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Review Article

CALCIUM OSCILLATIONS AND ITS FUNCTIONAL SIGNIFICANCE IN CHRONOBIOLOGY OF PANCREATIC β-CELLS: THE ART OF DISCOVERING SCIENCE

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Historical Perspectives

"They'll find I' th' physiognomies
O' th' planets, all men's destinies
They'll feel the pulses of the stars
To find out agues, cough, catarrhs;
And tell what crisis does divine".

Butler, Hudibra, I.

Throughout the annals of time, men even the prehistoric ones have been fascinated by the ever-existing rhythmic processes in living systems. However, this idea wasn't completely in the open till 1797 when Hufeland proposed rhythmic events of life in relation to 24 hours or solar day as a prime unit of functional chronology[1]. In Florida, nose and throat surgeons found that hemorrhages in throat operation were 82% higher during the second quarter of the cycle of the moon than at other times[2]. Similarly, insulin sensitivity index was found to be lower during winter than summer in Swedish population[3]. Seasonal variations of HbA_{1C} in diabetic subjects and glycemic variations in healthy subjects have also been reported[4-6]. Thus, it seems that periodic biological events are intimately related to the non-biological cycles, whether terrestrial, astronomical, physical, electrical or others. But certainly it has been realized by the early scientists that the universe is rhythmic and displays incessant movement in the form of periodicity. The capacity to follow them, to oscillate, would enhance the survival potential of a species, including we, the human beings.

In 1843, nearly half a century after Hufeland's Publication, Chossat presented his report of 20 years of study on the changes in cloacal temperature of pigeons under various experimental conditions as to environmental temperature and nutrition[1]. Further analyses on biological rhythms revealed that *'a close study of these rhythms should yield vital information concerning the construction of various biochemical reactions in the body, especially if cybernetic and thermodynamic principles are applied'*. Many enthusiastic scientists and clinicians then devoted themselves for basic understanding of the fundamentals of biological rhythms. In the 30's of the twentieth century, the periodic behavior of the normal blood glucose was characterized. In this case small meals were given regularly throughout the day and generally each meal produced a variable, transient increase. However, it was found that the glucose concentration often tended to drop somewhat at about 2 to 3 p.m. even if food was given[7]. The essential feature of this periodic behavior is that the blood glucose level is relatively stable, varying between 4.4 and 6.6 mM/L. It was discovered later, that under physiological conditions the blood glucose level is kept at around 5 mM/L in fasting mammals, including humans due to the pulsatile release of the glucostatic hormone insulin.

Pancreatic β-Cells and Calcium

Insulin is secreted from the pancreatic β-cells in a highly regulated fashion. Among the factors affecting insulin release, glucose is the most important physiological stimulant and is considered as the primary regulatory signal. The exact sequence of biochemical events involved in glucose stimulated insulin secretion (GSIS) has not yet been identified. Nevertheless, it is well documented that GSIS is manifested due to a rise of cytosolic Ca^{2+} ($[Ca^{2+}]_i$), which acts as the primary intracellular messenger that couples physiological or pharmacological insulin secretegogues to insulin release from stored granules[8]. A characteristic feature of the $[Ca^{2+}]_i$ response to glucose is its oscillatory nature observed both in

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individual pancreatic β-cells and in intact pancreatic islets[9-12]. Recent experiments have shown that the $[Ca^{2+}]_i$ oscillations correspond to pulsatile insulin secretion and electrical activity of β-cells and thus it has been suggested that the $[Ca^{2+}]_i$ oscillations may have important role in maintaining pulsatile release of insulin[13-18].

Pancreatic β-cell's response to glucose is oscillatory. When glucose enters the β-cells through high capacity glucose transporters, its metabolism through glycolysis and Kreb's cycle causes changes in the ATP/ADP ratio in the cytoplasm resulting in closure of the ATP-sensitive K^+ channels and thereby trigger membrane depolarization[19,20]. This leads to the opening of voltage dependent calcium channels (VDCCs), Ca^{2+} influx and to subsequent rise in $[Ca^{2+}]_i$ that promotes insulin secretion (Fig 1). Apart from the voltage-sensitive Ca^{2+} influx from extracellular space, another major source of $[Ca^{2+}]_i$ rise is mobilization of Ca^{2+} from intracellular stores[21-23]. Recent studies also suggest the existence of store operated Ca^{2+} entry in pancreatic β-cells[24-26]. However, once the $[Ca^{2+}]_i$ is elevated, to restore it to its basal level, the β-cells drive Ca^{2+} actively either out of the cell across the plasma membrane through calcium pump and Na/Ca exchanger or to various intracellular stores[27-29]. We can simply postulate that the upstroke of the oscillation is due to Ca^{2+} influx and/or the release and the descending phase involves stimulation of outward Ca^{2+} transport and/or intracellular sequestration. And oscillations are generated and maintained via dynamic interplay of discrete signaling cascades which provides complex feedback, as well enhances co-ordination that critically maintains the fine tuning of $[Ca^{2+}]_i$ fluctuations during different phases of the oscillation.

