Intercellular waves propagation in an array of cells coupled through paracrine signaling: A computer simulation study

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A linear chain of cells is considered in which calcium (Ca^{2+}) fluctuations within a cell are described by a simple minimal model. Cells are coupled together by bidirectional paracrine signaling via calcium oscillations. Two typical zones of propagation are observed: a transition zone and a regular zone. The transition zone exhibits the same phenomena that can be observed in single cells, pairs or triplets of cells. Within the regular zone, simple periodic oscillations of calcium propagate and the Ca^{2+} signal is similar from one cell to another (same amplitude and same frequency). But, the signals are separated by a slight phase shift characterizing the propagation of Ca^{2+} waves due to the type of coupling used. We also consider the colonization of the lattice by the abnormal oscillations of sick cells.

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I. INTRODUCTION

The internal regulation program of a biological cell can be affected by a variety of factors such as the availability of water and oxygen, and the nature of the substrate. It also responds to specific signals that modify its physiology by direct chemical intervention, such as the hormones, neurotransmitters, or Ca^{2+} ions [1] that are needed to ensure the proper function of the organ of which it is part. Following the observation of intracellular Ca^{2+} waves in medaka eggs [2], it has become clear that calcium often acts as a second messenger in living cells. It thus regulates multiple cellular functions including, e.g., muscle contraction and synaptic transmission [3], and the expression of genes that are essential for dendritic development [4]. The calcium signal initially employed in these processes consists of a transient increase in the intracellular concentration. This increase can arise either from influx through the plasma membrane of a cell caused by an agonist, or by calcium release from an internal store such as the endoplasmic reticulum (ER) via the opening of channels that themselves have calcium dependent kinetics.

A number of theoretical models have been proposed to explain the oscillatory behavior of calcium ions in a cell. Basically, there are two classes of model: those in which the calcium oscillation is due to the receptors in the membrane surrounding the intracellular Ca^{2+} pool (ER) [5,6], and those in which an agonist induces calcium oscillations in the cell via a mechanism in which important dynamic aspects are found in the respective receptor complexes of the plasma membrane [7–10]. Two mechanisms have been identified that support cell-to-cell communication of calcium signals in various cell types. The first mechanism involves the diffusion of a calcium-mobilizing messenger, or calcium itself, through gap junction channels [7,9–11]. The second mechanism relies on paracrine signaling [12] involving the release of a messenger, diffusion in the extracellular space, binding to receptors on neighboring cells, and activation of downstream signaling cascades ultimately leading to an increase of cytoplasmic free calcium in the target cells. Osipchuk and Cahalan [13] provided the first evidence that an extracellular messenger mediates the spreading of cell-to-cell calcium signals in mast cells. Many types of paracrine signaling exist depending on the type of secondary agonist release. In one type, a calcium spike in one cell causes the release of a secondary agonist such as Adenosine 5′-triphosphate/Uridine triphosphate (ATP/UTP), to the extracellular space, followed by the stimulation of nearby cells [14]. Another recent paracrine signaling scheme is based on the fact that the fluctuation of extruded calcium during intracellular calcium spiking in one cell can activate calcium-sensing receptors (CaRs) on the membrane of adjacent cells producing secondary spikes in these cells [15]. The calcium increase in the target cell might on its turn initiate a new cycle of calcium signal communication, i.e., cause regeneration of the cell calcium signaling events. This would produce regenerative calcium signal propagation [16,17], thereby spreading the signal over a larger population of cells as compared to the purely diffusive spread of calcium or calcium mobilizing messengers between cells.

In this paper, we extend the modeling approach to encompass the problem of intercellular calcium signaling. We present the results of an array consisting of a linear chain of cells in which calcium fluctuations are described by a simple model; the cells are coupled by paracrine signaling via calcium oscillations. In Sec. II, we present the model equation describing our chain of cells. The analysis of the model in Sec. III will focus on the conditions under which intercellular calcium wave can occur, and on how this wave propagates in the chain. Section IV gives results describing the propagation to the rest of the lattice of abnormal oscillations due to localized sick cells. We are aware that a detailed description of these processes would require a considerably more complex model. The present paper aims to elucidate the characteristics of the calcium propagation mechanism in

