M. J. 1993. Inositol trisphosphate and calcium signalling. *Nature (Lond.).* 361:315–325.

Bezprozvanny, I., J. Watras, and B. E. Ehrlich. 1991. Bell-shaped calcium-response curves of Ins(1,4,5)P_3- and calcium-gated channels from endoplasmic reticulum of cerebellum. *Nature (Lond.).* 351:751–754.

Blackman, C. F., L. S. Kinney, D. E. House, and W. T. Joines. 1989. Multiple power-density windows and their possible origin. *Bioelectromagnetics.* 10:115–128.

Byus, C. V., R. L. Lundak, R. M. Fletcher, and W. R. Adey. 1984. Alterations in protein kinase activity following exposure of cultured lymphocytes to modulated microwave fields. *Bioelectromagnetics.* 5:34–51.

Cadossi, R., F. Bersani, A. Cossarizza, P. Zucchini, G. Emilia, G. Torelli, and C. Franceschi. 1992. Lymphocytes and low frequency electromagnetic fields. *FASEB J.* 6:2667–2674.

Cobbold, P., K. S. R. Cuthbertson, and N. M. Woods. 1988. The generation of repetitive free calcium transients in a hormone-stimulated hepatocyte. *In* Hormones and Cell Regulation No. 12. J. Nunez, J. E. Dupont, and E. Carafoli, editors. INSERM/John Libbey Eurotext, Paris. 135–146.

Cockcraft, S. 1992. G-protein-regulated phospholipases C, D and A_2-mediated signalling in neutrophils. *Biochim. Biophys. Acta.* 1113:135–160.

Cuthbertson, K. S. R. 1989. Intracellular calcium oscillators. *In* Cell to Cell Signalling: From Experiments to Theoretical Models. A. Goldbeter, editor. Academic Press, London. 435–447.

Dupont, G., and A. Goldbeter. 1989. Theoretical insights into the origin of signal-induced calcium oscillations. *In* Cell to Cell Signalling: From Experiments to Theoretical Models. A. Goldbeter, editor. Academic Press, London. 461–474.

Dupont, G., and A. Goldbeter. 1992. Protein phosphorylation driven by intracellular calcium oscillations: a kinetic analysis. *Biophys. Chem.* 42: 257–270.

Fewtrell, C. 1993. Ca^{2+} oscillations in non-excitable cells. *Annu. Rev. Physiol.* 55:427–454.

Finch, E. A., T. J. Turner, S. M. Goldin. 1991. Calcium as a coagonist of inositol 1,4,5-trisphosphate-induced calcium release. *Science (Washington DC).* 252:443–446.

Frey, A. H. 1993. Electromagnetic field interactions with biological systems. *FASEB J.* 7:272–281.

Gardner, P., A. Alcover, M. Kuno, P. Moingeon, C. M. Weyand, J. Goronzy, and E. L. Reinherz. 1989. Triggering of T-lymphocytes via either T3-Ti or T11 surface structures opens a voltage-insensitive plasma membrane calcium-permeable channel: requirement for interleukin-2 gene function. *J. Biol. Chem.* 264:1068–1076.

Gardner, P. 1989. Calcium and T lymphocyte activation. *Cell.* 59:15–20.

Gardner, P. 1990. Patch clamp studies of lymphocyte activation. *Annu. Rev. Immunol.* 8:231–252.

Gilman, A. G. 1987. G-Proteins: transducers of receptor-generated signals. *Annu. Rev. Biochem.* 56:615–649.

Girard, S., and D. Clapham. 1993. Acceleration of intracellular calcium waves in *Xenopus* oocytes by calcium influx. *Science (Washington DC).* 260:229–232.

Goldbeter, A. (ed.) 1989. Cell to Cell Signalling: From Experiments to Theoretical Models. Academic Press, London.

Goldbeter, A., G. Dupont, and M. J. Berridge. 1990. Minimal model for signal-induced Ca^{2+} oscillations and for their frequency encoding through protein phosphorylation. *Proc. Natl. Acad. Sci. USA.* 87: 1461–1465.

Grundler, W., and F. Kaiser. 1992. Experimental evidence for coherent excitations correlated with cell growth. *Nanobiology.* 1:163–176.

Grundler, W., F. Kaiser, F. Keilmann, and J. Walleczek. 1992. Mechanisms of electromagnetic interaction with cellular systems. *Naturwissenschaften.* 79:551–559.