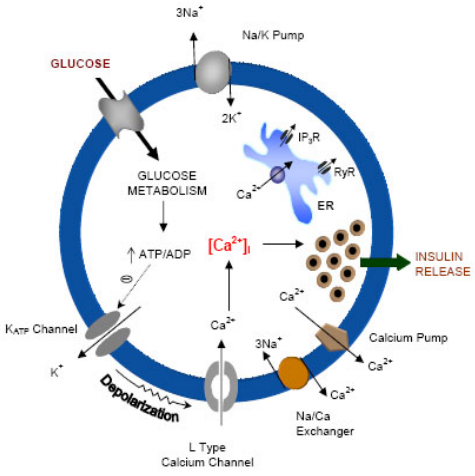

Figure 1. *Schematic representation of ionic events in 'glucose stimulated insulin secretion' from pancreatic B-cells. IC = intracellular compartments.*

Properties of $[Ca^{2+}]_i$ Oscillations

Oscillations in $[Ca^{2+}]_i$ are of different fundamental types, involving different mechanisms. However, in secretory cells two major kinds of $[Ca^{2+}]_i$ oscillations are seen – baseline transients or spikes and sinusoidal oscillations[30,31]. Spikes are characterized by transient increase in $[Ca^{2+}]_i$ that rise rapidly from a baseline of $[Ca^{2+}]_i$. The shape of transients may vary depending on agonist-type: they may be symmetrical or may have a relatively rapid rising phase with a slower falling phase (Fig 2). Sinusoidal oscillations generally appear as symmetrical oscillations superimposed on a sustained level of $[Ca^{2+}]_i$ usually above the pre-stimulus baseline level. They resemble more closely to true sine waves. These oscillations are generally considered insensitive to variations in agonist concentrations and may simply reflect how certain cells respond to a maintained elevation of calcium[30]. A less common $[Ca^{2+}]_i$ oscillatory pattern that seems to be distinct from the spiking and sinusoidal patterns has been described by Rooney and Thomas[32]. The oscillations are highly asymmetric, consisting of a rapid increase in $[Ca^{2+}]_i$ followed by a slow decline during which the next asymmetric oscillation is initiated. Such a pattern is extremely prominent in adrenal glomerulosa cells, in neutrophils and in mucosal mast cells[33].

In pancreatic islets, stimulatory glucose concentrations (>7mM) induce two types of $[Ca^{2+}]_i$ oscillations – fast and slow. Fast oscillations are transient spikes in which the $[Ca^{2+}]_i$ level rises sharply and then subsequently decreases along an exponential-like time course[10]. They oscillate at a frequency ranging from 2 to 5/min with duration of 3-11s (Fig 3). They are the direct consequence of β-cell bursting electrical activity, their duration depends on glucose concentration, and they are synchronous throughout the islet[34]. In contrast, slow oscillations are characterized by smooth rising and falling phase with duration of 1-3 min and frequency of 0.2-1/min. A mixed pattern of fast oscillations superimposed on the slow pattern is also a common observation. Both the slow and fast oscillations of $[Ca^{2+}]_i$ in pancreatic islets depend on periodic entry of Ca^{2+}. However, the fast ones somehow depend also on mobilization of Ca^{2+} from intracellular stores[35].