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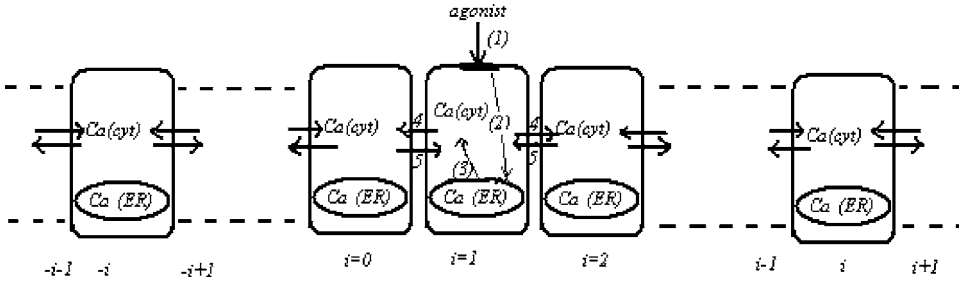

FIG. 1. Linear array of cells. Solid arrows depict reactions/transport steps. (1) Agonist link to extracellular side of a receptor bound to membrane, (2) second messenger (IP_3) binds to specific receptors in the membrane of an internal store of Ca^{2+} (endoplasmic reticulum), (3) large flux of calcium ions from the internal store into the cytosol, (4) calcium extruded to the extracellular neighborhood of the cell, which activate CaRs on the surfaces of the adjacent cells, (5) calcium extruded (coming from the neighborhood cells) by reaction to the CaRs activation.

an array of cells. The results may inform experimental studies and more detailed modeling approaches particular to specific systems.

II. MODEL

An important characteristic of the calcium oscillations is that Ca^{2+} signals can be propagated from one cell to another, thus providing an important means of intercellular communication. There are two aspects to such communication: the intracellular Ca^{2+} dynamics and the coupling between cells. Of the many models describing the intracellular dynamics of calcium, we choose the minimal one proposed by Dupont *et al.* [7] and used recently by Gracheva and Gunton [18]. It has been used to explain the results of an experimental model study in which intercellular communication is observed between two cells without any gap junction communication [15].

The model uses two variables: the cytosolic Ca^{2+} concentration and the calcium concentration of the internal store (ER). The calcium oscillation is induced in a single cell by the link of an agonist on its plasma membrane surface [19]. In our model (Fig. 1), we assume for the sake of simplicity that, when establishing the paracrine coupling between cells, the calcium extruded from one cell can stimulate its nearest neighbors. We further assume that the coupling involves the stimulus of the target cell being proportional to the cytosolic calcium content of its neighboring cells. This avoids modeling the complex dynamics involved in the calcium receptor (CaR) dynamic mechanism as introduced by Höfer *et al.* [15]. The assumption seems reasonable given that some of the cytosolic Ca^{2+} content in the cell is extruded into a small space near a CaR receptor. Therefore, cells are coupled together by the bidirectional paracrine coupling that has been demonstrated between astrocytes and endothelial cells in coculture [20,21]. This one-dimensional chaining-up can be observed in protozoa which can live in an isolated state, but can also agglomerate if necessary. For example, the acrasial amoeba can form a multicellular structure when life conditions become unfavorable. This multicellular structure organizes itself on a longer rod on which spherical cells are fixed. The aggregation process is due to a substance which has been recognized as acrasine or AMPc [1]. On certain approximations, our model could also explain how calcium propagates in multicellular structure such as epithelial cells, hepatocyte cells, liver cells, atrocytes, and others.

For the mathematical modeling, let us consider x_i as the calcium concentration in the cytosol of the ith cell and y_i its internal store calcium concentration. Therefore, the ith cell is described by the following set of equations:

$$\frac{dx_i}{dt} = a_i + \beta V_1(x_{i+1} - 2x_i + x_{i-1}) - V_{2,i} + V_{3,i} + k_f y_i - kx_i \tag{1}$$

$$\frac{dy_i}{dt} = V_{2,i} - V_{3,i} - k_f y_i \tag{2}$$

where

$$a_i = \begin{cases} V_0 + bV_1 & \text{if } i = 1, \\ V_0 & \text{if } i \neq 1, \end{cases}$$

$$V_{2,i} = \frac{V_{m2}x_i^2}{k_2^2 + x_i^2} \quad \text{and} \quad V_{3,i} = \frac{V_{m3}x_i^4 y_i^2}{(k_a^4 + x_i^4)(k_r^2 + y_i^2)}. \tag{3}$$

The term bV_1 represents a constant hormonal stimulus which is localized on the first cell of the array. β is the coupling between neighboring cells. We have considered an infinite chain of cells. Considering that a signal can be reproduced after N cells, however, the boundary conditions can be taken as cyclic and defined as

$$x_{i+N} = x_i$$

$$y_{i+N} = y_i. \tag{4}$$

The chain of cells is therefore described by the set of Eqs. (1)–(4).

