Selection of in-phase or out-of-phase synchronization in a model based on global coupling of cells undergoing metabolic oscillations

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On the basis of experimental observations, it has been suggested that glycolytic oscillations underlie the pulsatile secretion of insulin by pancreatic β cells, with a periodicity of about 13 min. If β cells within an islet are synchronized through gap junctions, the question arises as to how β cells located in different islets of Langerhans synchronize to produce oscillations in plasma levels of insulin. We address this question by means of a minimal model that incorporates the secretion of insulin by cells undergoing glycolytic oscillations. Global coupling and synchronization result from the inhibition exerted by insulin on the production of glucose, which serves as the substrate for metabolic oscillations. Glycolytic oscillations are described by a simple two-variable model centered on the product-activated reaction catalyzed by the allosteric enzyme phosphofructokinase. We obtain bifurcation diagrams for the cases in which insulin secretion is controlled solely by the product or by the substrate of the metabolic oscillator. Remarkably, we find that the oscillating cells in these conditions synchronize, respectively, in phase or out of phase. Numerical simulations show that in-phase and out-of-phase synchronization can sometimes coexist when insulin release is controlled by both the substrate and the product of the metabolic oscillator. The results provide an example of a system in which the selection of in-phase or out-of-phase synchronization is governed by the nature of the coupling between the intracellular oscillations and the secretion of the biochemical signal through which the oscillating cells are globally coupled. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2983753]

The coherent, pulsatile secretion of insulin by pancreatic β cells provides a striking illustration of synchronization in a population of coupled biological oscillators. Periodic insulin secretion appears to be controlled by metabolic oscillations within the cells. To explore the bases of the synchronization phenomenon, we consider a minimal model for pulsatile insulin secretion coupled to intracellular metabolic oscillations. Global coupling of the cellular oscillators occurs in the model through inhibition of glucose production in the liver by extracellular insulin. This inhibition controls the substrate input to the cells and thereby modulates their metabolic oscillations. Unexpectedly, the model indicates that synchronization of the oscillating cells occurs in phase or out of phase, depending on whether the release of insulin is coupled to periodic variations of the substrate or of the product of the reaction responsible for intracellular metabolic oscillations. Thus, the selection of in-phase versus out-of-phase synchronization is governed by the nature of the coupling between insulin release and the metabolic oscillator. We characterize this selection process by means of bifurcation diagrams and numerical simulations, and consider in turn the cases of two and N coupled oscillators. We extend the treatment to the case of a hybrid mechanism in which hormone release is controlled by both the substrate and the product of the metabolic oscillator, in proportions measured by a single parameter. At small or large values of this parameter, we recover the results about in-phase or out-of-phase synchronization, but at intermediate values the model can display coexistence between the two modes of synchronization. In the case of N coupled cells, we then observe a clustering phenomenon by which roughly half of the cells oscillate in phase and the rest of the cells oscillate with an opposite phase. Beyond the case of insulin secretion by pancreatic β cells, the results provide an example of a system of coupled oscillators in which the selection of in-phase or out-of-phase synchronization depends on the nature of the link between the intracellular oscillations and the secretion of the factor through which cells are globally coupled.

I. INTRODUCTION

The pulsatile secretion of insulin by pancreatic β cells involves a hierarchy of periodic processes.[1–5] High-frequency bursting oscillations of the membrane potential, with a period of the order of seconds, underlie the secretion of insulin. Oscillations of cytosolic Ca^{++} with a period of several minutes have also been reported. When measured in the plasma, insulin, as well as glucose, varies with a periodicity of the order of 13 min.[3,6] Defects in the 13-min pulsatility appear to be related to the occurrence of certain forms of type-2 diabetes.[3,7,8]

The mechanism of the pulsatile secretion of insulin remains an object of debate.[2,9] Some authors have suggested that pulsatile insulin release is associated with the occurrence of glycolytic oscillations in pancreatic β cells.[10,11] These metabolic oscillations, of a period of tens of seconds up to 20 min, have first been demonstrated in yeast cells and extracts, and later in muscle extracts.[12–15] Evidence for the oc-

currence of glycolytic oscillations has also been obtained in pancreatic β cells.[10,11] The ratio ATP/ADP varies periodically in the course of glycolytic oscillations. Pulsatile secretion of insulin could be brought about by the periodic variation of this ratio through the negative control exerted by ATP on a K^+ conductance. The periodic closure of the ATP-dependent K^+ channel would trigger the periodic depolarization of β cells, followed by Ca^{++} entry, which would itself elicit insulin secretion.[2,4,5]

Within the pancreas, β cells are organized in Langerhans islets, which contain a few thousand coordinated β cells.[4] If glycolytic oscillations underlie the pulsatile secretion of insulin, the coupling of β cells through gap junctions may explain how pulsatile insulin secretion by many cells within an islet is coordinated.[16,17] The question remains, however, as to whether and how cells from different islets of Langerhans may be coordinated.[2,4] In the absence of coupling, islets would oscillate independently with different phases so that no clearcut overall insulin pulsatility would be detected. The observation of global oscillations of insulin in plasma with 13-min period suggests that coupling leading to synchronization of different islets indeed takes place *in vivo*. One possible coordination mechanism rests on neural communications between different islets within the pancreas.[4] The isolated pancreas remains, however, capable of secreting insulin in a pulsatile manner.[18]

The purpose of the present paper is to explore an alternative mechanism for synchronization in a population of insulin-secreting pancreatic β cells. By means of a theoretical model for pulsatile insulin secretion, we show that synchronization might readily originate from global coupling due to the fact that each β cell secretes insulin and that the level of the hormone consequently oscillates in the extracellular medium.[1,7,8,10] Because insulin inhibits glucose production in the liver, the periodic variation of insulin in the extracellular medium results in the periodic variation of glucose input to β cells. Periodic glucose infusion was shown to be capable of synchronizing insulin-secreting cells within the pancreas.[3,7,19] Thus, the oscillations of the output of each cell in the population result in oscillations in the input to these cells, which in turn might cause their synchronization. The present results indicate that such self-synchronization through global coupling by circulating insulin could underlie the coherent pulsatile secretion of insulin in pancreatic β cells.

Synchronization of coupled oscillators represents a central issue in many areas of physics, chemistry, and biology,[20–24] particularly in the fields of neurosciences[25–27] and insulin-secreting pancreatic cells.[28–30] In the latter case, because cells from different islets are coupled through a common intermediate that they secrete into the extracellular medium, the mechanism considered belongs to the class of globally coupled oscillators. Other examples of global coupling of oscillating cells include cAMP oscillations in *Dictyostelium* amoebae,[31] synchronization through "quorum sensing" of glycolytic oscillations in yeast cell suspensions,[32–34] synchronization of GnRH secreting cells in the hypothalamus,[35] circadian oscillations generated by coupled neurons in the suprachiasmatic nucleus,[36–38] and other genetic oscillators.[39]

To address the synchronization of pancreatic islets via global coupling through insulin modulation of the glucose input to β cells, we consider a minimal model for pulsatile insulin secretion coupled to intracellular glycolytic oscillations in these cells. More detailed models incorporating the role of gap junctions, Ca^{++} signaling, variations of the membrane potential, and metabolic oscillations have been proposed by Bertram and co-workers for the pulsatile release of insulin by β cells.[40–43] These authors consider a more complex model for glycolytic oscillations. These oscillations control variations of the membrane potential through an ATP-dependent K^+ channel. Such variations in turn control the level of cytosolic Ca^{++}, which is the main determinant of insulin secretion. In the present, minimal model, we consider that in each cell, glycolytic oscillations are produced by a two-variable system[14,44,45] describing the time evolution of the substrate and product of the product-activated reaction catalyzed by the allosteric enzyme phosphofructokinase (PFK), which is responsible for glycolytic oscillations.[14,15] A similar two-variable model was retained for the coupling of insulin release to glycolytic oscillations, in a study that did not address explicitly intercellular coupling.[46] There, the authors considered an indirect, intricate link between glycolytic oscillations and insulin, which was assumed to modulate glucose input to the cells through an intermediary variable.

