Role of Oscillations in Membrane Potential, Cytoplasmic Ca^{2+}, and Metabolism for Plasma Insulin Oscillations

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A model for the relationship between ionic and metabolic oscillations and plasma insulin oscillations is presented. It is argued that the pancreatic β-cell in vivo displays two intrinsic frequencies that are important for the regulation of plasma insulin oscillations. The rapid oscillatory activity (2–7 oscillations [osc] per minute), which is evident in both ionic and metabolic events, causes the required elevation in cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) for the exocytosis of insulin granules. This activity is important for regulation of the amplitude of plasma insulin oscillations. The frequency of the rapid oscillatory ionic activities is regulated by glucose and allows the β-cell to respond in an analogous way, with gradual changes in $[Ca^{2+}]_i$ and insulin release in response to the alterations in glucose concentration. The slower oscillatory activity (0.2–0.4 osc/min), which is evident in the metabolism of the β-cell, has a frequency corresponding to the frequency observed in plasma insulin oscillations. The frequency is not affected by changes in the glucose concentration. This activity is suggested to generate energy in a pulsatile fashion, which sets the frequency of the plasma insulin oscillations. It is proposed that the slow oscillations in $[Ca^{2+}]_i$ observed in vitro are a manifestation of the metabolic oscillations and do not represent an in vivo phenomenon. *Diabetes* 51 (Suppl. 1):S171–S176, 2002

Regular variations in the blood concentration of insulin with typical durations of several minutes are present in humans (1,2). The frequency of the insulin pulses is important for the action of the hormone. Significantly less insulin is required when administered in a pulsatile fashion rather than as a bolus injection (3–7), which may result in different expression of the insulin receptor on target tissue (8). Indeed, a contributing cause to the glucose intolerance in type 2 diabetes may be related to the loss of the regular accentuated plasma insulin oscillations (9,10), which could aggravate insulin resistance by receptor downregulation.

The oscillatory behavior seems to be intrinsic of the β-cell. Oscillations in the β-cell membrane potential, cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$), and metabolism, with similar frequencies as the plasma insulin oscillations, have been described and suggested to be responsible for the pulsatile release of insulin (11–19). However, observations and conclusions made from experiments with isolated β-cells or islets do not necessarily translate into the in vivo situation. When evaluating the possible effects of oscillatory membrane potential, $[Ca^{2+}]_i$, and metabolism on plasma insulin oscillations, oscillatory activities with different frequencies have to be considered. In this context, the oscillatory activity with a frequency of 2–7 oscillations (osc) per minute and that with a frequency of 0.2–0.4 osc/min will be discussed. Experimental observations of these rapid and slow islet oscillatory activities in vivo and in vitro are reviewed, together with an in vivo model of the β-cell, where the role of the oscillatory activities in membrane potential, $[Ca^{2+}]_i$, and metabolism for the generation of plasma insulin oscillations are presented and discussed.

IN VITRO OSCILLATIONS OF ISLET MEMBRANE POTENTIAL AND $[Ca^{2+}]_i$

Rapid oscillations in membrane potential and $[Ca^{2+}]_i$ (2–7 osc/min) have been observed and characterized in the isolated islet (14,15,20–29). The membrane potential measurements consist of "slow waves," which are periods of depolarization when accumulations of action potentials ("bursts") promote influx of Ca^{2+}, interspersed with periods of hyperpolarization, when no Ca^{2+} influx is present. These slow waves give rise to the rapid oscillations in $[Ca^{2+}]_i$. The glucose concentration affects both the duration of the bursts and the time relationship between periods of depolarization and hyperpolarization, i.e., the frequency of the slow waves (30).

