A Minimal Generic Model of Bacteria-Induced Intracellular Ca^{2+} Oscillations in Epithelial Cells

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ABSTRACT The toxin α-hemolysin expressed by uropathogenic *Escherichia coli* bacteria was recently shown as the first pathophysiologically relevant protein to induce oscillations of the intracellular Ca^{2+} concentration in target cells. Here, we propose a generic three-variable kinetic model describing the Ca^{2+} oscillations induced in single rat renal epithelial cells by this toxin. Specifically, we take into account the interplay between 1), the cytosolic Ca^{2+} concentration; 2), IP_3-sensitive Ca^{2+} channels located in the membrane separating the cytosol and endoplasmic reticulum; and 3), toxin-related activation of production of IP_3 by phospholipase C. With these ingredients, the predicted response of cells exposed to the toxin is in good agreement with the results of experiments.

INTRODUCTION

The calcium ion, Ca^{2+}, is one of the most versatile and universal cellular signaling agents in biological systems (Berridge et al., 1998, 2003; Orrenius et al., 2003). Regulation of the Ca^{2+} concentration in cells involves Ca^{2+} transport via the plasma membrane and Ca^{2+} exchange between the cytosol and intracellular compartments, including the endoplasmic reticulum (ER) and mitochondria. The Ca^{2+} fluxes depend in a nonlinear fashion on Ca^{2+} concentration levels and profiles as well as on the concentration of the second messenger, inositol 1,4,5-trisphosphate (IP_3). The intracellular Ca^{2+} concentration often exhibits a periodic temporal behavior (Goldbeter, 1996; Schuster et al., 2002). The oscillatory features of a Ca^{2+} signal provide a general mechanism for cells to control and distinguish between different Ca^{2+}-regulated intracellular events, and the temporal increase in Ca^{2+} also enables cells to avoid the cytotoxic effects that prolonged increases of the intracellular Ca^{2+} concentration otherwise would exert on cells. In various human, animal, and plant cells, the frequency of Ca^{2+} oscillations ranges from 10^{-3} to 1 Hz.

A large variety of mathematical mean-field models have been developed to describe Ca^{2+} oscillations (Goldbeter, 1996; Schuster et al., 2002; Falcke, 2004; for Monte Carlo simulations, see Zhdanov, 2002). The majority of the available experimental and theoretical studies are focused on situations when the Ca^{2+} oscillations are not complicated by perturbations. There are also a few treatments of the effect of perturbations on the Ca^{2+}-supporting network. In particular, Li et al. (2004) proposed a model that describes several aspects of agonist-induced Ca^{2+} signaling in single pituitary gonadotrophs. The model is based on the agonist-mediated regulation of the Ca^{2+} channels between the cytosol and ER. Parthimos et al. (1999) analyzed the interplay of intracellular and membrane Ca^{2+} oscillators.

In nature, intracellular Ca^{2+} balance can be influenced by the intrusive processes related to viruses or bacteria. In vivo or in vitro, such processes may induce or suppress Ca^{2+} oscillations, as was recently shown in the case of interaction between uropathogenic *Escherichia coli* and rat renal epithelial cells (Uhlén et al., 2000; Laestadius et al., 2002). In this article, we construct a minimal generic model mimicking Ca^{2+} oscillations in this system.

OUTLINE OF THE EXPERIMENTAL DATA

The experiments (Uhlén et al., 2000) were performed in a culture of primary tubule epithelial cells from rat kidney. The intracellular cytosolic Ca^{2+} concentration was recorded in single cells loaded with Fura-2/AM (in the cytoplasm, the AM-group is cleaved off by cytosolic esterases making Fura-2 active and hydrophilic, so that it does not penetrate into organelles like ER). The fluorescence patterns revealed that addition of the pyelonephritogenic *E. coli* strain ARD6 to the cells induced Ca^{2+} oscillations whereas the control medium did not. The oscillatory response began ~30 min after the cells were exposed to bacteria and it was sustained for at least 60 min (in separate experiments, oscillations were observed for as long as 2.5 h). Immunohistochemistry studies and a gentamycin assay showed no bacterial attachment and intracellular proliferation at this stage, suggesting that the inducer of Ca^{2+} oscillations is secreted out from the bacteria. Accordingly, when cells were exposed to filtered *E. coli* supernatants, Ca^{2+} oscillations were induced within a few minutes. Using a set of defined bacterial mutants, it was eventually shown that the toxin α-hemolysin (Hly) is responsible for induction of Ca^{2+} oscillations, and this occurs most probably via interaction of Hly with a receptor located in the plasma membrane. Experimental studies aiming to