In coupling insulin release to glycolytic oscillations, we will assume, without considering intermediate steps, that release of the hormone is directly triggered either by the substrate or by the product of the PFK reaction. We further consider that extracellular insulin inhibits the production of glucose in the liver,[47] and thereby modulates the substrate input to β cells. Variations in the input rate of the glycolytic substrate affect the dynamics of metabolic oscillations produced by the PFK reaction within the cells. For simplicity, we consider that the inhibitory effect of insulin on glucose production in the liver is instantaneous. The regulatory effects of insulin, however, are both direct and indirect,[47] and it is likely that even for direct effects, a delay occurs before the level of insulin at a given time affects hepatic glucose production. The consequences of such a delay on the dynamic patterns of synchronization will be investigated in a subsequent publication.

Unexpectedly, we observed that synchronization of oscillating cells in the population occurs in phase or out of phase, depending on whether the release of insulin is coupled to periodic variations of the substrate or of the product of the PFK reaction. Thus, the selection of in-phase versus out-of-phase synchronization is governed by the nature of the coupling between insulin release and the intracellular metabolic oscillator. We characterize this selection process by means of bifurcation diagrams and numerical simulations, and discuss its possible physiological implications. We first treat the case of two coupled cells and consider thereafter the case of N coupled cells. We extend the treatment to the case of a hybrid mechanism in which hormone release is controlled by both the substrate and the product of the metabolic oscillator, in various proportions measured by a single parameter, θ. Upon

TABLE I. Parameter values.

Parameter	Definition	Value
e	Ratio of kinetic constants	0.9
v_e	External glucose input	0 s^{-1} (in presence of periodic forcing, see Fig. 8)
v_{mr}	Maximum rate of insulin release	0.1 μM s^{-1}
k_d	Rate constant for insulin degradation	0.05 s^{-1}
v_{max}	Maximum rate of glucose input	0.078 s^{-1}
K_I	Threshold constant for inhibition of glucose input by insulin	2 μM
K_a	Threshold constant for activation of insulin release by the substrate or product of the PFK reaction	20
L	Allosteric constant of phosphofructokinase (PFK)	7.5×10^6
c	Nonexclusive binding coefficient of the substrate to PFK	0.01
σ_j	Maximum rate of the oscillatory PFK reaction	70 s^{-1} ($\forall j$)
k	Product degradation rate constant	0.014 s^{-1}
n	Hill coefficient characterizing the activation of insulin release by the substrate or product of the PFK reaction	2
m	Hill coefficient characterizing the inhibition by insulin of glucose synthesis in the liver	2

increasing θ from zero (full control by the substrate) to unity (full control by the product), we show that the system successively passes through three distinct modes of dynamic behavior in parameter space: synchronization out of phase, birhythmicity in the form of a coexistence of in-phase or out-of-phase synchronization, and finally in-phase synchronization. In the case of birhythmicity in a system of N coupled cells, we observe a clustering phenomenon by which roughly half of the cells oscillate in phase and the rest of the cells oscillate with an opposite phase.

Beyond the case of insulin secretion by pancreatic β cells, the results provide an example of a system in which the selection of in-phase or out-of-phase synchronization is governed by the nature of the coupling between the intracellular oscillations and the secretion of the biochemical signal through which the oscillating cells are globally coupled.

II. MINIMAL MODEL FOR PULSATILE INSULIN SECRETION BY PANCREATIC β CELLS UNDERGOING GLYCOLYTIC OSCILLATIONS

The model describing, for cell j, insulin release coupled to glycolytic oscillations is governed by the following system of kinetic equations:

$$\frac{d\alpha_j}{dt} = v_i + v_e - \sigma_j \phi_j, \tag{1a}$$

$$\frac{d\gamma_j}{dt} = \sigma_j \phi_j - k\gamma_j, \tag{1b}$$

$$\frac{dI}{dt} = v_{mr}\xi - k_d I \tag{1c}$$

with

$$v_i = v_{max}\frac{K_I^m}{K_I^m + I^m}, \tag{2a}$$

$$\phi_j = \frac{\alpha_j e(1+\alpha_j e)(1+\gamma_j)^2}{L(1+\alpha_j c)^2 + (1+\alpha_j e)^2(1+\gamma_j)^2} \tag{2b}$$

for $j=1,\ldots,N$, where N denotes the number of coupled cells.

Equations (1a) and (1b) pertain to the mechanism generating glycolytic oscillations in each cell. These equations are similar to those of the allosteric model proposed for the product-activated PFK reaction.[14,44,45] Equation (1c) pertains to the secretion of insulin by cells undergoing glycolytic oscillations. In these equations, α and γ denote the normalized concentrations of substrate and product in the product-activated PFK reaction. Parameters are defined in Table I. The substrate input v_i is defined by Eq. (2a), which expresses cooperative inhibition exerted by insulin on glucose synthesis in the liver. Function ϕ defines the rate function of the product-activated allosteric enzyme reaction (see Refs. 14 and 44 for further details).

Function ξ defines the coupling of insulin secretion to glycolytic oscillations. We will consider two modes of coupling. Either insulin secretion is activated in a cooperative manner by the substrate

$$\xi = \xi_a = \frac{1}{N}\sum_j \frac{\alpha_j^n}{K_a^n + \alpha_j^n} \tag{3a}$$

or it is activated by the product

$$\xi = \xi_b = \frac{1}{N}\sum_j \frac{\gamma_j^n}{K_a^n + \gamma_j^n}. \tag{3b}$$

These two possibilities can be considered as the extreme cases of a single, hybrid mechanism (Fig. 1) in which insulin

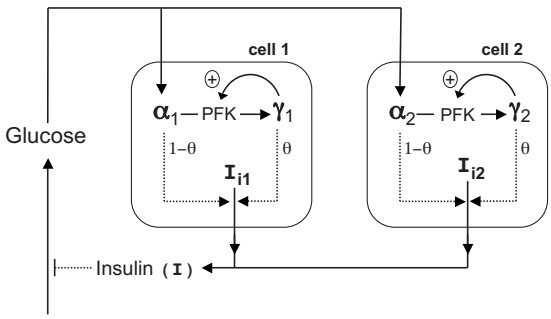

FIG. 1. Minimal model for the synchronization through global coupling of two cells undergoing metabolic oscillations. In the case of insulin, the two oscillators represent two islets of Langerhans, each of which contain thousands of β cells synchronized through gap junctions. Each cell then represents the behavior of a single islet and the model applies to the synchronization of different islets. Intracellular glycolytic oscillations are produced by the enzyme phosphofructokinase (PFK), which is activated by its reaction product (γ). The release of insulin (I) is assumed to be coupled to variations in substrate (α) and product, in proportions measured by parameter θ. Extracellular insulin exerts a negative feedback on glucose production in the liver. Such a modulation of glucose production in the liver affects the input of substrate to the oscillatory PFK reaction within each cell.

secretion is activated simultaneously by both the substrate and product of the reaction generating glycolytic oscillations,

$$\xi = \xi_h = \frac{1}{N}\left((1-\theta)\sum_j \frac{\alpha_j^n}{K_a^n + \alpha_j^n} + \theta \sum_j \frac{\gamma_j^n}{K_a^n + \gamma_j^n} \right). \qquad (3c)$$

The relative contribution of substrate and product of the metabolic oscillator to the activation of insulin release is measured by parameter θ. Then the cases of pure activation by the substrate ($\xi_h = \xi_a$) or by the product ($\xi_h = \xi_b$) correspond to $\theta = 0$ and 1, respectively.