Slow oscillations (0.2–0.4 osc/min) in $[Ca^{2+}]_i$ (15,23,26–28,31) and in membrane potential activity (32–34) have been recorded and characterized in the isolated islet. The frequency of these oscillations is not affected by the glucose concentration (23,27). The slow oscillations of $[Ca^{2+}]_i$ are primarily observed in cultured islets. As the culture period is extended, the rapid $[Ca^{2+}]_i$ oscillations disappear in favor of the slow ones, which also disappear after prolonged culture (31). The glucose concentration during culture is also decisive for the $[Ca^{2+}]_i$ oscillatory pattern (26). Although a low-culture glucose concentration makes the islet incapable of responding to glucose-induced $[Ca^{2+}]_i$ oscillations, an intermediate-culture glucose concentration is compatible with subsequent glucose-

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$[Ca^{2+}]_i$, cytoplasmic Ca^{2+} concentration; osc, oscillations.

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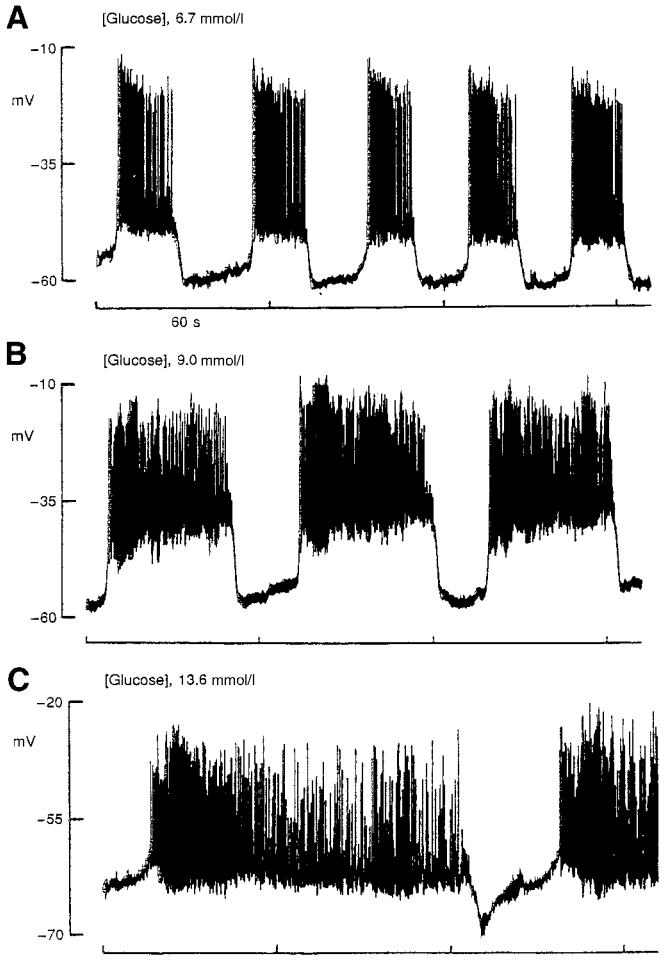

FIG. 1. Patterns of electrical activity for different glycemic levels. Specimen intracellular records of the electrical activity recorded in vivo from islets of Langerhans of different animals are shown (*A–C*). The blood glucose concentration measured during the electrical recordings is indicated in each trace. Reproduced with permission from Sanchez-Andres et al. (40).

induced rapid and slow $[Ca^{2+}]_i$ oscillations. The dependence of the $[Ca^{2+}]_i$ oscillatory pattern on culture period and glucose concentration is related to islet cAMP content (35–38). Indeed, when the content of cAMP was increased in the isolated islet, the regular electrical activity was promoted (39) and the slow $[Ca^{2+}]_i$ oscillations were replaced by the rapid oscillatory pattern (15,29). Freshly isolated islets predominantly exhibited the rapid $[Ca^{2+}]_i$ oscillations (23,24,28). Also, membrane potential recordings from freshly microdissected islets showed the rapid oscillatory pattern of β-cell electrical activity (20,21, 23–25).

IN VIVO OSCILLATIONS OF ISLET MEMBRANE POTENTIAL AND $[Ca^{2+}]_i$

Oscillations in β-cell membrane potential and islet $[Ca^{2+}]_i$ have been monitored in vivo (40–42). The membrane potential recordings showed glucose-dependent high-frequency oscillations (Fig. 1). These rapid oscillations were also observed in the $[Ca^{2+}]_i$ measurements (Fig. 2). No evidence of low-frequency oscillatory activity has been found so far using membrane potential or $[Ca^{2+}]_i$ recording techniques.