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identify the nature of the receptor are currently in progress (C. Oxhamre and A. Richter-Dahlfors, unpublished). In all three cases (with bacteria, filtered supernatant, and purified Hly) the period of oscillations was 12 ± 0.7 min. As a rule, the oscillations were slightly irregular, and with no apparent synchronization between adjacent cells. Inside separate cells, the Ca^{2+} gradients were observed primarily near the nucleus, whereas in other cellular regions, the Ca^{2+} distribution was nearly uniform.

Hly, which plays the key role in inducing Ca^{2+} oscillations, has a dual physiological function (Uhlén et al., 2000; Laestadius et al., 2002). At high concentration, 1), the toxin is cytolytic due to its pore-forming activity in the plasma membrane. However, at sublytic concentrations, 2), the toxin interacts with the cell membrane, probably via a specific receptor interaction, thus inducing intracellular Ca^{2+} oscillations. Such oscillations are commonly dependent on cellular production of inositol 1,4,5-trisphosphate (IP_3), which is generated upon activation of phospholipase-C (PLC). IP_3 is a second messenger that binds to and thereby activates the IP_3 receptor (IP_3R) of Ca^{2+} channels located in the ER membrane. Obviously, IP_3 is important for Hly-induced Ca^{2+} oscillations, since experimental data show that inhibition of PLC (by U73122) and IP_3R (by 2-aminoethoxydiphenyl borate, a cell-permeable IP_3R agonist) both abrogate the Ca^{2+} oscillations (Uhlén et al., 2000). Control experiments showed that addition of the Ca^{2+} ionophore ionomycin, which produces nonregulated Ca^{2+} pores in the cell membrane, resulted in persistent rise of the Ca^{2+} concentration in the cytosol, but no oscillations. Activation of voltage-operated L-type calcium channels with Bay K 8644 or by depolarization through an increase of the $[K^+]$ in the medium also resulted in a persistent rise in $[Ca^{2+}]$, but not oscillations. Thus, for induction of Ca^{2+} oscillations, factor *2* (above) is more important compared to *1* (above).

Additional experiments were performed (Uhlén et al., 2000) to clarify the role of other various specific factors in the Ca^{2+} response. In particular, blocking voltage-operated L-type calcium channels by nifedipine was found to abolish the oscillatory Ca^{2+} response.

The findings outlined above and reported in detail by Uhlén et al. (2000) and Laestadius et al. (2002) suggest that a model mimicking the oscillations under consideration can be constructed by complementing one of the existing models, which is capable of predicting spontaneous intracellular Ca^{2+} oscillations, with additional terms describing the toxin interaction with the receptors responsible for, or influencing, the IP_3 production. The parameters should then be chosen so that the constructed model does not exhibit oscillations in the absence of the toxin-receptor interaction.

MODEL

In the literature (see the review by Schuster et al., 2002), one can find three types of minimal two-variable models predicting spontaneous oscillations of the cytosolic Ca^{2+} concentration in combination, respectively, with 1), the Ca^{2+} concentration in ER; 2), the variable describing the IP_3R Ca^{2+} channels in ER; or 3), the concentration of Ca^{2+}-binding proteins. Although we cannot unequivocally exclude scenarios *1* and *3*, approach *2* appears to be more relevant as a basis for construction of our minimal generic model for the following two reasons. First, as already noted, the experiments (Uhlén et al., 2000) clearly indicate that the IP_3R Ca^{2+} channels play an important role in the oscillations under consideration, because inhibition of PLC (by U73122) and IP_3R (by 2-aminoethoxydiphenyl borate) suppresses the oscillations. Secondly, the receptor for Hly is a protein that provides structural support to the plasma membrane (C. Oxhamre, unpublished), and this protein has not previously been reported to elicit any intracellular signaling cascades. Thus, we do not know of any other effects of the activation of this receptor (e.g., on the Ca^{2+}-binding protein network), except for those on PLC and IP_3R. Thus, we employ a model of type *2* to describe the toxin-cell interaction, i.e., our two variables are the Ca^{2+} concentration in the cytosol, c, and the variable p_3 (see below), describing the IP_3R Ca^{2+} channels located in the membrane of ER.