III. THE CASE OF TWO COUPLED CELLS: SYNCHRONIZATION IN PHASE OR IN ANTIPHASE

We shall consider in turn the case in which insulin secretion is activated by the substrate or by the product of the oscillatory PFK reaction. The hybrid case in which insulin secretion is controlled by both substrate and product will be considered in Sec. VI below. Here we focus on the coupling of two cells characterized by identical or different parameter values. The case of a larger number of cells will be dealt with in the next section.

A. Control of insulin release by substrate or product leads to antiphase or in-phase oscillations

When insulin release is controlled solely by substrate α, as described by Eq. (3a), numerical integration of Eqs. (1a)–(1c) for the case of two identical cells ($N=2$) always leads to the situation in which cell 1 and cell 2 oscillate in antiphase [Fig. 2(a)]. In contrast, when insulin release is controlled solely by product γ as described by Eq. (3b), numerical integration of Eqs. (1a)–(1c) for the case of two identical cells always leads to the situation in which cell 1 and cell 2 oscillate in phase [Fig. 2(b)]. As a result, the net variation of the insulin level oscillates with reduced amplitude and with half of the period in Fig. 2(a) as compared to Fig. 2(b).

To permit the evolution to the asymptotic solution, the initial conditions for cells 1 and 2 are slightly different (see the caption to Fig. 2). Indeed, if the initial conditions were exactly the same for cells 1 and 2 in the case of Fig. 2(a), the two cells would oscillate in phase, even though this periodic solution is unstable, instead of evolving to the stable oscillations in antiphase.

We established the bifurcation diagram for the two cases (control of insulin release by substrate α or product γ) as a function of the maximum rate of glucose input, $v_{\max}$. The results are shown in Figs. 3(a) and 3(b), respectively. In each case—as previously reported for glycolytic oscillations, without coupling to insulin release[14,44,45]—sustained oscillations of the limit cycle type occur in a domain bounded by two critical values of the substrate input. However, in the coupled two-cell system, when hormone release is controlled by the substrate, the oscillations in antiphase are stable while the in-phase oscillations are unstable [Fig. 3(a)]. We observe precisely the opposite phenomenon in the case in which insulin release is controlled by the product [Fig. 3(b)]. These results agree with those obtained by numerical integration in Figs. 2(a) and 2(b).

B. Coupling two oscillators: Effect of an asymmetry and of coupling strength

In Sec. III A, we coupled two identical cells. The question arises as to how the dynamic behavior of the coupled system is affected by an asymmetry between the two cells. Here we explore the effect of such an asymmetry by changing the value of parameter σ_2, at a fixed value $\sigma_1 = 70$ (in s^{-1}), for different values of the inhibition constant K_I. The latter parameter provides a measure of coupling strength: as K_I increases, the rate of glucose synthesis goes to its maximum value $v_{\max}$ and becomes independent of the insulin level in the extracellular medium. In contrast, as K_I decreases and tends to zero, the inhibition of glucose synthesis by insulin becomes stronger and the effect of intercellular coupling becomes more significant. We focus on the case in which insulin release is controlled by product γ.

The bifurcation diagram in Fig. 4(a) shows the different types of behavior observed as a function of K_I and σ_2. Let us first consider the case $\sigma_2 = \sigma_1 = 70$. Then, decreasing K_I from a large initial value, we observe that the two cells at first oscillate in phase, in a periodic manner, until a critical value of K_I is reached below which the oscillations become chaotic. At even lower value of K_I (very large coupling strength), the system reaches a stable steady state.

When $\sigma_2 \neq \sigma_1$, the decrease of K_I from a large initial value produces successively quasiperiodic oscillations [region QP in Fig. 4(a); see Fig. 4(b) for the time series and the phase plane trajectory corresponding to point (b)], in-phase synchronization [region S in Fig. 4(a); see Fig. 4(c) corresponding to point (c)], chaos [region KO in Fig. 4(a); see Fig. 4(d) corresponding to point (d)], and a stable steady state (region SS). In Fig. 4(c), the asymmetry seen in the decreasing phase of the limit cycle for α_1 and α_2 is not apparent in the time series, because the decrease is very fast, but it can be seen upon enlarging the temporal evolution in Fig. 4(c) (see inset).