Harootunian, A. T., J. P. Y. Kao, S. Paranjape, and R. Y. Tsien. 1991. Generation of calcium oscillations in fibroblasts by positive feedback between calcium and IP_3. *Science (Washington DC).* 251:75–78.

Haverstick, D. M., and L. S. Gray. 1993. Increased intracellular Ca^{2+} induces Ca^{2+} influx in human T lymphocytes. *Mol. Biol. Cell.* 4:173–184.

Himpens, B., H. De Smedt, G. Droogmans, and R. Casteels. 1992. Differences in regulation between nuclear and cytoplasmic Ca^{2+} in cultured smooth muscle cells. *Am. J. Physiol.* 263:C95–C105.

Hoth, M., and R. Penner. 1992. Depletion of intracellular calcium stores activates a calcium current in mast cells. *Nature (Lond.).* 355:353–356.

Iino, M., and M. Endo. 1992. Calcium-dependent immediate feedback control of inositol 1,4,5-trisphosphate-induced Ca^{2+} release. *Nature (Lond.).* 360:76–78.

Kaiser, F. 1977. Limit cycle model for brain waves. *Biol. Cybern.* 27: 155–163.

Kaiser, F. 1987. The role of chaos in biological systems. *In* Energy Transfer Dynamics. T. W. Barrett and H. A. Pohl, editors. Springer, Berlin. 224–236.

Kaiser, F. 1988. Theory of nonlinear excitations. *In* Biological Coherence and Response to External Stimuli. H. Fröhlich, editor. Springer, Berlin. 25–48.

Kaiser, F., and C. Eichwald. 1991. Bifurcation structure of a driven, multi-limit-cycle van der Pol oscillator. *Int. J. Bifurcation Chaos.* 1:485–491.

Kaiser, F. 1992. Biophysical models related to Fröhlich excitations. *Nanobiology.* 1:149–161.

Khan, A. A., J. P. Steiner, M. G. Klein, M. F. Schneider, and S. H. Snyder. 1992. IP_3 receptor: localization to plasma membrane of T cells and cocapping with the T cell receptor. *Science (Washington DC).* 257:815–818.

Kraus, M., and B. Wolf. 1993. Cytosolic calcium oscillators: critical discussion and stochastic modelling. *Biol. Sign.* 2:1–15.

Lewis, R. S., and M. D. Cahalan. 1989. Mitogen-induced oscillations of cytosolic Ca^{2+} and transmembrane Ca^{2+} current in human leukemic T cells. *Cell Regul.* 1:99–112.

Li, Y-X., and A. Goldbeter. 1989. Oscillatory isozymes as the simplest model for coupled biochemical oscillators. *J. Theor. Biol.* 138:149–174.

Lückhoff, A., and D. E. Clapham. 1992. Inositol 1,3,4,5-tetrakisphosphate activates an endothelial Ca^{2+}-permeable channel. *Nature (Lond.).* 355: 356–358.

Lugiato, L. A. 1984. Theory of optical bistability. *In* Progress in Optics XXI. E. Wolf, editor. North-Holland, Amsterdam. 68–213.

Lyle, D. B., X. Wang, R. D. Ayotte, A. R. Sheppard, and W. R. Adey. 1991. Calcium uptake by leukemic and normal T-lymphocytes exposed to low frequency magnetic fields. *Bioelectromagnetics.* 12:145–156.

Markin, V. S., D. Liu, M. D. Rosenberg, and T. Y. Tsong. 1992. Resonance transduction of low level periodic signals by an enzyme: an oscillatory actication barrier model. *Biophys. J.* 61:1045–1049.

Mason, M. J., M. P. Mahaut-Smith, and S. Grinstein. 1991. The role of intracellular Ca^{2+} in the regulation of the plasma membrane Ca^{2+} permeability of unstimulated rat lymphocytes. *J. Biol. Chem.* 266: 10872–10879.

Merkle, D., and F. Kaiser. 1991. Dynamics of the passive optical ring resonator: stability analysis and the onset of self-pulsing. *Phys. Lett. A.* 153: 95–100.

Meyer, T., D. Holowka, and L. Stryer. 1988. Highly cooperative opening of calcium channels by inositol 1,4,5-trisphosphate. *Science (Washington DC).* 240:653–655.

Meyer, T., and L. Stryer. 1988. Molecular model for receptor-stimulated calcium spiking. *Proc. Natl. Acad. Sci. USA.* 85:5051–5055.