For the Ca^{2+} concentration in the cytosol, we have

$$dc/dt = F_{ch} + F_{leak} - F_{pump}, \tag{1}$$

where F_{ch} is the Ca^{2+} flux into the cytosol from ER via the IP_3R channels, F_{leak} the leak flux into the cytosol, and F_{pump} the flux out of the cytosol to ER via Ca^{2+}-dependent pumps.

In general, the leak flux, F_{leak}, may occur from ER and also from the extracellular space through L-type calcium channels (Schuster et al., 2002). In the minimal model described here, the other two Ca^{2+} fluxes, F_{ch} and F_{leak}, are related to ER. This means that in the case of stable oscillations, F_{leak} should represent the flux from ER, because in this situation the three fluxes should be balanced. If, however, the model is aimed at the transient regimes, F_{leak} may contain both components.

In addition to IP_3 binding sites, the IP_3R Ca^{2+} channels usually contain a few activating and inhibiting Ca^{2+} binding sites (Sneyd and Dufour, 2002). In the simplest case, adopted here, there are two such sites, i.e.,

$$F_{ch} = F^0_{ch} p_1 p_2 p_3, \tag{2}$$

where F^0_{ch} is the maximum flux, p_1 is the parameter describing the effect of IP_3 on the channel function, and p_2 and p_3 are the Ca^{2+} site-related probabilities that a channel is open. Assuming that IP_3 and Ca^{2+} binding to and detachment from the activating regulatory sites are rapid and employing the steady-state approximation for these sites, we have

$$p_1 = p_{11} + p_{12} n/(K_1 + n) \tag{3}$$

and

$$p_2 = c/(K_2 + c), \quad (4)$$

where n is the IP_3 concentration in the cytosol, p_{11} is the parameter corresponding to the channel performance in the absence of IP_3, p_{12} is the IP_3-related increment of the probability that the channel is not blocked, $n/(K_1 + n)$ and $c/(K_2 + c)$ are the probabilities that the activating regulatory sites are occupied respectively by IP_3 and Ca^{2+}, and K_1 and K_2 are the corresponding equilibrium constants.

Ca^{2+} binding to the inhibiting regulatory sites of the IP_3R Ca^{2+} channels is considered to be relatively slow and described by the Langmuir equation,

$$dp_3/dt = -k_{31}cp_3 + k_{32}(1 - p_3), \quad (5)$$

where p_3 and $1 - p_3$ are the probabilities that a site is vacant or occupied, and k_{31} and k_{32} are the attachment and detachment rate constants, respectively. (Note that $k_{32} = k_{31}K_3$, where K_3 is the equilibrium rate constant.)

The flux F_{leak} is considered to be independent of c and n. For the flux corresponding to the Ca^{2+}-dependent pumps, we use the simplest expression based on the steady-state approximation (compare to Eq. 4),

$$F_{pump} = F^0_{pump}c/(K_{pump} + c), \quad (6)$$

where F^0_{pump} is the maximum flux, and K_{pump} is the corresponding equilibrium constant.

The equations above with $n = const$ make it possible to describe the toxin-free case. In this respect, our model is similar to that proposed by Atri et al. (1993) for mimicking Ca^{2+} oscillations in the *Xenopus* oocyte. The main difference is that we use the Langmuir equation (Eq. 5) instead of a more formal phenomenological equation employed by Atri and co-workers. In addition, in our case, each IP_3R Ca^{2+} channel has one inhibiting Ca^{2+} site instead of two such sites assumed by Atri et al. (1993).

In the presence of Hly, we should, in addition, introduce the terms describing the toxin-mediated activation of PLC, which generates IP_3. In principle, we should also take into account an increase of F_{leak} due to the toxin-induced activation of the L-type voltage-gated Ca^{2+} channels (Uhlén et al., 2000). This factor seems, however, to be minor compared to activation of PLC, because in the absence of activation the experiment and our model (see below) do not exhibit oscillations with an increase of F_{leak}. For this reason, we ignore this factor in the bulk of our calculations and focus our attention on the toxin-mediated production of IP_3 and activation of IP_3R.