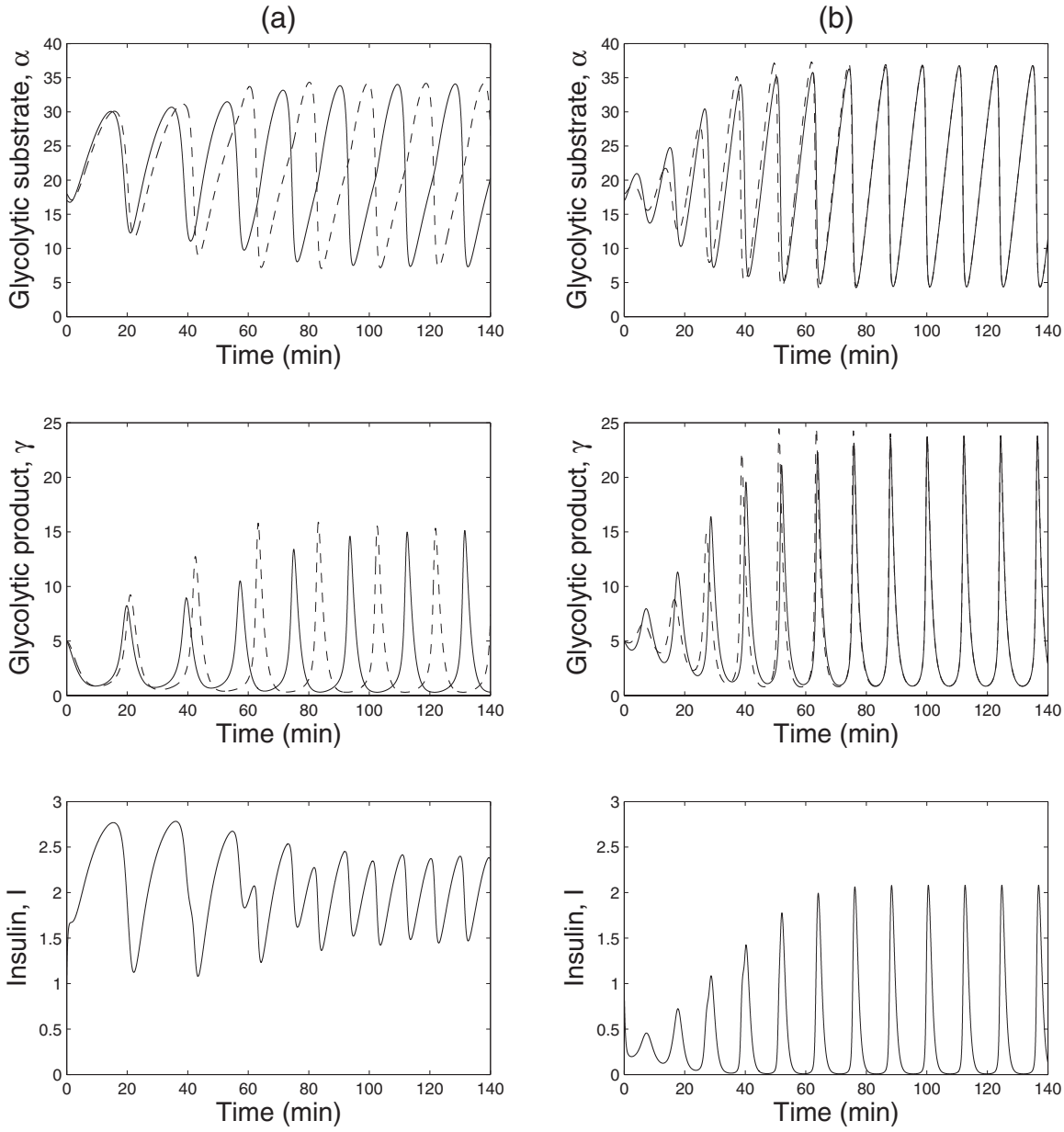

FIG. 2. Different modes of synchronization in the case of two coupled oscillators. (a) When insulin release is controlled solely by the glycolytic substrate, α, as described by Eq. (3a), the oscillations are in antiphase. (b) When insulin release is controlled solely by the glycolytic product, γ, as described by Eq. (3b), the oscillations are in phase. The curves have been obtained by numerical integration of Eqs. (1) with $N=2$. Parameter values are listed in Table I, except $v_{mr}=0.2\ s^{-1}$. Similar results are obtained for $v_{mr}=0.1\ s^{-1}$, but the transients are much longer. The curves represent the time course of the variables of cell 1 (α_1, γ_1, continuous line), cell 2 (α_2, γ_2, dashed lines), and the extracellular level of insulin (I). In (a), initial conditions must be slightly different for the two cells to observe the evolution to synchronization in antiphase. Indeed, if the two cells are indistinguishable, they will oscillate on the unstable limit cycle corresponding to in-phase synchronization (see Fig. 3).

IV. THE CASE OF *N* COUPLED CELLS: SYNCHRONIZATION IN PHASE OR OUT OF PHASE

A. In-phase versus out-of-phase oscillations

We now extend the analysis to the general case of N coupled oscillators ($N>2$) and consider again the two extreme cases in which the release of insulin into the extracellular medium is controlled only by the substrate or only by the product of the oscillatory PFK reaction (Fig. 5). As in the case $N=2$, numerical integration of the kinetic equations (1a)–(1c) shows that synchronization occurs out of phase [Fig. 6(a)] or in phase [Fig. 6(b)], depending on whether the release of insulin is controlled by the substrate or by the product. The level of insulin in the extracellular medium oscillates with much reduced amplitude when the cells oscillate out of phase with each other, as compared with the case in which they synchronize in phase.

B. Coupling *N* oscillators: Effect of an asymmetry and of coupling strength

We focus again on the case in which insulin release is controlled by product γ and consider the coupling of 10 cells with σ_i ($i=1,\ldots,10$) values randomly chosen in the range $65-75\ s^{-1}$. Then, cells synchronize in phase when the coupling strength is large (e.g., for $K_I=0.5$; see Fig. 7, right

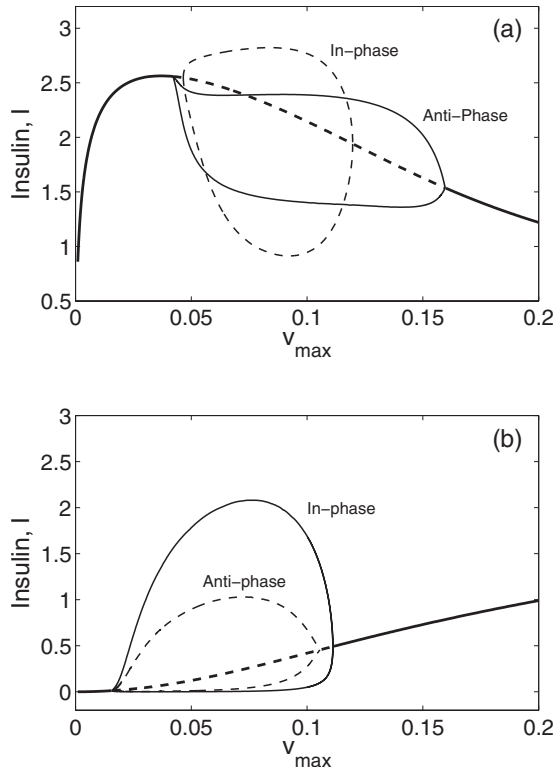

FIG. 3. Bifurcation diagram as a function of the maximum rate of glucose input (v_{max}) into the cell. The curves show the steady state as well as the envelope (minimum and maximum) of the oscillations of extracellular insulin, I. (a) When insulin release is controlled solely by the glycolytic substrate, α, as described by Eq. (3a), the stable limit cycle regime (solid line) corresponds to antiphase oscillations, while the unstable limit cycle regime (dashed line) corresponds to in-phase oscillations. (b) When insulin release is controlled solely by the glycolytic product, γ, as described by Eq. (3b), the situation is reversed: the stable limit cycle regime corresponds to in-phase oscillations and the unstable limit cycle regime corresponds to antiphase oscillations. Parameter values are listed in Table I. The curves have been obtained by means of the program AUTO (Ref. 50).

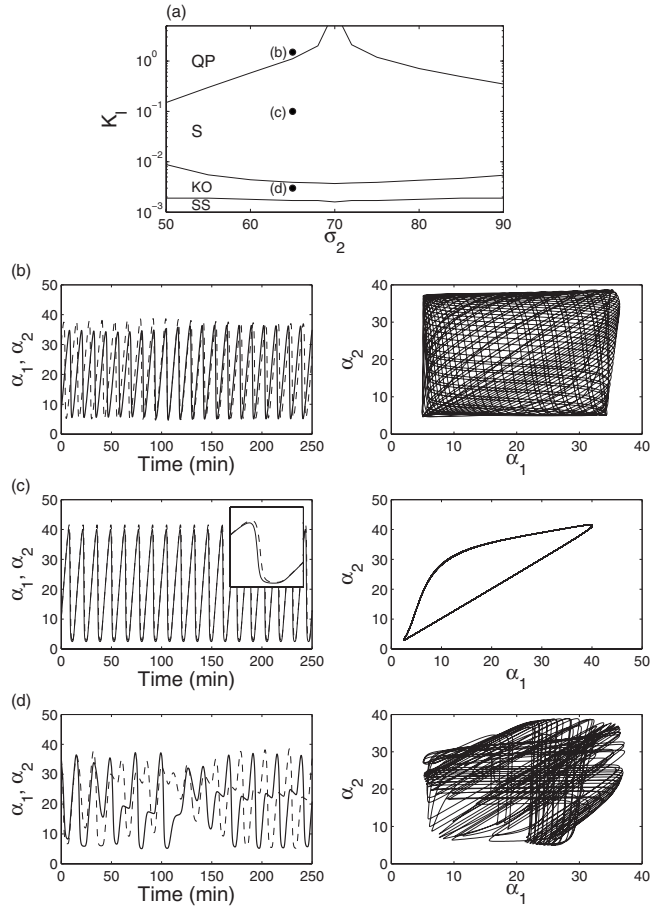

FIG. 4. Coupling between two oscillators: effect of an asymmetry in the reaction rate σ. Insulin secretion is coupled here to the glycolytic product, γ. Panel (a) shows the behavior observed as a function of σ_2, which measures the level of asymmetry, with σ_1 being fixed to 70 s^{-1}, and of K_I, which measures insulin inhibition of glucose synthesis and controls coupling strength. The lower K_I, the larger the coupling strength. The symbols refer to quasiperiodicity (QP), synchronization (S), chaos (KO), and stable steady state (SS). Rows (b)–(d) illustrate three examples of dynamical behavior obtained for σ_2=65 s^{-1} and corresponding to points b–d in panel (a), with K_I=1.1 mM, 0.2 mM, and 0.0015 mM, respectively: (b) quasiperiodic oscillations, (c) in-phase synchronized oscillations, and (d) chaotic oscillations. For each behavior the left panel shows the time series of the concentration of the glycolytic substrate, α_1 (continuous line) and α_2 (dashed lines), in the two cells, and the right panels displays the projected trajectory in the (α_1, α_2) phase plane. The inset in panel (c) is an enlargement of the oscillations, showing that α_1 and α_2 decrease abruptly in time with a slight phase shift, which explains why the limit cycle corresponding to in-phase synchronization does not tightly follow the diagonal. Parameter values are listed in Table I.

column) while they fail to synchronize and oscillate in a quasiperiodic manner when the coupling is weak (e.g., for K_I=2; see Fig. 7, left column). Only when cells synchronize in phase does the extracellular insulin level oscillate with large amplitude.