Meyer, T., and L. Stryer. 1990. Transient calcium release induced by successive increments of inositol 1,4,5-trisphosphate. *Proc. Natl. Acad. Sci. USA.* 87:3841–3845.

Meyer, T., and L. Stryer. 1991. Calcium spiking. *Annu. Rev. Biophys. Biophys. Chem.* 20:153–174.

Missiaen, L., C. W. Taylor, and M. J. Berridge. 1991. Spontaneous calcium release from inositol trisphosphate-sensitive calcium stores. *Nature (Washington DC).* 352:241–244.

Miyazaki, S., M. Yuzaki, K. Nakada, H. Shirakawa, S. Nakanishi, S. Nakade, and K. Mikoshiba. 1992. Block of Ca^{2+} wave and Ca^{2+} oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster eggs. *Science (Washington DC).* 257:251–255.

Nemeth, E. F., and E. Carafoli. 1990. The role of extracellular calcium in the regulation of intracellular calcium and cell function. *Cell Calcium.* 11:319–321.

Nishizuka, Y. 1992. Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science (Washington DC).* 258:607–614.

Payet, M. D., L. Bilodeau, J. Héroux, G. Harbec, and G. Dupuis. 1991.

Spectrofluorimetric and image recordings of spontaneous and lectin-induced cytosolic calcium oscillations in Jurkat T cells. *Cell Calcium.* 12:325–334.

Polk, C. 1992. Dosimetric extrapolations of extremely low-frequency electric and magnetic fields across biological systems. *Bioelectromagnetics Suppl.* 1:205–208.

Putney, J. W., Jr. 1986. A model for receptor-regulated calcium entry. *Cell Calcium.* 7:1–12.

Restrepo, D., T. Miyamoto, B. P. Bryant, and J. H. Teeter. 1990. Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish. *Science (Washington DC).* 249:1166–1168.

Rooney, T. A., E. J. Sass, and A. P. Thomas. 1989. Characterization of cytosolic calcium oscillations induced by phenylephrine and vasopressin in single fura-2-loaded hepatocytes. *J. Biol. Chem.* 264:17131–17141.

Snyder, P. M., K-H. Krause, and M. J. Welsh. 1988. Inositol trisphosphate isomers, but not inositol 1,3,4,5-tetrakisphosphate, induce calcium influx in *Xenopus laevis* oocytes. *J. Biol. Chem.* 263:11048–11051.

Somogyi, R., and J. W. Stucki. 1991. Hormone-induced calcium oscillations in liver cells can be explained by a simple one pool model. *J. Biol. Chem.* 266:11068–11077.

Tse, A., F. W. Tse, W. Almers, and B. Hille. 1993. Rhythmic exocytosis stimulated by GnRH-induced calcium oscillations in rat gonadotropes. *Science (Washington DC).* 260:82–84.

Tsien, R. W., and R. Y. Tsien. 1990. Calcium channels, stores, and oscillations. *Annu. Rev. Cell Biol.* 6:715–760.

Wakui, M., Y. V. Osipczuk, and O. H. Petersen. 1990. Receptor-activated cytoplasmic Ca^{2+} spiking mediated by inositol trisphosphate is due to Ca^{2+}-induced Ca^{2+} release. *Cell.* 63:1025–1032.

Walleczek, J., and R. P. Liburdy. 1990. Nonthermal 60 Hz sinusoidal magnetic-field exposure enhances $_{45}Ca_{2+}$ uptake in rat thymocytes: dependence on mitogen activation. *FEBS Lett.* 271:157–160.

Walleczek, J. 1992. Electromagnetic field effects on cells of the immune system: the role of calcium signalling. *FASEB J.* 6:3177–3185.

Walleczek, J., and T. F. Budinger. 1992. Pulsed magnetic field effects on calcium signalling in lymphocytes: dependence on cell status and field intensity. *FEBS Lett.* 314:351–355.

Weaver, J. C., and R. D. Astumian. 1990. The response of living cells to very weak electric fields: the thermal noise limit. *Science (Washington DC).* 247:459–462.

Woods, N. M., K. S. R. Cuthbertson, and P. H. Cobbold. 1986. Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. *Nature (Lond.).* 319:600–602.

Yule, D. I., and D. V. Gallacher. 1988. Oscillations of cytosolic calcium in single pancreatic acinar cells stimulated by acetylcholine. *FEBS Lett.* 239:358–362.