Assuming the IP_3 production and degradation are rapid and using the steady-state approximation for these processes, we have

$$n = W_{IP3}/k_d, \quad (7)$$

where W_{IP3} is the IP_3-production rate, and k_d the degradation rate constant. W_{IP3} should increase with increasing the number of membrane-bound toxins. This is possible if the IP_3-producing PLC becomes directly or indirectly activated by the Hly receptor in the plasma membrane. In both cases, W_{IP3} can be represented as

$$W_{IP3} = W^0_{IP3}[P + \kappa(1 - P)], \quad (8)$$

where W^0_{IP3} is the toxin-free IP_3-production rate, P and $1 - P$ are the probabilities that PLC is out of and in direct (or indirect) contact with a toxin, and κ is the ratio of the corresponding IP_3-production activities. Substituting Eq. 8 into Eq. 7 yields

$$n = n_0[P + \kappa(1 - P)], \quad (9)$$

where $n_0 \equiv W^0_{IP3}/k_d$ is the IP_3 concentration in the toxin-free case.

Assuming in addition that the toxin binding to the membrane is irreversible, we have

$$dP/dt = -k_bNP, \quad (10)$$

where k_b is the binding rate constant, and N is the toxin concentration. To describe the experiment (Uhlén et al., 2000), we consider that N rapidly becomes constant. In this case, Eq. 10 can be integrated as $P = \exp(-k_bNt)$. Substituting this expression into Eq. 9 yields

$$n = n_0[\exp(-k_bNt) + \kappa(1 - \exp(-k_bNt))]. \quad (11)$$

Thus, to describe the toxin-induced Ca^{2+} oscillations, we have to integrate Eqs. 1 and 5 in combination with the expressions in Eqs. 2–4, 6, and 11. The initial conditions for Eqs. 1 and 5 should correspond to the steady-state toxin-free regime. In our calculations, the model parameters for the toxin-free case were fixed as shown in Table 1. In addition, we have two parameters, κ and k_bN, describing evolution of IP_3 and PLC. These parameters were varied as outlined below to illustrate various types of the cellular response to the toxin-membrane interaction.

TABLE 1 Parameters for the toxin-free case

Parameter	Value	Dimension
Channel		
F^0_{ch}	8.0	μM/min
Site 1		
p_{11}	0.2	—
p_{12}	0.8	—
K_1	5.0	μM
n_0	1.0	μM
Site 2		
K_2	0.7	μM
Site 3		
k_{31}	0.5	min^{-1}
K_3	0.7	μM
Leak		
F_{leak}	0.5	μM/min
Pump		
F^0_{pump}	2.0	μM/min
K_{pump}	0.1	μM/min

MODEL PARAMETERS

Our choice of the parameter values was based on several requirements:

1. In the toxin-free case described by Eqs. 1–6 (with $n = n_0$), the cell should be in a stable steady state.
2. For $n = n_0$, the model should not predict oscillations with increasing F_{leak}.
3. The species concentrations, c and n, have to be in the physiologically reasonable range (the basal level of Ca^{2+} is 0.10 ± 0.01 μM; in stimulated cells exhibiting Ca^{2+} oscillations, the Ca^{2+} concentration is increased up to 0.5–1.5 μM (Uhlén et al., 2000)).
4. The timescale, characterizing Hly-induced Ca^{2+} oscillations, has to be $\sim$10 min.

These requirements make it possible to determine the scale and ratio of the parameter values. The choice of the specific values of the parameters is, however, not unique—i.e., one can slightly change some of the parameters. The important point is that the oscillations can be observed in a limited range of the parameter values. For this reason, with variation of one of the parameters (in Table 1), one should usually change at least one other parameter to get oscillations.