III. NUMERICAL SIMULATION RESULTS

We perform numerical simulations of the complete model by integrating Eqs. (1)–(4) using the fourth order Runge-Kutta algorithm. We find in general that when Ca^{2+} oscillations appear in a cell, they propagate over the chain. The

TABLE I. Typical simulation constants for the minimal model.

Parameter	Value
K	6 s^{-1}
K_f	1.0 s^{-1}
K_2	1.0 μm
K_a	0.9 μm
K_r	2.0 μm
V_0	1.3 μm s^{-1}
V_1	7.3 μm s^{-1}
V_{m1}	65.0 μm s^{-1}
V_{m2}	500.0 μm s^{-1}
γ	0.50

number of cells is large: typically $N=1500$. All cells are identical and the parameters chosen for the simulations are listed in Table I. The concentrations of Ca^{2+} in the cytosol and in the internal store of each cell of the chain at rest are taken to be $x_i(0)=0.2275$ μM *and* $y_i(0)=2.121\,96$ μM. Let us consider the cell receiving the external agonist as the first cell of the chain, $i=1$, i.e., the excited cell (Fig. 1). We use b and β, i.e., the terms characterizing the concentration of the agonist and the coupling parameter, respectively, as the bifurcation and control parameters. For a given choice of parameters, there is a birth and propagation of a calcium wave signal in the chain when the concentration of the agonist exceeds a minimal value b_{min}. This minimum value depends mainly on the parameters V_0 and V_1 as shown previously for this model [7,18,19]. Note that the maximum value of the bifurcation parameter which has been found for this minimal model, for a cell [7,19] and a for couple of cells [18], and over which cells show over stimulation phenomena is obtained just for the first three neighbors of the excited cell, the calcium wave signal taking birth for a higher concentration of agonist in the number four cell of the array. Figure 2 shows the minimal agonist concentration required for the Ca^{2+} waves to occur and propagate in the array when the coupling parameter β varies. This minimal value decreases with the increase of the coupling parameter between cells. This can be explained by the fact that the Ca^{2+} wave birth in a cell depends on the number of calcium sensing receptors (CaRs) which can be activated on the surface of this cell. So, if the intercellular spacing is small, or if the chemical interaction strength is strong such that the local extracellular fluctuations are sufficiently large, calcium sensing receptors (CaRs) on the surfaces of adjacent cells can be activated,

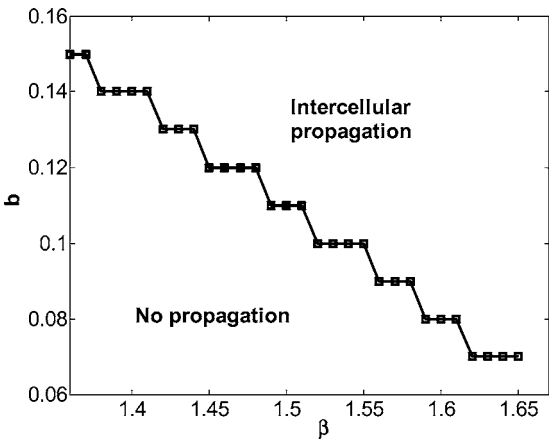

FIG. 2. Minimal external hormonal stimulus required for calcium oscillations to take birth and propagate in the chain when the coupling parameter varies.

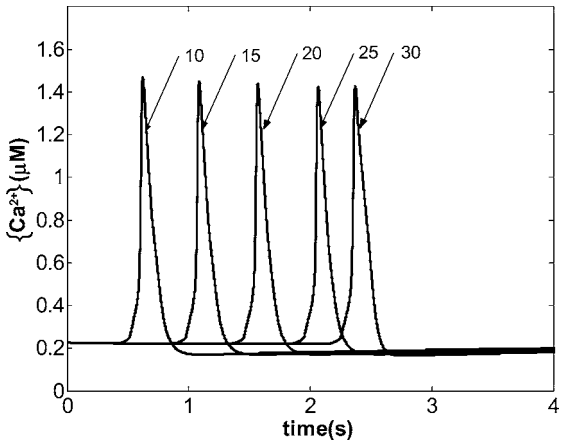

FIG. 3. Calcium signal birth in cells no 10, 15, 20, 25, 30, when $b=0.4$.