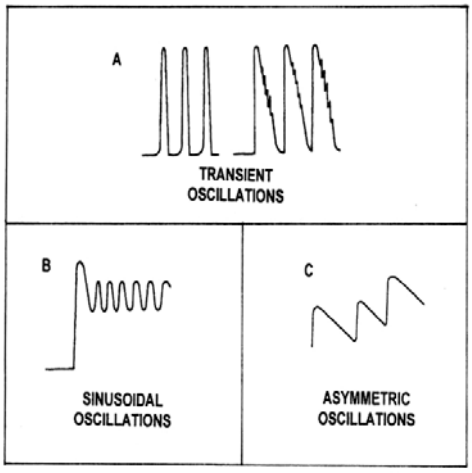

Figure 2. *Different patterns of calcium oscillations. (A) Transient oscillations or spikes. Symmetrical transients (left) and transients with slower recovery phase (right). (B) Sinusoidal oscillations. (C) Asymmetric oscillations.*

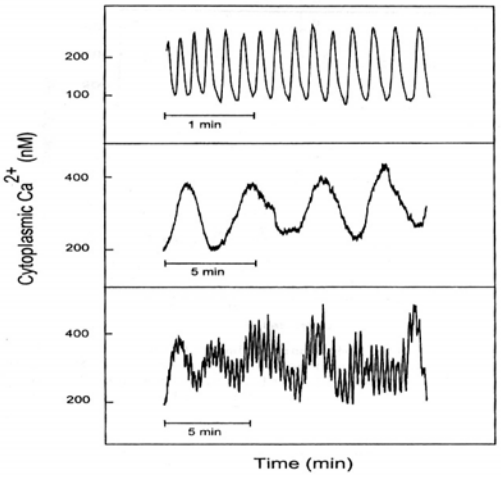

Figure 3. *Traces showing different oscillatory patterns of (Ca^{2+}), in pancreatic islets. Stimulation of pancreatic islets with 11 mM glucose can produce fast (upper trace), slow (middle trace) or mixed (lower trace) patterns of (Ca^{2}), oscillations.*

Individual pancreatic β-cells exhibit different types of $[Ca^{2+}]_i$ oscillations[36]. *Type a or slow $[Ca^{2+}]_i$* oscillations are sinusoidal which usually appear at glucose concentration of 7-20 mM with different thresholds for the individual cells (Fig 4). These oscillations have typical frequencies of 0.05-0.5/min, starting from the basal level with amplitudes of 300-500 nM[37,38]. The initial response of individual β-cells to glucose is characterized by a transient initial lowering of $[Ca^{2+}]_i$, due to sequestration of Ca^{2+} into intracellular compartments[39-41], followed by a sharp rise of Ca^{2+} (Fig 5). The slow $[Ca^{2+}]_i$ oscillations are strictly dependent on extracellular Ca^{2+} and disappear in the presence of the voltage-dependent Ca^{2+} channels (VDCC) blockers[42]. The slow $[Ca^{2+}]_i$ oscillatory response is elicited not only by glucose as well leucine[43], isoleucine[44], ±-ketoisocaproate[45] and tolbutamide[46]. Various mechanisms have been proposed to explain the generation of this $[Ca^{2+}]_i$ fluctuations at single cell level including oscillations in glucose metabolism[47-51], fluctuations of inositol 1,4,5 trisphosphate production[52], oscillations of Ca^{2+} in the endoplasmic reticulum[53], periodic Ca^{2+} influx during bursting electrical activity[42,54] and cyclical periods of Ca^{2+} induced Ca^{2+} release[55]. But still it is an ongoing problem and no definitive conclusion has been reached so far.

Type b or fast $[Ca^{2+}]_i$ oscillations usually appear as superimposed on the slow oscillations or on a sustained level of elevated $[Ca^{2+}]_i$. They occur at a frequency of 2-8/min with a duration of approximately 10s and amplitudes of 70-250 nM (Fig 4). The proportion of β-cells responding to glucose with the type b oscillations is higher in cells analyzed shortly after isolation than in those kept in culture for 1-2 days[36]. A critical cAMP concentration may be required for the appearance of these type b oscillations[38,56].

Type c oscillations are irregular $[Ca^{2+}]_i$ transients with a duration of < 10s and sometimes observed during glucose stimulation alone but becomes more frequent when cells are exposed to high concentrations of glucagon or when the adenylate cyclase activity has been stimulated with forskolin. The $[Ca^{2+}]_i$ transients are independent of voltage dependent Ca^{2+} influx and disappear after addition of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) blocker, thapsigargin, indicating that the mobilization of Ca^{2+} from intracellular stores is responsible for their generation[57].