V. EFFECT OF PERIODIC FORCING ON THE SYNCHRONIZATION OF COUPLED CELLS

It is interesting to determine the effect of periodic forcing on the dynamics of the coupled system, because the synchronization of pancreatic cells by a pulsatile glucose signal has been studied experimentally [3,7] and theoretically.[19,30] We first address this issue in the case of two coupled cells. When the two cells are identical, in the absence of forcing, synchronization occurs in antiphase [Fig. 2(a)] or in phase [Fig. 2(b)] depending on whether the release of insulin is controlled by the substrate or by the product of the metabolic oscillator. As shown in Figs. 8(a) and 8(b), in the presence of external forcing by a periodic signal of glucose, expressed by the

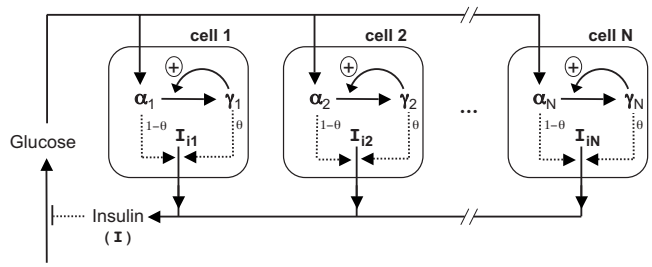

FIG. 5. Model for the global coupling of N cellular oscillators. The scheme represents an extension to N cells of the model for two coupled oscillators represented in Fig. 1.

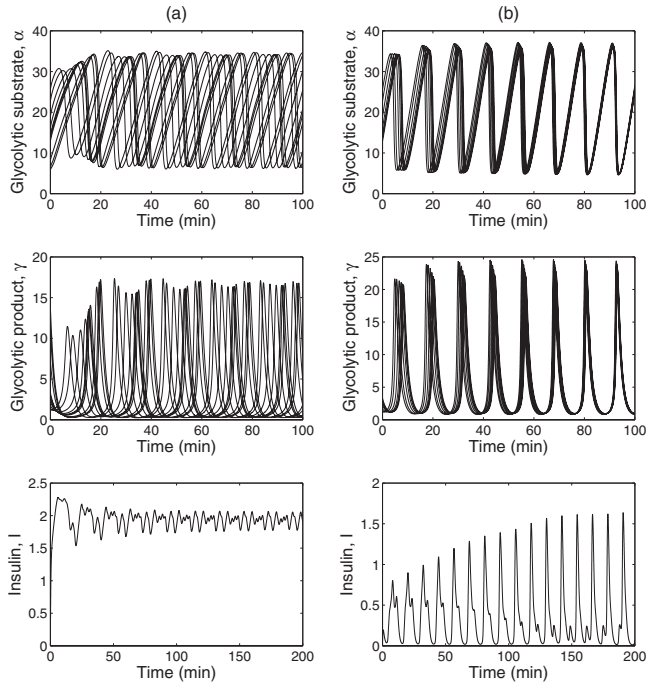

FIG. 6. Oscillations obtained for the global coupling of ten oscillating cells ($N=10$). (a) When insulin release is controlled solely by the substrate, α, as described by Eq. (3a), oscillations are out-of-phase. (b) When insulin release is controlled solely by the product, γ, as described by Eq. (3b), the oscillations are in phase. Parameter values are given in Table I.

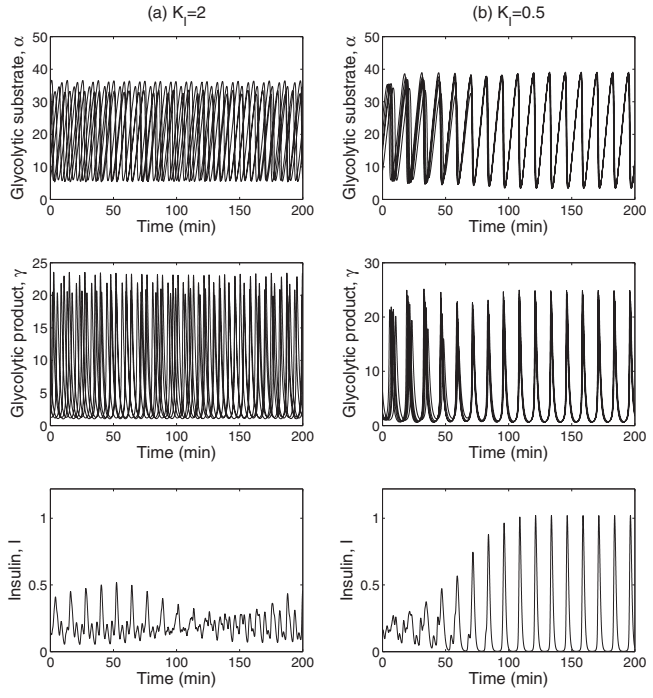

FIG. 7. Oscillations obtained for the ten-coupled oscillator model when the cells differ by the value of their maximum reaction rate, σ, at values of K_I corresponding to a small or large coupling strength. The value of σ is chosen randomly in the interval [65–75]. Insulin release is controlled solely by the product, γ, according to Eq. (3b). In the left column, the coupling strength ($K_I=2$ mM) is not sufficient to induce synchronization, whereas in the right column, the coupling strength is large enough ($K_I=0.5$ mM) to induce in-phase synchronization. Other parameter values are as in Table I.

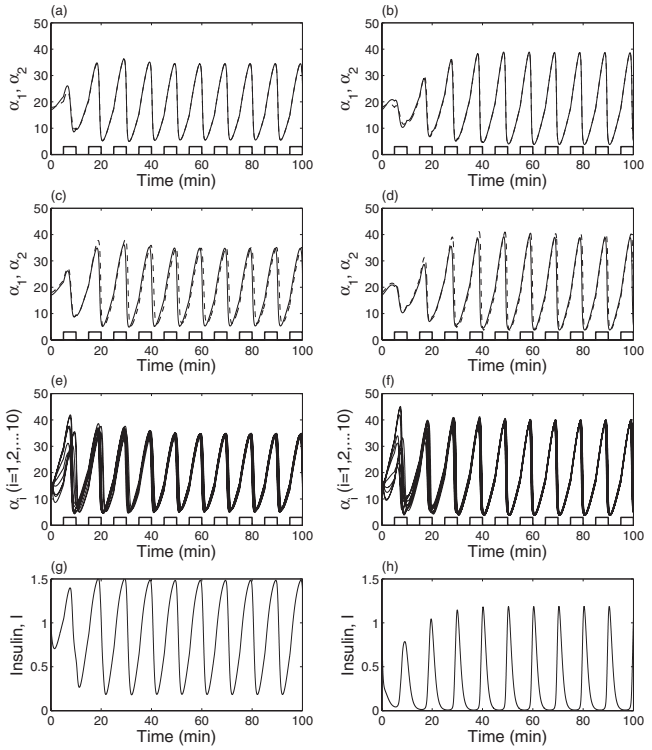

FIG. 8. Oscillations obtained in the presence of periodic forcing of the substrate input. Insulin release is controlled solely by the glycolytic substrate, α (left column) or by the product, γ (right column). [(a) and (b)] The two oscillators are identical ($\sigma_1=\sigma_2=70$ s^{-1}), except for initial conditions, which are slightly different. [(c) and (d)] The two oscillators are slightly asymmetrical ($\sigma_1=70$ s^{-1}, $\sigma_2=65$ s^{-1}). [(e)–(h)] Ten oscillators with σ_j value randomly chosen in the interval [65–75] s^{-1}. The square wave shows the periodic glucose input v_e, which oscillates between 0 and 3 s^{-1} with a period of 10 min. In panels (a)–(f), the curves represent the time course of glycolytic substrate, α_1 (continuous line) and α_2 (dashed lines), in the two cells. In panels (e) and (f), the curves show the evolution of the substrate in each of the ten cells. In panels (g) and (h), the curves represent the time course of extracellular insulin. Other parameter values are as in Table I.

term v_e in Eq. (1a), the two cells oscillate in phase in the two cases, regardless of the nature of the control of insulin release. A similar result is observed when the two cells differ by the value of parameter σ [Figs. 8(c) and 8(d)].