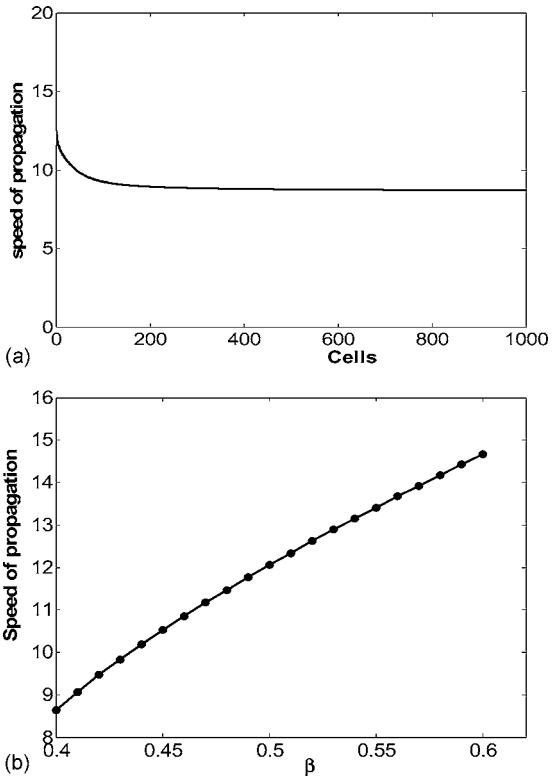

FIG. 4. Speed of Ca^{2+} wave signal propagation in the chain; initial conditions are given by $y_1(i)=0.2275$; $y_2(i)=2.12196$; (a) Speed of Ca^{2+} wave signal propagation. (b) Speed of Ca^{2+} wave signal propagation in the chain when varying the coupling parameter.