IN VITRO OSCILLATIONS OF ISLET METABOLISM

Isolated islets also showed oscillations in metabolism with both the high- and low-frequency component. The low-frequency oscillations were observed in measurements of oxygen tension (pO_2) in individual islets (18,19,43) and groups of islets (13), lactate production in groups of islets (44), and ATP/ADP ratio in suspensions of mouse β-cells (17). The high-frequency oscillations were observed in pO_2 in individual islets and were synchronous with the rapid changes in $[Ca^{2+}]_i$ (18,43). Activation of the mitochondrial Ca^{2+}-dependent dehydrogenases (45,46) may well serve as linkage between the two oscillatory phenomena, irrespective of whether oscillations in $[Ca^{2+}]_i$ initiate changes in pO_2 or vice versa. The slow oscillations in pO_2 were ascribed to oscillations in glycolysis (13), electrical activity (11), and Ca^{2+} entry through the voltage-independent Ca^{2+} channels (47). In favor of a glycolytic oscillator, pulses of insulin release have been found at low glucose concentrations when $[Ca^{2+}]_i$ was low and stable, but pO_2 oscillated in synchronous measurements of insulin release and $[Ca^{2+}]_i$ (48) and insulin release and pO_2 (19). Also, when using the activity of the ATP-sensitive K^+ channel as an indicator of the ATP/ADP ratio, oscillations in the channel activity were recorded in β-cells with a stable and low $[Ca^{2+}]_i$ (49).

IN VIVO OSCILLATIONS OF ISLET METABOLISM

When modified Clark-like electrodes were inserted into islets of the living rat, rapid and slow oscillations in islet pO_2 were demonstrated (Fig. 3) (P.B., J. Westerlund, P. Liss, P.O. Carlsson, unpublished data).

MODEL OF IN VIVO OSCILLATORY MEMBRANE POTENTIAL, $[Ca^{2+}]_i$, pO_2, AND INSULIN RELEASE

The proposed relationship between oscillations in membrane potential, $[Ca^{2+}]_i$, and metabolism (pO_2) is outlined in Fig. 4, together with how the oscillatory activities relate to the pulsatile release of insulin from the pancreatic β-cell

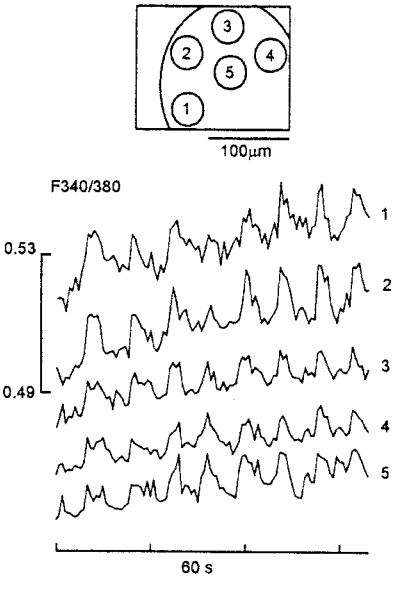

FIG. 2. Spatial analysis of the in vivo calcium oscillations in an islet of Langerhans. The zones depicted in the diagram correspond to the zone of the islet with sufficient fluorescence at 340 nm. Reproduced with permission from Fernandez and Valdeolmillos (42).