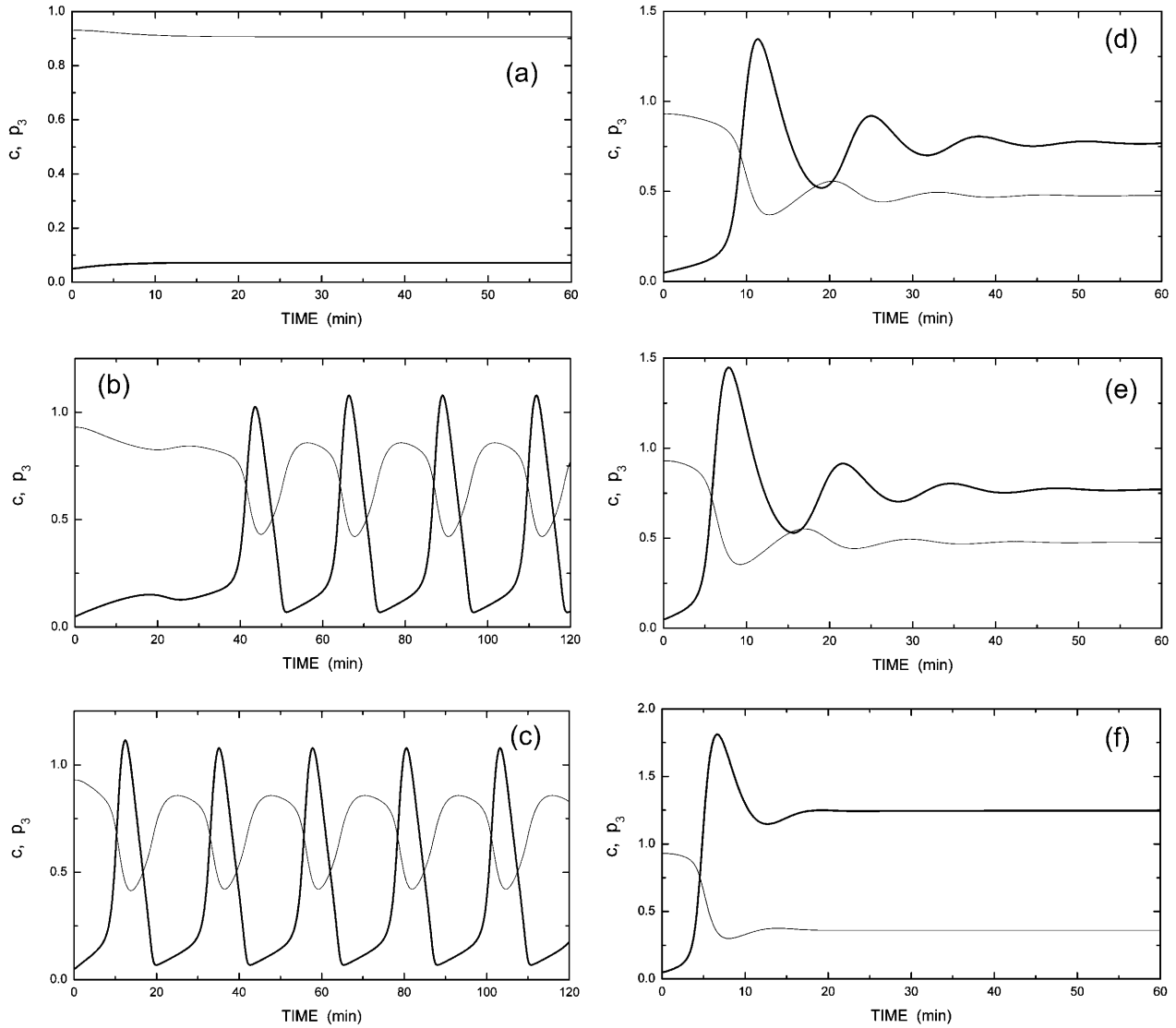

FIGURE 1 Cytosolic Ca^{2+} concentration (*thick solid line*), c (μM), and the probability that an inhibiting Ca^{2+} site of the IP_3R Ca^{2+} channels is vacant (*thin solid line*) as a function of time for the toxin-induced perturbation with (*a*) $\kappa = 3.0$ and $k_bN = 0.25$ min^{-1}, (*b*) $\kappa = 5.0$ and $k_bN = 0.25$ min^{-1}, (*c*) $\kappa = 5.0$ and $k_bN = 0.5$ min^{-1}, (*d*) $\kappa = 6.0$ and $k_bN = 0.25$ min^{-1}, (*e*) $\kappa = 6.0$ and $k_bN = 0.5$ min^{-1}, and (*f*) $\kappa = 10.0$ and $k_bN = 0.25$ min^{-1}. For the other parameters, see Table 1. The initial conditions correspond to the steady-state toxin-free regime.