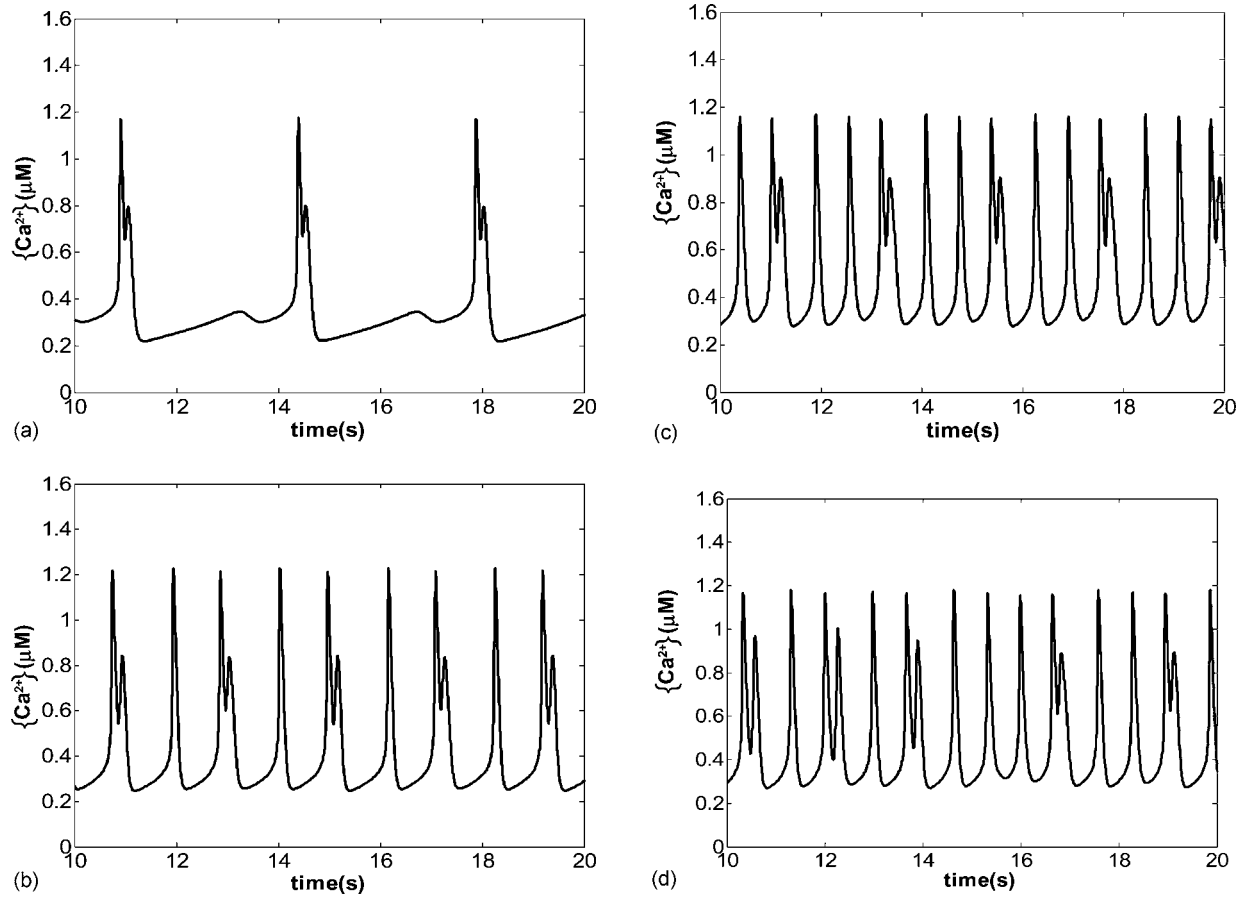

FIG. 5. Ca^{2+} oscillations in the excited cell (cell no 1 of the Fig. 1 when the hormonal excitation increases. (a) Complex periodic oscillations ($b=0.18$). (b) Appearance of one spike of simple oscillation between two spikes of complex oscillations ($b=0.3$). (c) Appearance of two spikes of simple oscillations between two spikes of complex oscillations ($b=0.38$) (d) appearance of chaotic behavior ($b=0.385$).

producing secondary spikes in these cells. An increase of the coupling parameter between cells can therefore be seen as the space decreasing, or the chemical interaction increasing, between them; there is an associated increase in the number of activated CaRs. Figure 2 shows a pronounced staircase structure. This can be understood by the discrete nature of the lattice. There are ranges of discrete parameter β requiring the same intensity of hormonal stimulus for the wave propagation. The behavior appearing in Fig. 2 is similar to that presented in Ref. [10], where the authors obtain a critical curve for the gap-junctional permeability as function of the maximal rate of calcium-induced calcium release. This critical curve separates the domain of propagation of intercellular waves to the domain of no wave propagation (see Fig. 6 of Ref. [10]).

To observe the propagation of the Ca^{2+} signal in the chain, we illustrate in Fig. 3 the birth of the Ca^{2+} wave signal in some cells of the array. One sees that the calcium wave signal appears firstly in cells neighboring the excited cell, and then propagates further. Our interest centers on the speed of Ca^{2+} wave propagation in the overall chain. It is evident from the results of Fig. 4(a) that the intercell propagation speed decreases in the first few cells of the chain, and then remains constant. However, the overall propagation speed in the chain increases when the intercell coupling increases [Fig. 4(b)]. This can be explained by the fact that, as the birth of the Ca^{2+} oscillations in a cell depends on the number of CaRs activated, an increase of the coupling parameter causes an increase in the number of CaRs affected in adjacent cells, resulting in an increase in the speed of the Ca^{2+} wave propagation. The results of Figs. 2 and 4(b) present behavior similar to what was obtained in Ref. [22], where the modeling of calcium channel dynamics is considered. Indeed, the authors of Ref. [22] found that the propagation of the front wave

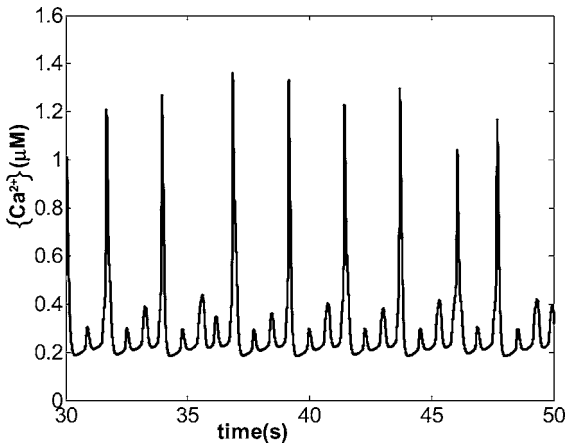

FIG. 6. Variation of the amplitude of oscillations in a cell exhibiting chaotic behavior in the transition zone $b=0.60$.

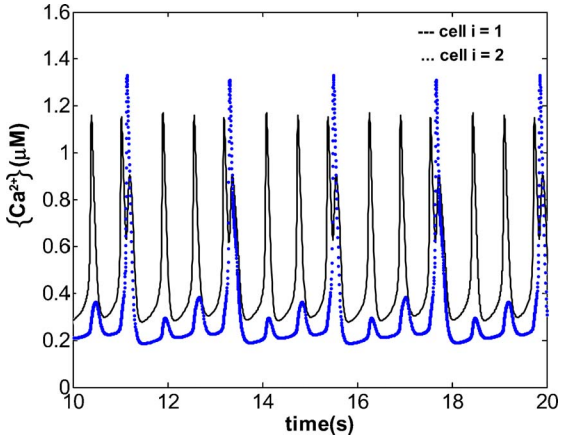

FIG. 7. (Color online) 3:1 rhythm between the first and the second cells ($b=0.40$) in the transition zone.

depends on the diffusion constant and the probability factor of activation or inhibition (parameters playing the same role as β and b in this paper). Considering the two quantities as controlling parameters, they found many regions including the forward propagation region separated from the pinning region. In the propagation region the average front speed increases with the diffusion coefficient. However, for a small diffusion coefficient, the discreteness of the lattice causes the front to become pinned as the probability factor becomes smaller than the threshold value for the excitation probability.