We next consider the coupling of a larger number of cells ($N=10$), characterized by values of σ_i ($i=1,\ldots,10$) randomly chosen between 65 and 75 s^{-1}. When insulin release is controlled solely by the product, the ten cells synchronize in phase in the presence of external forcing by a periodic glucose signal [Fig. 8(f)], while in the absence of forcing, for the same coupling strength, i.e., $K_I=2$, cells oscillate out of phase [Fig. 7(a)]—only when the coupling strength increases, e.g., for $K_I=0.5$, do the cells synchronize in phase in the absence of forcing [Fig. 7(b)]. When insulin release is controlled solely by the substrate, the ten cells synchronize partially in phase in the presence [Fig. 8(e)] but not at all in the absence of external forcing. As a result, the level of insulin oscillates with large amplitude [Fig. 8(g)], which is even larger in the case of full in-phase synchronization [Fig. 8(h)].

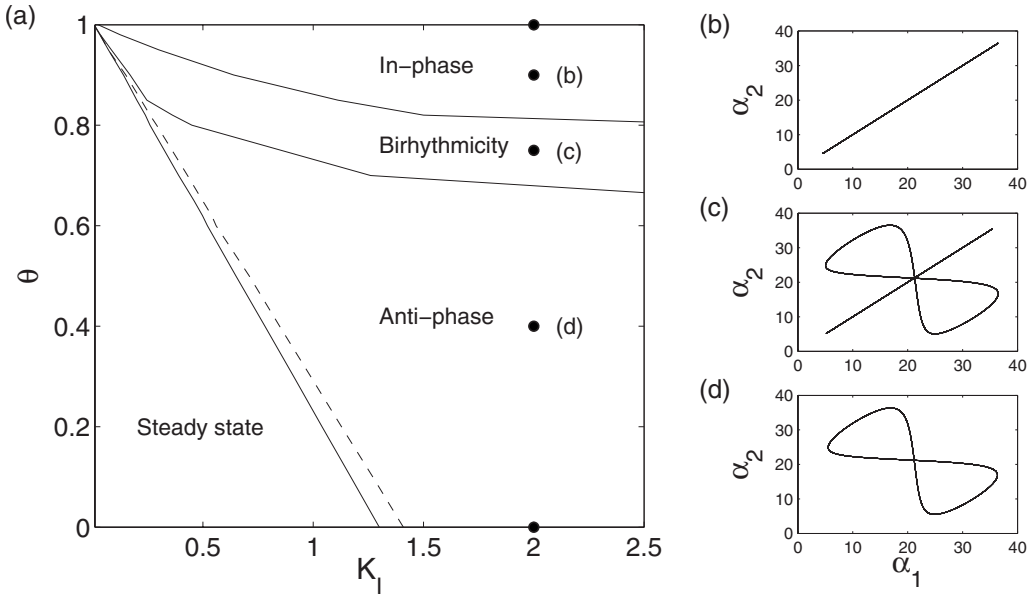

FIG. 9. (a) Stability diagram showing the stable modes of dynamic behavior obtained for two coupled oscillators with the hybrid insulin release mechanism defined by Eq. (3c), as a function of the coupling strength K_I and of parameter θ. The dashed line represents the bifurcation of the unstable solution corresponding to in-phase oscillations. Points (b), (c), and (d) correspond to the three panels in the right part of the figure, which illustrate in the phase plane the cases of in-phase synchronization (θ=0.9), the coexistence between in-phase and antiphase synchronization (θ=0.75), and synchronization in antiphase (θ=0.4). The points on the axes for θ=0 and 1 correspond to the situations illustrated in Figs. 2(a) and 2(b), respectively. Other parameter values are listed in Table I.

VI. THE HYBRID CASE: INSULIN SECRETION CONTROLLED BY BOTH THE SUBSTRATE AND PRODUCT OF THE PFK REACTION

As noted in Sec. II, the conditions in which insulin release is controlled solely by the substrate or by the product of the metabolic oscillator represent the extreme cases of a general, hybrid mechanism in which both the substrate and the product contribute to the control of insulin release, in proportions measured by a single parameter, θ, defined in Eq. (3c).

Shown in Fig. 9 is the bifurcation diagram established as a function of θ and K_I for the case of two coupled identical cells. The cases in which insulin release is controlled only by the substrate (θ=0) or by the product (θ=1) considered in Figs. 2(a) and 2(b) correspond to the dots at the bottom and at the top of the diagram, for K_I=2. The bifurcation diagram indicates the existence of several domains of dynamic behavior of the coupled system:

(i) At low values of K_I, the system evolves to a stable steady state. Thus, oscillations may vanish when the coupling is very strong, regardless of whether insulin release is controlled by the substrate or the product (this domain increases, however, when θ decreases,

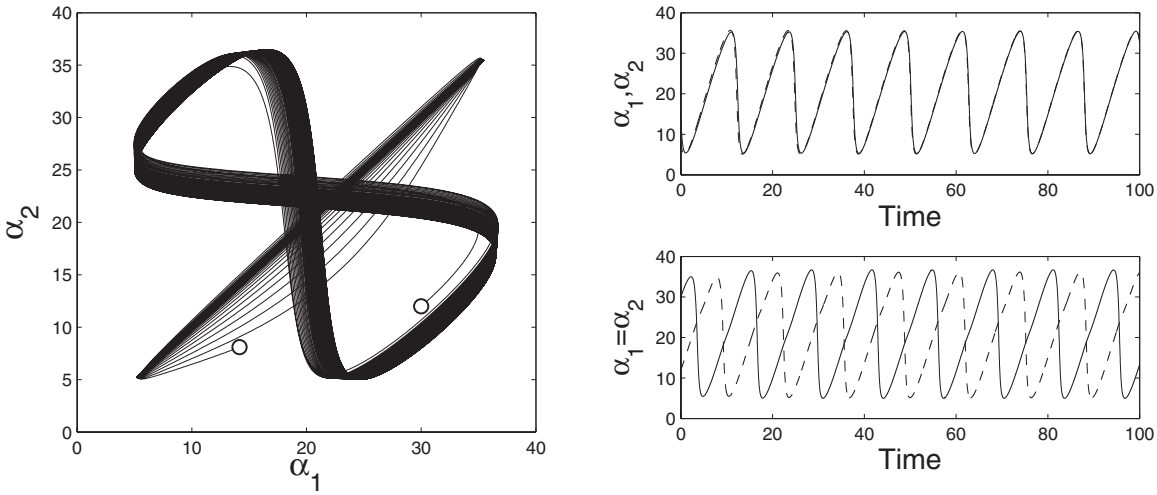

FIG. 10. Birhythmicity obtained in the case of two coupled oscillators with the hybrid mechanism: coexistence between synchronization in phase or in antiphase at intermediate coupling strength. The curves have been obtained for K_I=1 and θ=0.75. In the phase plane (left panel), the two circles represent two distinct initial conditions, one leading to oscillations in phase (α_1=14, γ_1=19, α_2=8, γ_2=22, I=1), the other one leading to oscillations in antiphase (α_1=30, γ_1=1, α_2=12, γ_2=3, I=1). Other parameter values are as in Table I.

i.e., when insulin release becomes controlled primarily by the substrate).