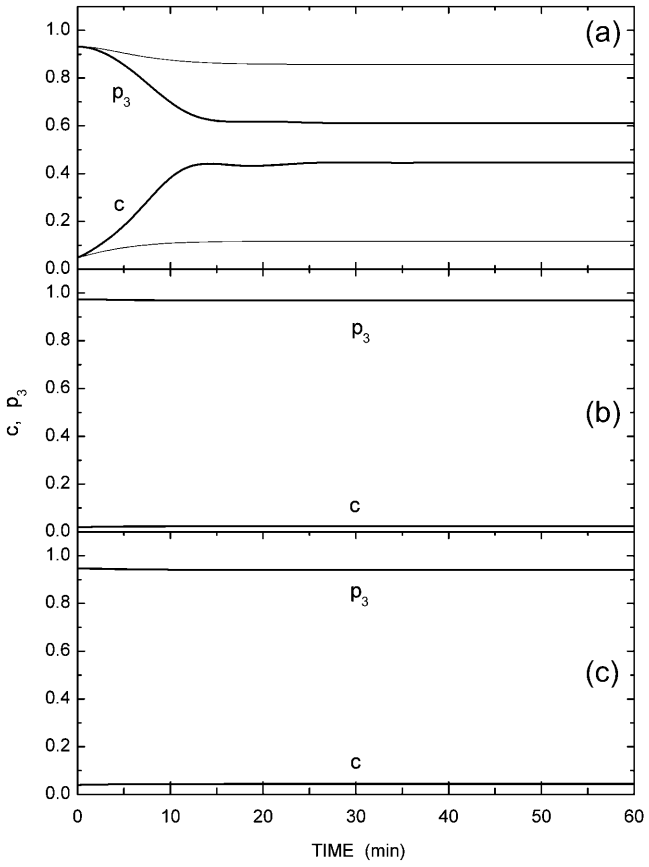

FIGURE 2 Cytosolic Ca^{2+} concentration, c (μM), and the probability that an inhibiting Ca^{2+} site of the IP_3R Ca^{2+} channels is vacant as a function of time. (*a*) Toxin-free case (for the parameters, see Table 1) with the perturbation, defined by Eq. 12 with $k_1 = 0.25\ \text{min}^{-1}$ and $\kappa_1 = 1.5$ and (*thin lines*) and 2 (*thick lines*). In this case, the model mimics the formation of additional pores by using the Ca^{2+} ionophore ionomycin or the activation of voltage-operated L-type calcium channels with Bay K 8644. (*b*) For the toxin-induced perturbation with $\kappa = 3.0$ and $k_bN = 0.25\ \text{min}^{-1}$ (the other parameters are as in Table 1, except $F_{\text{leak}} = 0.25\ \mu$M/min). (*c*) For the toxin-induced perturbation with $\kappa = 3.0$ and $k_bN = 0.25\ \text{min}^{-1}$ (the other parameters are as in Table 1, except $F^0_{\text{ch}} = 4.0\ \mu$M/min). In the latter two cases (*b* and *c*), the model mimics blocking voltage-operated L-type calcium channels by nifedipine. The initial conditions correspond to the steady-state toxin-free regime.

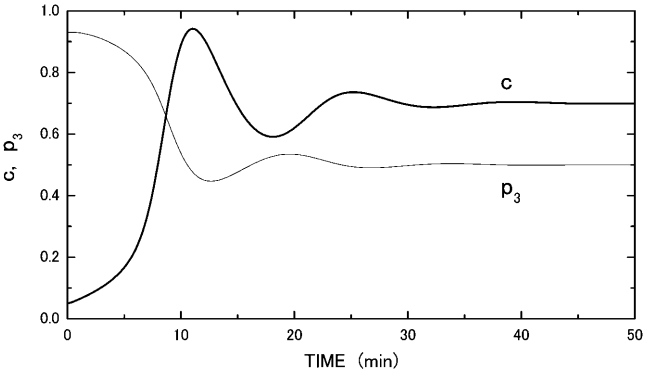

FIGURE 3 Cytosolic Ca^{2+} concentration, c (μM), and the probability that an inhibiting Ca^{2+} site of the IP_3R Ca^{2+} channels is vacant as a function of time for the toxin-induced perturbations defined by Eq. 11 with $\kappa = 3.0$ and $k_bN = 0.25\ \text{min}^{-1}$ and Eq. 12 with $\kappa_1 = 1.5$ and $k_1 = 0.25\ \text{min}^{-1}$.