Type d oscillations are seen when β-cells, stimulated with glucose, are exposed to extracellular ATP or charbachol, which results in a series of $[Ca^{2+}]_i$ transients of decreasing amplitude and increasing duration (Fig 4). It reflects mobilization of Ca^{2+} from intracellular stores mediated by activation of inositol 1,4,5-trisphosphate receptors and/or ryanodine receptors. These transients exhibit characteristic patterns, making it possible to identify individual β-cells by their $[Ca^{2+}]_i$ 'fingerprints'[58].

Significance of oscillatory $[Ca^{2+}]_i$ signals

In pancreatic β-cells, Ca^{2+} is the *'naturally selected'* second messenger[59,60] that decodes signals from different stimuli and relays messages to the biochemical machinery within the cell. But why does $[Ca^{2+}]_i$ oscillate? Does it really need to oscillate for proper signal transduction in pancreatic β-cells? Although results of some experiments intriguingly suggest that $[Ca^{2+}]_i$ oscillations are no more effective in insulin release than a sustained signal in pancreatic β-cell[61-63], certainly $[Ca^{2+}]_i$ oscillations confer positive functional advantages. In the following sections we will focus on the functional significance of oscillatory $[Ca^{2+}]_I$ signals in the pancreatic β-cells.

Regulation of insulin secretion. Oscillations in $[Ca^{2+}]_i$ permit a finer control of secretion than a sustained elevation of $[Ca^{2+}]_i$ as prolonged stimulation of cellular processes can cause desensitization[64,65]. It is anticipated that the various steps involved in exocytosis are also sensitive to distinct aspects of $[Ca^{2+}]_i$ signals, eg, kinetics, multiple spikes, amplitude, and localization[66]. In pancreatic β-cells, KCl alone induces a sustained $[Ca^{2+}]_i$ increase but causes transient insulin secretion[67]. In contrast, when glucose concentration is raised from basal to stimulatory, it induces $[Ca^{2+}]_i$ oscillations and a continuous oscillatory insulin release from intact islets and individual β-cells[16,17,68].

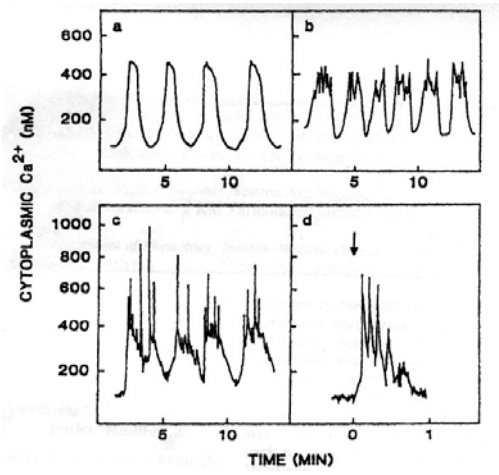

Figure 4. *(Ca2+)1 oscillations in individual pancreatic B-cell. Differentt types have been reffered to as a-d. (Reproduced with permission from Elsevier Science Publishers. Hellman B. Gylfe E, Grapengiesser E, Lund PE, Berts A. Cytoplasmic Ca2+ oscillations in pancreatic B-cells. Biochem Biophys Acta 1992, 1113:295-305).*

Regulation of gene expression. Kinetics of oscillatory $[Ca^{2+}]_i$ signaling exhibit significant variation in patterns and mechanisms of recognition[69,70]. It has been suggested that calcium spiking behavior permits information to be encoded and detected over a much broader range of signaling levels than with sustained $[Ca^{2+}]_i$ increases[31]. Thus, oscillations of $[Ca^{2+}]_i$ might regulate cellular processes other than insulin secretion, eg, gene expression. Glucose increases insulin gene expression both at transcriptional and translational levels[71]. The glucose induction of insulin transcription was inhibited by VDCC blocker suggesting that the stimulatory effect observed is mediated by Ca^{2+}. Thus, glucose-induced oscillatory $[Ca^{2+}]_i$ signal acts as a common pathway for effectively stimulating both the synthesis and release of insulin. Experimental results have clearly shown that $[Ca^{2+}]_i$ oscillations and their frequencies are specific for gene activation, both in terms of efficiency and selectivity[72]. Li *et a1.*[73] and Dolmetsch *et a1.*[74] provided ample evidence for oscillatory $[Ca^{2+}]_i$ signals to be more effective to activate Ca^{2+}-dependent transcription factors than a single, prolonged increase.