(ii) At larger values of K_I, e.g., for $K_I \geq 1.5$, when increasing progressively θ from the value $\theta=0$, the two oscillating cells at first synchronize in antiphase, then birhythmicity occurs in the form of a coexistence between stable oscillations in phase or in antiphase (see Fig. 10), and finally the two cells synchronize to oscillate in phase when θ approaches the value $\theta=1$.

(iii) The bifurcation line for the oscillations synchronized in antiphase (dashed line) is located slightly to the right of the bifurcation locus for the oscillations in antiphase (solid straight line) in the coupled system. This situation is reminiscent of the bifurcation diagram established as a function of the maximum substrate input rate $v_{\max}$ in Figs. 3(a) and 3(b), where the bifurcation of the antiphase solution precedes that of the in-phase solution for $\theta=0$ [Fig. 3(a)] and follows it for $\theta=1$ [Fig. 3(b)].

When extending the analysis of the hybrid mechanism to the case of a larger number of oscillating cells, we observe another form of birhythmicity. Thus, in the case of 10 identical oscillators, we find that at intermediate coupling strength, e.g., for $\theta=0.9$, the ten oscillators synchronize in phase [Fig. 11(a)], while for $\theta=0.4$ they synchronize out of phase [Fig. 11(c)]. For $\theta=0.75$, we observe the coexistence between stable patterns of in-phase and out-of-phase synchronization. The latter corresponds to a situation in which the cells are grouped in two clusters, each of which contains five oscillators synchronized in phase, with the two clusters oscillating in antiphase with respect to each other [Fig. 11(b)].

The formation of clusters does not require an even number of oscillators. We indeed observe the same behavior with 11 coupled oscillators. In this case, at intermediary values of θ, besides the full synchronization with all oscillators synchronized in phase, clusters of 5 and 6 oscillators, in antiphase with respect to each other, can be obtained. Similarly, with 13 oscillators it is possible to obtain clusters containing 6 and 7 oscillators, respectively. The formation of clusters is likely not an artifact due to the hypothesis that all oscillators are identical. Using 10 oscillators differing by their reaction rate σ_i randomly chosen between 65 and 75 s^{-1}, we also observe, at intermediary value of θ (e.g., $\theta=0.9$), the formation of two clusters of five oscillators, more or less in-phase in each cluster, with the two clusters oscillating in antiphase with respect to each other [Fig. 11(d)]. The possibility cannot be excluded that in these various instances of coexistence between multiple patterns of synchronization, additional types of clustering may be observed depending on the number of oscillators and on the choice of initial conditions.

VII. DISCUSSION

Experimental observations suggest[10,11] that the pulsatile release of insulin by pancreatic β cells is controlled by metabolic oscillations that occur in glycolysis as a result of the allosteric regulation of the enzyme phosphofructokinase. Glycolytic oscillations have been observed in yeast cells more than four decades ago, and continue to serve as a prototypic system to study oscillations and synchronization at the cellular level.[12–15] Thus, synchronization of yeast cells oscillating in suspensions has been studied both experimentally and theoretically.[32–34] In yeast, intercellular communication leading to synchronization by a global external signal is mediated by acetaldehyde, a glycolytic intermediate secreted by cells oscillating in suspension.[32] Such synchronization through a quorum-sensing mechanism has also been explored for other oscillating systems such as a population of model genetic oscillators known as Repressilators.[39]

In pancreatic β cells, synchronization occurs at two distinct levels. Within an islet of Langerhans, β cells appear to be coupled through gap junctions, which allow for intercellular synchronization.[16,17] How different islets within the pancreas become synchronized remains unknown.[2,4] It has been suggested that insulin secreted by β cells could lead to synchronization through the modulation by insulin of the glucose input to the cells.[10] In this work, we explored such a mechanism by considering a minimal model for the periodic secretion of insulin coupled to glycolytic oscillations in pancreatic β cells. We assumed, furthermore, that insulin secreted by the cells into the extracellular medium inhibits the production of glucose in the liver. This in turn affects the rate of input of glucose into the cells. Because glucose serves as a substrate for glycolysis, such a mechanism allows for the modulation of the input of glycolytic substrate by the glycolytic oscillator, which thus controls its own substrate input via the pulsatile release of insulin. A similar approach was followed by Pedersen *et al.*,[30] who showed that this mechanism may indeed lead to in-phase synchronization of different islets, using a more detailed model for insulin secretion coupled to oscillations in Ca^{++}, membrane potential, and glycolysis. Inter-islet synchronization was also achieved in response to periodic forcing by glucose.[30] The authors of that study, however, did not discuss the possibility of different modes of synchronization.

The minimal model considered here contains two variables for each cell, describing the glycolytic oscillator, as well as a global variable, insulin, which is secreted by the cells into the extracellular medium. We considered different possibilities for the coupling of insulin release to these metabolic oscillations. We first discussed the dynamics of this system in a system of two coupled cells, before dealing with the coupling of N oscillating cells. The analysis shows that the nature of the coupling between insulin release and the variables of the metabolic oscillator has a marked influence on the characteristics of the synchronization process. Thus, the oscillating cells (from different islets in the case of pancreatic secretion of insulin, if we assume that cells are synchronized through gap junctions within an islet) synchronize in phase or out of phase depending on whether insulin release is controlled by the substrate or by the product of the intracellular metabolic oscillator. When considering a mixed mechanism in which insulin release is controlled by both the substrate and the product in relative proportions measured by a single parameter, θ, the transition between in-phase and out-of-phase synchronization could be expressed as a function of this parameter. Upon increasing θ from zero (hor-