An increase in the strength of the external hormonal stimulus (i.e., in the parameter b), from the minimal value $b_{min}=0.18$, causes the excited cell (cell no 1) to exhibit a series of complex periodic oscillations. They display two different maxima: a main spike closely followed by a smaller secondary one [Fig. 5(a)]. A further increase in b results in an alternation of such structures with simple oscillations [Fig. 5(b)], their alternation with pairs of simple oscillations [Fig. 5(c)], or with triplets or larger numbers of simple oscillations (not shown). These simple oscillations appear between the double maxima generated in the excited cell. Finally, the sequence yields oscillations that exhibit, not only irregular numbers of simple spikes among complex double-maxima, but also irregular spacing between the latter. This corresponds to the occurrence of chaotic oscillations [Fig. 5(d)]. Calcium oscillations appear in the nearest neighbor of the excited cell exactly when complex oscillations take place in the excited cell. Two zones of propagation can thus be observed. A first zone is constituted of cells in the neighborhood of the excited cell, which we will refer to as the *transition zone*. Within it, the Ca^{2+} signal shows diverse and particular phenomena such as the disappearance of the complex spiking oscillations, period-doubling from one cell to the next, leading to the blocking phenomenon, the variation of the oscillation amplitude, and chaotic behavior (Fig. 6). The phenomenon of blocking in the transition zone occurs

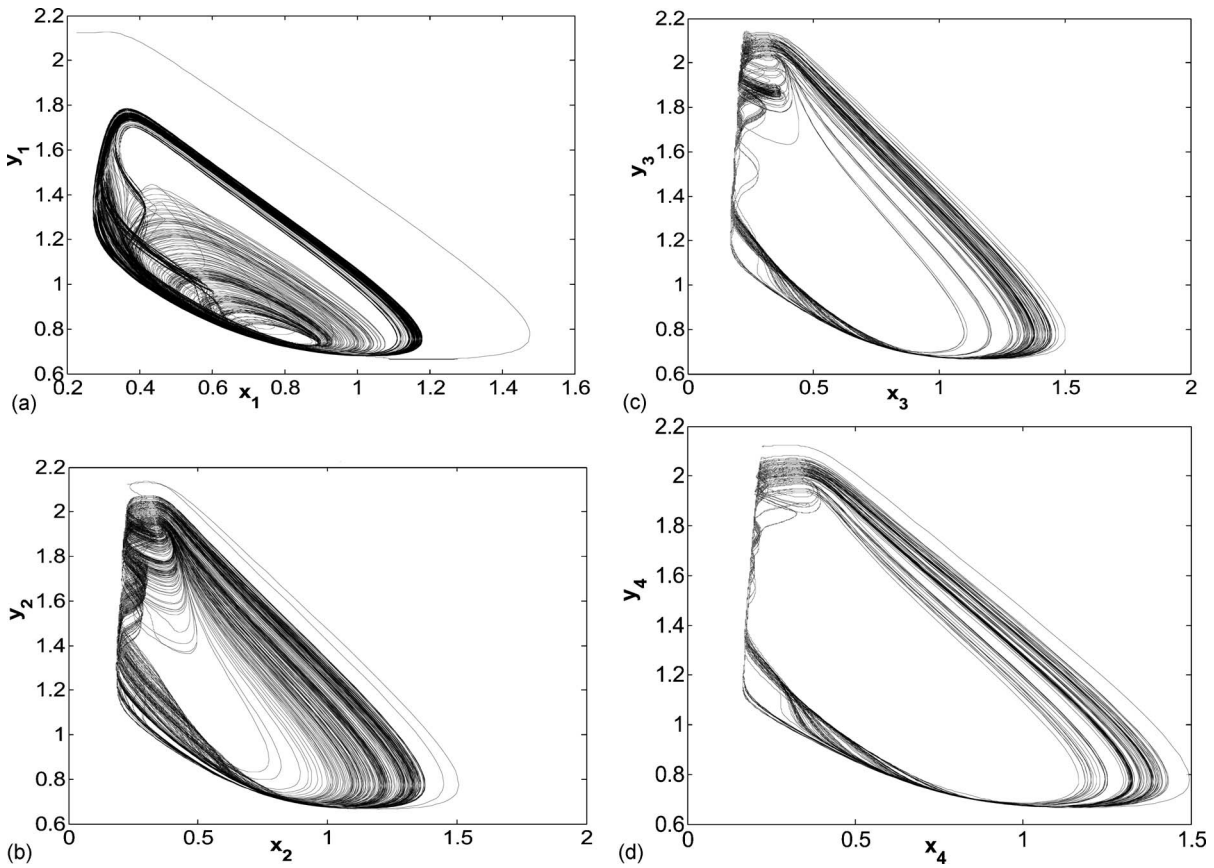

FIG. 8. Phase plot diagram of the cells in the transition zone when the first cell exhibit chaotic behaviour ($b=0.385$): (a) cell no 1 (excited cell), (b) cell no 2, (c) cell no 3, (d) cell no 4.