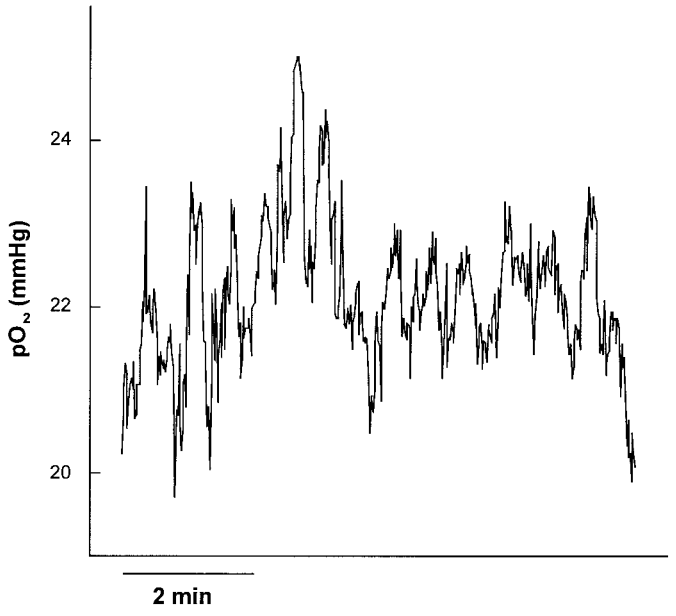

FIG. 3. In vivo recording of islet pO_2 from an anesthetized rat. Oxygen tension was measured with a modified Clark-like electrode. The blood glucose concentration was 6.4 mmol/l during the recording (P.B., J. Westerlund, P. Liss, P.O. Carlsson, unpublished data).

in vivo. It is suggested that the slow oscillatory activity is a metabolic (pO_2) manifestation, which gives rise to the slow oscillations in insulin release. Furthermore, it is suggested that the rapid oscillatory activity can be seen in membrane potential, $[Ca^{2+}]_i$, pO_2, and insulin, where glucose-dependent membrane potential changes cause oscillations in $[Ca^{2+}]_i$ via periodic entry of Ca^{2+}. These rapid $[Ca^{2+}]_i$ oscillations are linked to changes in metabolism (pO_2) and initiate the rapid secretory events. At normoglycemia (Fig. 4*A*), one slow oscillation in pO_2 and insulin is shown (approximate duration 5 min) with superimposed rapid oscillations. At hyperglycemia (Fig. 4*B*), glucose-induced amplification of the amplitude of the slow oscillatory activities in pO_2 and insulin is observed together with a lengthening of the periods of action potential (membrane potential).

DISCUSSION OF THE MODEL

Role of rapid oscillatory activity in vivo. The rapid oscillatory ionic activities set the $[Ca^{2+}]_i$ of the β-cell. The rapid oscillatory ionic and metabolic events are instrumental for the amplitude regulation of plasma insulin oscillations in setting the $[Ca^{2+}]_i$, which plays a key role in exocytosis of insulin (50). This is achieved in a glucose-dependent manner by changing the duration of the bursts and frequency of the slow waves, which determine rapid $[Ca^{2+}]_i$ events. The alterations in duration of the bursts and the frequency of the slow waves do not depend on a certain glucose concentration but seem to be continuously mirroring changes in the ambient glucose concentration in vivo (40). In isolated islets, the relationship between duration of the bursts and glucose concentration is strikingly similar to the relationship between insulin release and glucose concentration (30). It is reasonable to assume that a similar relationship exists between the rapid oscillatory ionic activity and insulin release in vivo (40–42), which has also been suggested (42). Such a relationship implies a situation in which the secretory response of the β-cell can vary continuously, i.e., is analogous, in a glucose-dependent manner. Another model of explaining the graded response to variations in the glucose concentration is the digital model, where the existence of different populations of β-cells, which become actively secreting, are "recruited" at different glucose concentrations (51). The existence of such subpopulations has been demonstrated in dispersed β-cells (52). As a consequence, the concept of the β-cell as the fuel sensor (53) would require a set of β-cells with varying numbers of β-cells with different glucose sensitivities covering the physiological range. With the analogous model, this set of β-cells is not required because the fuel-sensing capacity would reside in each β-cell. The observations that glucose-induced changes in insulin release are not accompanied by changes in $[Ca^{2+}]_i$ oscillatory pattern in the isolated islet (27) and that β-cells in the same islet and islets in the same pancreas respond with similar alterations in the oscillatory activity to changes in the blood glucose concentration (42,54) seem to favor the analogous model. In this context, it should be noted that the rapid oscillatory changes are