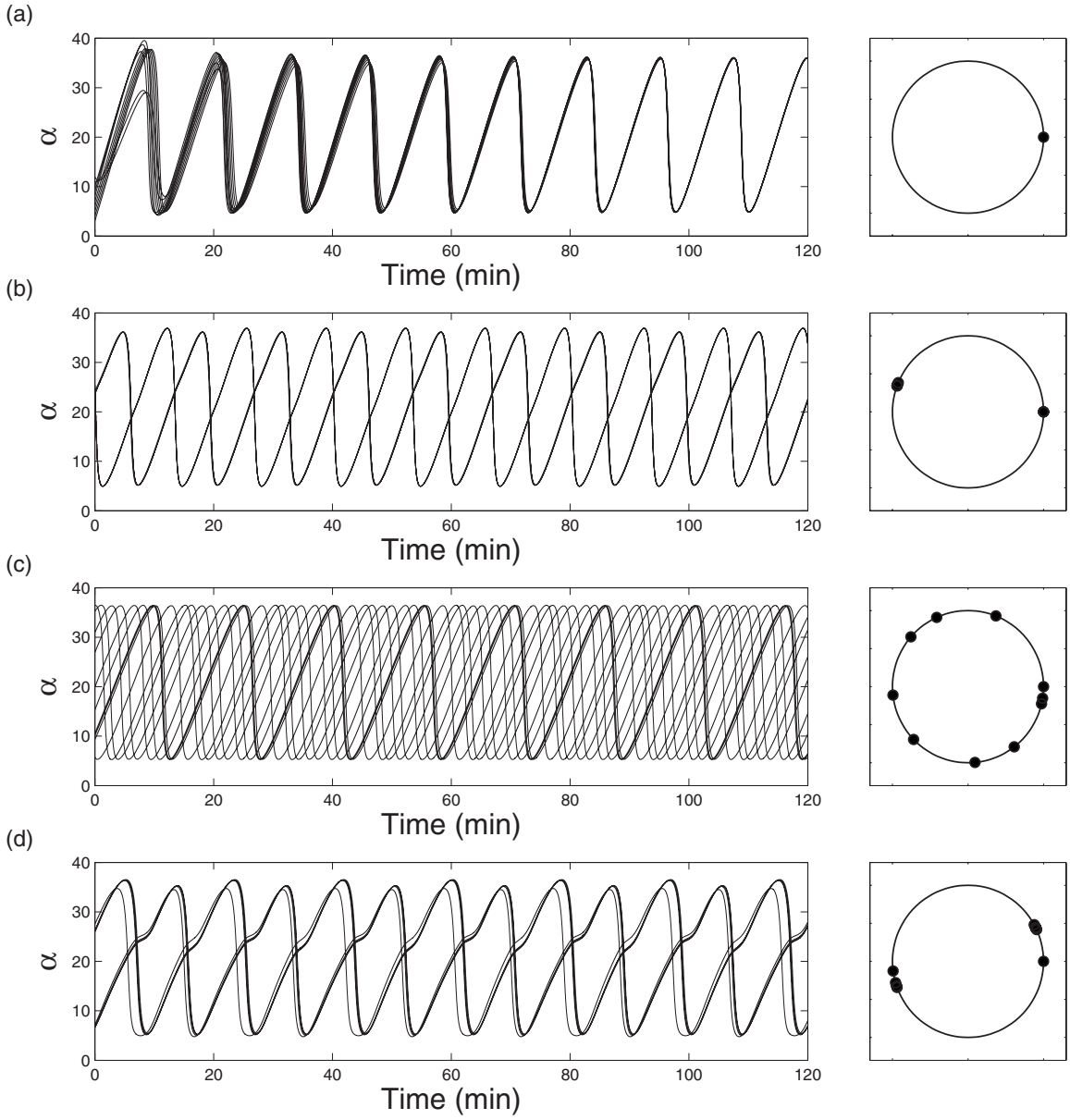

FIG. 11. Global coupling of ten oscillators with the hybrid release mechanism, as a function of the parameter θ. (a) $\theta=0.9$: regardless of the initial conditions, the ten oscillators synchronize in phase. (b) $\theta=0.75$: coexistence between in-phase (not shown) and out-of-phase (shown) synchronization. In the latter case, two clusters, each containing five oscillators synchronized in phase, synchronize in antiphase with respect to each other. The selection of the synchronization regime depends on the initial conditions. In-phase synchronization is observed, for example, for the following initial conditions: $\alpha_1=11$, $\alpha_2=11.1$, $\alpha_3=11.2$, $\alpha_4=11.3$, $\alpha_5=11.4$, $\alpha_6=11.5$, $\alpha_7=11.6$, $\alpha_8=11.7$, $\alpha_9=11.8$, $\alpha_{10}=11.9$, $\gamma_1=5$, $\gamma_2=5.1$, $\gamma_3=5.2$, $\gamma_4=5.2$, $\gamma_5=5.4$, $\gamma_6=5.5$, $\gamma_7=5.6$, $\gamma_8=5.7$, $\gamma_9=5.8$, $\gamma_{10}=5.9$, $I=1$; whereas out-of-phase synchronization is obtained for the following initial conditions: $\alpha_1=11$, $\alpha_2=12$, $\alpha_3=13$, $\alpha_4=14$, $\alpha_5=15$, $\alpha_6=16$, $\alpha_7=17$, $\alpha_8=18$, $\alpha_9=19$, $\alpha_{10}=20$, $\gamma_1=1$, $\gamma_2=2$, $\gamma_3=3$, $\gamma_4=4$, $\gamma_5=5$, $\gamma_6=6$, $\gamma_7=7$, $\gamma_8=8$, $\gamma_9=9$, $\gamma_{10}=10$, $I=1$. (c) $\theta=0.4$: regardless of the initial conditions (provided that they are not identical for all oscillators), the ten oscillators synchronize out of phase, without forming clusters. In (a)–(c), the ten oscillators are identical. (d) $\theta=0.9$ and the ten oscillators differ by the maximum reaction rate σ_j randomly chosen in the interval $[65-75]$ s^{-1}. For the latter case, the coupling strength is $K_I=0.5$ mM, and two clusters are observed. Initial conditions are $\alpha_1=11$, $\alpha_2=12$, $\alpha_3=13$, $\alpha_4=14$, $\alpha_5=15$, $\alpha_6=16$, $\alpha_7=17$, $\alpha_8=18$, $\alpha_9=19$, $\alpha_{10}=20$, $\gamma_1=1$, $\gamma_2=2$, $\gamma_3=3$, $\gamma_4=4$, $\gamma_5=5$, $\gamma_6=6$, $\gamma_7=7$, $\gamma_8=8$, $\gamma_9=9$, $\gamma_{10}=10$, $I=1$. For each case, plotted on the right panels is the phase distribution of the oscillators. The phase difference between the maximum of α_i $(i=1,\ldots,N)$ and the maximum of α_1 is computed, converted into a phase angle, and plotted on the unit circle. Other parameter values are listed in Table I.

mone release fully controlled by the substrate) to unity (hormone release fully controlled by the product), we observed the passage from out-of-phase to in-phase synchronization, with an intermediate domain where the two modes of stable synchronization coexist (Figs. 9–11). Coexistence between in-phase and out-of-phase oscillations has also been reported in models for neuronal coupling.[48]

Why is there such a difference in the pattern of synchronization between the cases in which insulin release is controlled by the substrate or by the product of the intracellular metabolic oscillator? The difference might be linked to the distinct waveforms that characterize oscillations in the two metabolites: the substrate concentration increases progressively, as a ramp, due to its accumulation at a constant rate of

infusion, while the product increases much more abruptly, in a spike, due to the cooperative activation of the allosteric enzyme by its reaction product. The product spike could provide a stronger signal leading to in-phase synchronization, which might not be achieved by the more sluggish increase in substrate.

Other modes of complex behavior such as chaos have been observed in the model in some conditions, e.g., in the case of coupling between two oscillating cells (see Fig. 4). The occurrence of aperiodic oscillations is not surprising, given the control exerted on the substrate input to oscillating cells by their oscillatory output, insulin. For the parameter values considered, we only found simple periodic oscillations in the case of a single cell (see Fig. 3). However, we cannot exclude the possibility of chaotic behavior in the case of a single oscillating cell when its substrate input is modulated periodically by its pulsatile output. This situation is indeed reminiscent of the simple or complex modes of oscillatory behavior observed in a three-variable model for Ca^{++} oscillations in which a parameter controlling oscillatory behavior is modulated periodically by the oscillating level of Ca^{++}.[49]

More realistic models for insulin secretion coupled to membrane potential bursting, Ca^{++} oscillations, and glycolytic oscillations have been proposed for pancreatic β cells.[40–43] These models are much more complex than the simple model considered here. It would be of interest to analyze the patterns of synchronization in these models. Here, we considered a minimal model, which does not incorporate the link between insulin secretion and Ca^{++} signaling or membrane potential oscillations, nor the role of gap junctions. It is likely that some of these processes affect the patterns of synchronization of pancreatic β cells. The selection between in-phase or out-of-phase synchronization between different islets might not occur in more detailed models.

The present results go beyond the particular case of insulin oscillations in pancreatic β cells, and pertain, more generally, to oscillating cells coupled in a global manner through the secretion into the extracellular medium of an intermediate affecting the dynamics of intracellular oscillations. The system considered here provides a simple model for studying as a function of a single parameter the selection between in-phase and out-of-phase synchronization in a population of coupled cellular oscillators.

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