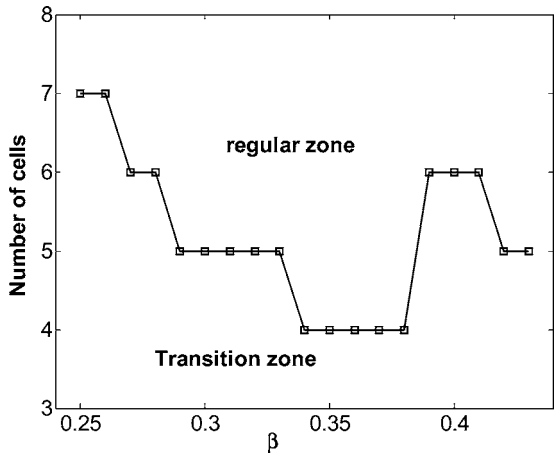

FIG. 9. Boundary between the transition zone and the regular zone as one varies the hormonal stimulus.

between the excited cell and its neighbors. Figure 7 shows a 3:1 rhythm between the first cell ($i=1$) and its nearest neighbor ($i=2$); the number of stimuli arising from the first cell is 3 and the number of responses of the second cell is 1. This phenomenon has already been observed in the study of this minimal model between a sensor cell and a donor cell coupled by a unidirectional paracrine signal [18]. It has also been found earlier by Chay *et al.* [23] when studying a model of intracellular calcium oscillations in which the hormonal stimulus was modeled by a sequence of square pulses. As one varies b, we observe that as in the previous study of this model by Gracheva and Gunton [18], the cell $i=2$ passes through a sequence of $N:M$ phase-locked regimes in response to the oscillatory stimuli from the first cell. Note that this phenomenon can also be observed in all other cells in the transition zone.

When the excited cell exhibits chaotic behavior, the same phenomenon occurs throughout the transition zone as shown in Fig. 8 which presents a phase plot of the cells in the transition zone corresponding to the chaotic behavior of Fig. 5(d). As this chaotic signal propagates from one cell to the next, it becomes more regular and, by the fourth cell, has adopted regular behavior that persists throughout the second zone, which we will refer to as the *regular zone*. Here, the Ca^{2+} wave signal is identical from one cell to another (same amplitude and frequency of oscillation). However, there is a slight phase shift from the spike of one cell to the spike in the next one (not shown) characteristic of wave propagation phenomena. Figure 9 shows the boundary between the two zones as one varies the hormonal stimulus. It illustrates the number of cells exhibiting particular behaviors within the transition zone for a given external hormonal stimulus. This number of cells decreases with β in a given interval until it reaches a limit number where there are only four cells in the transition zone. It then suddenly returns to an elevated number of cells and starts to decrease again as the bifurcation parameter increases.

IV. EFFECTS OF LOCALIZED DISEASES

The capacity of a pathogenic microorganism to cause disease is conditioned by its ability to colonize a given niche