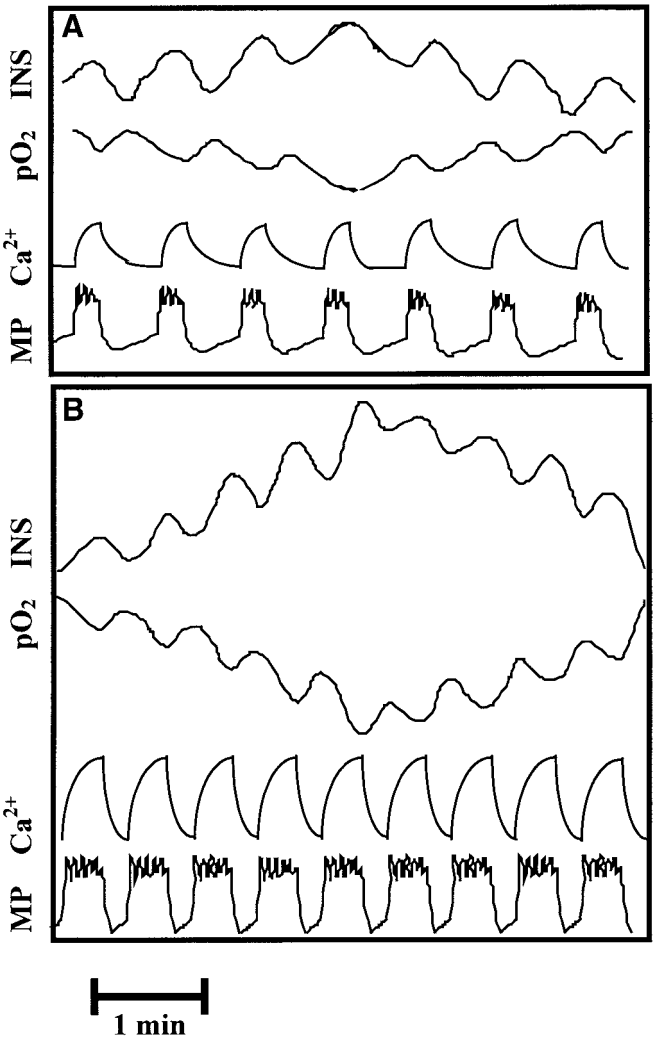

FIG. 4. Model of in vivo oscillatory membrane potential (MP), $[Ca^{2+}]_i$, pO_2, and insulin (INS) release. The relationship between rapid and slow oscillatory activities at normoglycemia (*A*) and hyperglycemia (*B*) is shown.

synchronized between β-cells in the islet but not between islets in the pancreas (42,54).

The interrelationship between the rapid oscillatory activity in metabolism and ionic movements is complex; both ionic changes have been proposed to drive metabolism by activating dehydrogenases (55,56) and oscillatory metabolic changes to precede the ionic movements (17,57). Irrespective of causality, it can be assumed that the rapid regular ionic and metabolic events have their secretory counterpart also in vivo, as has been demonstrated in the isolated islet (14,25,26,58–61). However, it is not clear if these rapid secretory events are detectable by plasma insulin measurements because there is no synchronization of the oscillations in electrical activity of different islets from the same pancreas in vivo (54).

Role of the slow oscillatory activity in vivo. The slow oscillatory pattern, which is suggested to be a metabolic phenomenon in vivo, closely follows that of secretion (13,19) and seems to be responsible for the plasma insulin oscillations. Indeed, the recently observed slow in vivo oscillations in islet pO_2 were synchronous with plasma insulin oscillations in the portal vein (P.B., J. Westerlund, P. Liss, P.O. Carlsson, unpublished data). The frequency of these slow metabolic and plasma insulin oscillations corresponds to that of the plasma insulin oscillations found in humans (2).

The metabolic oscillations have been proposed to depend on the autocatalytic role of phosphofructokinase to initiate glycolytic oscillations (16). This mechanism has, however, not been demonstrated in the β-cell, and the way in which the regular variations in metabolism are achieved in the β-cell is still elusive. Nevertheless, variations in the ATP/ADP ratio have been observed (17) and could explain the slow insulin oscillations because the exocytotic process, apart from requiring elevation of $[Ca^{2+}]_i$, is also ATP-dependent (62). In contrast to the rapid oscillatory activity, changes in the glucose concentration do not alter the frequency of the slow oscillations (15,23,27,63).