Regulation of metabolism. Oscillations in $[Ca^{2+}]_i$ are also integrated at the level of the metabolic response. In hepatocytes, vasopressin-induced $[Ca^{2+}]_i$ oscillation with the frequency of 0.5/min induces mitochondrial redox responses that were effectively maintained close to the peak response[75]. By contrast, sustained $[Ca^{2+}]_i$ increases induced by maximal vasopressin doses were associated with only a single transient increase of NADH. Thus, a Ca^{2+} response system, in this case mitochondrial energy metabolism, can be tuned to the oscillatory change of $[Ca^{2+}]_i$ signaling and actually tuned out by sustained $[Ca^{2+}]_i$ signal[31]. In pancreatic β-cells, KCl induced a sustained $[Ca^{2+}]_i$ increase and transient $[Ca^{2+}]_m$ increase, while glucose induced $[Ca^{2+}]_i$ oscillations and an oscillatory $[Ca^{2+}]_m$ increase, suggesting that repetitive transients of $[Ca^{2+}]_m$ associated with $[Ca^{2+}]_i$ oscillations are necessary for continuous stimulation of mitochondrial metabolism and thereby continuous secretion of insulin[76,77]. It is also possible that oscillations prevent mitochondrial calcium overload and damage in chronically stimulated cells[60,78]. The Na^+-dependent carriers, which discharge Ca^{2+} from mitochondrial matrix, are inhibited by increasing the extra-mitochondrial Ca^{2+} concentration within the physiological range[79]. Thus, the sustained increase in $[Ca^{2+}]_i$ induced by KCl may attenuate the movement of Ca^{2+} from the mitochondrial matrix and consequently prolong the time course of the $[Ca^{2+}]_m$ decline[77].

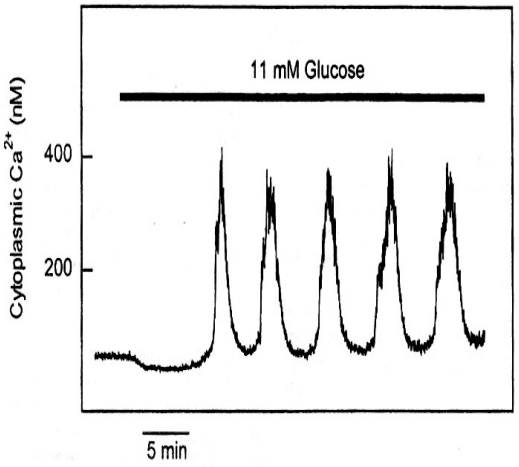

Figure 5. *Effect of raising glucose concentration from 3 to 11 mM on (Ca^{2+}), of a single pancreatic β-cell. The horizontal bar indicates the period with the higher glucose concentration.*

Regulation of apoptosis. Long-lasting sustained elevations of $[Ca^{2+}]_i$ activates Ca^{2+}-dependent degradative enzymes, e.g., protein kinases, endonucleases, proteases, and phospholipases,[80,81] whose prolonged activation can result in extensive catabolism of cellular constituents and lethal injury. Oscillatory $[Ca^{2+}]_i$ signals prevent these potentially damaging effects of Ca^{2+}-dependent enzymes. McCormack *et a1*.[82] have shown that induction of thymocytes apoptosis by glucocorticoid hormones are dependent on an early, receptor-mediated, sustained increase in $[Ca^{2+}]_i$ concentrations. In hepatoma 1c1c7 cells low ATP concentrations (1-10 μM) stimulate a transient, receptor mediated Ca^{2+} response whereas high concentrations of ATP (mM) can also cause a sustained increase in the $[Ca^{2+}]_i$[80,83]. It appeared that treatment of the hepatoma cells with high levels of ATP could activate Ca^{2+}-dependent, enzymatic DNA cleavage and contribute to cell killing[80], Uncontrolled steady-state rise of $[Ca^{2+}]_i$ can also induce Ca^{2+}-dependent activation of several genes that characterize many types of acute lethal injury. These genes can be induced within 15 min or less, as in the case of c-fos[66] and c-jun, to trigger additional events, such as the ced 3/ICE protease family members, which appear to be close to the final phase of cell death[81]. In pancreatic β-cells, increased cell death has been reported at elevated glucose concentrations when intracellular Ca^{2+} is not oscillating[84]. Recently, Iwakura *et al*.[85] have shown that sustained enhancement of Ca^{2+} influx induced by continuous exposure to glibenclamide caused apoptotic cell death in rat insulinoma cell line (RINm5F cells). These results are consistent with the concept that oscillatory $[Ca^{2+}]_i$ signals in pancreatic β-cells prevent cellular damage. In pancreatic β-cells, increased cell death has been observed at elevated glucose concentrations when $[Ca^{2+}]_i$ is not exhibiting oscillations[84]. Recently, it has been shown that sustained enhancement of Ca^{2+} influx induced by continuous exposure to glibenclamide caused apoptotic cell death in rat insulinoma cell line, RINm5F cells[85]. These results are consistent with the idea that oscillatory Ca^{2+} signals in pancreatic β-cells prevent cellular damage.