RESULTS OF CALCULATIONS

Typical results of our calculations illustrating the cell response to Hly are shown in Fig. 1. If activation of PLC by Hly is relatively weak, $\kappa = 3.0$, and $k_bN = 0.25\ \text{min}^{-1}$ (Fig. 1 *a*), the model predicts transition to a new steady state which is only slightly different compared to the initial toxin-free steady state. For larger values of κ, the limit-cycle Ca^{2+} oscillations first arise and then disappear via sharp supercritical and subcritical Hopf bifurcations, respectively (this classification is based on our careful analysis of the dependence of oscillatory kinetics on the governing parameters and initial conditions). For $\kappa = 5.0$ and $k_bN = 0.25\ \text{min}^{-1}$, e.g., stable oscillations (Fig. 1 *b*) are predicted to occur after ~30 min as experimentally observed (Uhlén et al., 2000) in the case of bacterial interaction with cells. For $\kappa = 5.0$ and $k_bN = 0.5\ \text{min}^{-1}$ (Fig. 1 *c*), stable oscillations appear within a few minutes due to Hly-cell interaction as observed in the case of using filtered supernatant or purified toxin. For $\kappa = 6.0$ and $k_bN = 0.25$ and 0.5 (Fig. 1, *d* and *e*), the transition from the initial state to the final stable state is accompanied by a few oscillations as also often observed in the experiment (see, e.g., Fig. 3 in Uhlén et al., 2000). For $\kappa = 10.0$ and $k_bN = 0.25$ (Fig. 1 *f*), the model predicts only one spike during the transition to the final stable state.

To complement the results presented in Fig. 1, it is instructive to show the model behavior in two other situations that were studied experimentally (Uhlén et al., 2000):

1. The control experiments showed that the formation of additional pores by using the Ca^{2+} ionophore ionomycin or the activation of voltage-operated L-type calcium channels with Bay K 8644 resulted in persistent rise of the Ca^{2+} concentration in the cytosol, but no oscillations. The transient kinetics corresponding to these experiments may be mimicked by employing $W_{\text{IP3}} = W^0_{\text{IP3}}$ and increasing the leak flux in analogy with Eq. 11,

$$F_{\text{leak}} = F^0_{\text{leak}}[\exp(-k_1 t) + \kappa_1(1 - \exp(-k_1 t))], \qquad (12)$$

   where F^0_{leak} is the flux in the perturbation-free case, κ the ratio of the final and initial fluxes, and k_1 the constant characterizing the perturbation process. In agreement with the experiments, oscillations are lacking in this case (Fig. 2 *a*).
2. According to the experiment, blocking voltage-operated L-type calcium channels by nifedipine abolishes the oscillatory Ca^{2+} response. This effect may be related, e.g., to reduction of the Ca^{2+} flux from the extracellular space and/or drain of ER. Although our model does not allow us to analyze these cases in detail, we may quali-

tatively mimic the former situation simply by appreciably reducing F_{leak}. The latter case can be mimicked by reducing F^0_{ch}. In agreement with the experiment, the model predicts in both cases that the Ca^{2+} response to the toxin-related perturbation is non-oscillatory (Fig. 2, *b* and *c*).

In addition, it is instructive to show the model behavior in the case when the toxin-related perturbation described earlier is combined with an appreciable increase of the Ca^{2+} leak flux given by Eq. 12 (the latter may mimic the Hly pore-forming activity). If, for example, the model parameters are the same as in the case of Fig. 1 *a*, the increase of the leak flux results in conversion of non-oscillatory kinetics to transient oscillations (Fig. 3). This example illustrates that the model may predict oscillations in this case as well even if we do not adjust the model parameters. This means that the role of the Hly-induced pores in oscillations is not crucial.

CONCLUSION

In summary, we have constructed the first generic kinetic model describing Ca^{2+} oscillations induced in single cells by the action of a bacterial toxin. The model incorporates the key biophysical factors resulting in oscillations. The predicted response of cells to toxin exposure is in agreement with that observed in experiments where Hly-producing *E. coli* bacteria interact with rat renal epithelial cells (Uhlén et al., 2000).

Finally, it is appropriate to note that although spontaneous intracellular Ca^{2+} oscillations (without toxin) have long been observed in various cells (Goldbeter, 1996; Schuster et al., 2002), only recently has anyone begun to understand the physiological role of this second messenger response (Berridge et al., 1998, 2003; Orrenius et al., 2003). Oscillations occurring at periodicities in the minute range are known to affect gene expression (Dolmetsch et al., 1998). Thus, the model described here may help us to understand how a bacterial toxin can fine-tune gene expression in the eukaryotic cells, which are of major importance for the host's inflammatory response.

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