For regular plasma insulin variations to occur, coordination of the secretory activities of the islets of Langerhans in the pancreas is required (9,64–67). Insulin release from the isolated perfused pancreas is pulsatile (68), which implies that factors within the pancreas are capable of coordinating the secretory activities. Some potential factors with such a coordinating role were recently reviewed (67). In this context, the slow metabolic oscillations may also contribute to the synchronization because oscillations in pO_2 of the exocrine pancreas have a similar frequency to those of the endocrine pancreas (P.B., J. Westerlund, P. Liss, P.O. Carlsson, unpublished data).

Are slow $[Ca^{2+}]_i$ oscillations absent in vivo? It could be argued that the reported absence of slow $[Ca^{2+}]_i$ oscillations in vivo is the result of technical limitations, which made the duration of the recordings too short to observe the oscillations (42). In such case, it is just a matter of time and technical development before we will also be able to see them in vivo. However, there are reasons to believe that the rapid $[Ca^{2+}]_i$ oscillations alone represent the in vivo $[Ca^{2+}]_i$ manifestations.

The in vitro results with progression from rapid oscillations in $[Ca^{2+}]_i$ in freshly isolated islets (23,24,28) and islets with enhanced cAMP content (15,29) via islets showing a mixture of rapid and slow oscillations in $[Ca^{2+}]_i$ (15,23,26,27,29,31) to islets with only slow oscillations in $[Ca^{2+}]_i$ after extended culture (31) are intriguing. The recordings of membrane potential also support the idea of the rapid oscillatory activity as the sole ionic oscillatory pattern in vivo. Freshly microdissected islets show an oscillatory pattern of β-cell electrical activity (20–22) almost identical to the recordings observed in vivo (40,41).

What are slow oscillations in membrane potential and $[Ca^{2+}]_i$? The slow oscillations in $[Ca^{2+}]_i$ have been explained by regular variations in the ATP/ADP ratio, which control the conductance of ATP-dependent K^+ channels leading to depolarization and periodic influx of Ca^{2+} (69). The frequency of these slow $[Ca^{2+}]_i$ oscillations coincides with the frequency of the slow metabolic oscillations present both in vivo and in vitro. If the proposed model of oscillatory activities of the β-cell is correct, the slow oscillations in $[Ca^{2+}]_i$ as well as in membrane potential are manifestations of the slow metabolic oscillations.

Why are rapid but not slow $[Ca^{2+}]_i$ oscillations present in vivo? The rapid oscillatory activities fine-tune the $[Ca^{2+}]_i$ to the ambient glucose concentration by modulating both the duration of the bursts and the frequency of the slow waves. This arrangement allows the β-cell to continuously monitor and respond to changes in the glucose concentration. In contrast, the slow $[Ca^{2+}]_i$ oscillations are not elicited until a certain threshold concentration of the glucose is reached (51). Although it is argued that this threshold concentration may vary between β-cells when explaining glucose-induced changes in insulin release (52), in most islets, 11 mmol/l glucose or more is needed to elicit these slow $[Ca^{2+}]_i$ oscillations (15,23,26–28,31). The need of the islet to reach such a high threshold concentration before it responds with $[Ca^{2+}]_i$ oscillations is clearly not satisfactory in terms of requirements for blood glucose handling, and one wonders how such islets would function in vivo.

CONCLUSIONS

The pancreatic β-cell displays a rapid and a slow oscillatory activity. The rapid ionic activity increases $[Ca^{2+}]_i$, which is critical for the amplitude regulation of plasma insulin oscillations. The rapid oscillations are regulated by glucose and allow the β-cell to respond with gradual changes in $[Ca^{2+}]_i$ in response to the alterations in the ambient glucose concentration. The slow metabolic activity has a frequency corresponding to the frequency observed in the plasma insulin oscillations. The slow metabolic oscillations generate energy in a pulsatile fashion, which sets the frequency of the plasma insulin oscillations.

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