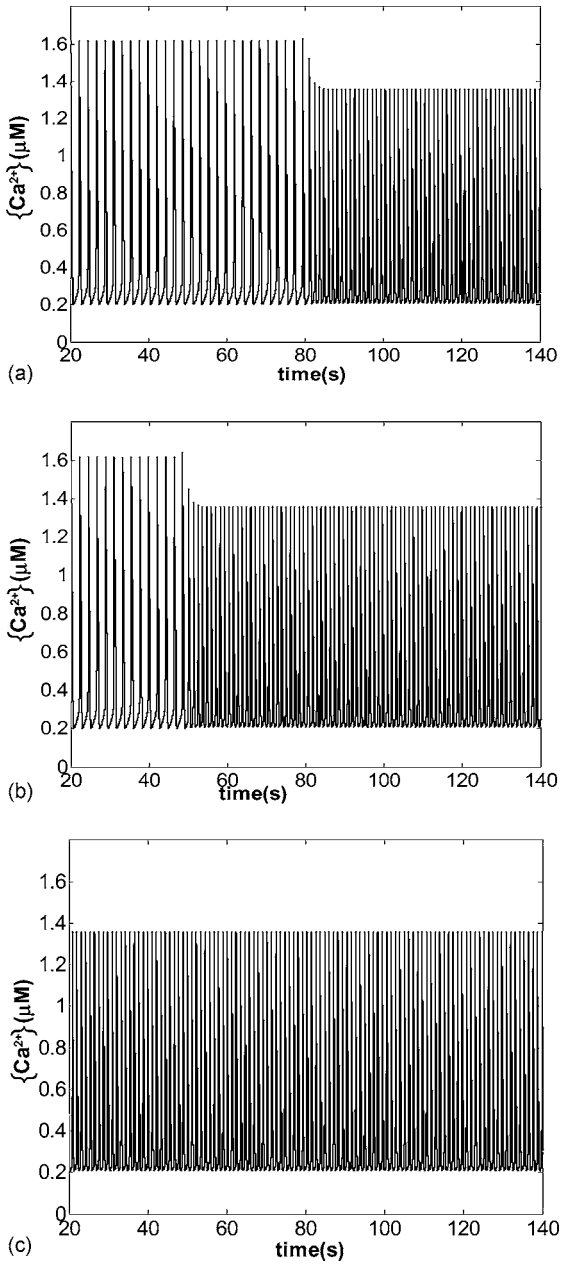

FIG. 10. Calcium oscillation of the chain of cells at the neighborhood of the source of infection, for the sick cells $k=2\ s^{-1}$. (a) Behavior of the cell $i=350$. (b) Behavior of the neighbouring cell $i=400$. (c) Behavior of the sick cell $i=450$.

and it is this that determines, e.g., the virulence factor. Disease can be induced by bacteria that replicate extracellularly and alter the cell mucosa by producing toxins. By perturbing the Ca^{2+} homeostasis, these toxins may affect the cytoskeletal architecture and alter the barrier function of the cell. Some bacterial toxins are cytotoxic because they induce the formation of large pores in host cell membranes. For example, *listeria listeriolysin O* (LLO), an enteroinvasive pathogen responsible for meningitis and encephalitis, forms pores in host cell membranes that oscillate between an open and a closed state, but show little ion specificity [24]. The formation of transient pores of this kind allows a Ca^{2+} influx that leads to long-lasting Ca^{2+} response oscillations. LLO

induced Ca^{2+} influx appears independent of endogenous Ca^{2+} channels, because it is not inhibited by cadmium which is known to be a broad spectrum inhibitor of voltage gated channels [25]. To characterize the Ca^{2+} influx in our study, we have used the term kx_i in Eq. (1) representing the influx of Ca^{2+} from the cytosol to the extracellular space. We infer that when transient pores are formed in a cell, a large quantity of Ca^{2+} passes into the extracellular space independently of the calcium channels. Therefore, the coefficient k increases, characterizing the large spreading of bacterial toxins in the given niche. By adding ten sick cells (i=440 to 450) in a healthy chain of N=1000 cells, we have studied the propagation or colonization of healthy cells of the chain by the disease effects (Fig. 10). We note that the cells neighboring the sick ones perceive the effect of the liberated toxin. At the neighborhood of the source of infection, as time increases, the chain exhibits oscillations similar to those of the sick cells (same frequency) as shown in Fig. 10; this is due to the expansion of toxins liberated by the sick cells.

V. CONCLUSION

It is now well known that at least three mechanisms of intercellular calcium signaling can be present in a multicellular system. They may play different roles, depending on the mode of external stimulation, and whether intercellular signaling involves the synchronization of oscillations or wave propagation. For synchronization, junctional calcium diffusion appears crucial, while waves may involve InsP3 diffusion, paracrine signals, or calcium diffusion [8]. We have shown here that the wave propagation in a chain of cells coupled by a bidirectional paracrine signal occurs in two zones: a transition zone and a regular zone. In the transition zone, most of the phenomena observed when studying a single cell or a set of cells coupled by the paracrine signal are observed. For example, a blocking phenomenon, where the response in a target cell is blocked to that of the sensor cell, is observed. In the regular zone, the calcium signal presents a slight phase shift from one cell to the next characterizing the propagation of the wave due to the type of coupling, and the speed of Ca^{2+} wave propagation is constant from one cell to other. Moreover, we have found that the abnormal behavior of sick cells propagates or quickly colonizes the whole cellular lattice.

For further studies, one can include in our model the membrane plasma receptor dynamic similar to Riccobene *et al.* [26], and oscillations in IP_3 [27,28]. One should also study others models, such as those involving the gap junction coupling, to see if all the phenomena observed in this minimal model can also be obtained with them.

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