Role in energy homeostasis. Oscillations are less costly for maintenance of cell homeostasis[65] considering that elevation of $[Ca^{2+}]_i$ activates energy-consuming processes for extrusion of the ion[33,60], while shortening of the time with a raised $[Ca^{2+}]_i$ will conserve energy[64]. As an example of the efficiency of oscillatory system in conserving energy, the calculated results suggest that the dissipation of free energy is reduced by 5-10% in oscillatory glycolysis[86].

Synchronization of cellular processes. Oscillations can be integrated in single cell or tissue level. $[Ca^{2+}]_i$ oscillations that result in asynchronous pulsatile responses in individual cells or groups of cells will be integrated into a smooth and continuous response in the total output of the tissue[31]. For example, response of single β-cell to glucose is heterogeneous – some cells display $[Ca^{2+}]_i$ oscillations, others show a sustained rise, whereas a small proportion appear unresponsive[87-89]. However, a consistent oscillatory $[Ca^{2+}]_i$ response is observed in clusters of 5-8 mouse pancreatic β-cells stimulated with 15 mM Glucose[37,65]. The heterogeneous response of isolated cells to glucose is masked when they are organized in the whole islets as a result of a very efficient coupling mechanism, which leads to synchronous glucose-induced oscillations of $[Ca^{2+}]_i$[34,35]. Analysis of $[Ca^{2+}]_i$ oscillations in different regions of a single glucose-stimulated islet also showed that they may be of variable amplitude but are always synchronous. Simultaneous measurements of $[Ca^{2+}]_i$ and insulin secretion in single mouse islets show that each $[Ca^{2+}]_i$ oscillation is accompanied by an oscillation of secretion[90]. This synchrony persists when the frequency of $[Ca^{2+}]_i$ oscillations is modified by a change in glucose concentration[91]. Thus, pulsatile insulin secretion, triggered by highly integrated $[Ca^{2+}]_i$

oscillations, from each islet is ultimately responsible for integrated pulsatile secretion by the whole pancreas and the generation of plasma insulin oscillations which are important for optimal action of the hormone[65].

Temporal control of cellular activity. In the biological targets the $[Ca^{2+}]_i$ signal responds to the frequency of $[Ca^{2+}]_i$ spikes rather than to the amplitude of $[Ca^{2+}]_i$ change. This has given rise to the concept of frequency-modulated $[Ca^{2+}]_i$ signaling[78,86]. However, it has also been reported that Ca^{2+} oscillations can be modulated both in frequency and amplitude[92-94]. Frequency encoding results in enhanced precision of control; it is particularly resistant to distortion by background noise[86]. Frequency dependent control systems also succeed in environments where amplitude dependent controls fail. If the $[Ca^{2+}]_i$ is to be used as an amplitude-dependent signal, in which case its level would have to be maintained at a higher concentration for prolonged periods, it would cause calcium toxicity[95]. In pancreatic β-cell the frequencies of the $[Ca^{2+}]_i$ oscillations vary as a function of agonist concentration[58]. For example, 25 μM carbamylcholine produced transients approximately every 15s, while at 200 μM transients occurred at ~10s intervals. Furthermore, Gilon *et a1*.[91] explicitly demonstrated that when the concentration of glucose is raised, the peak of $[Ca^{2+}]_i$ oscillations did not change significantly, but the frequency of $[Ca^{2+}]_i$ oscillations clearly increased. This may result from glucose capacity to increase the efficacy with which frequency-encoded Ca^{2+} signals activates the exocytotic process and increases insulin release.

Spatial control of cellular activity. Oscillations of $[Ca^{2+}]_i$ show spatial order, which has indeed functional advantage for periodicity in biological control[86]. A distinctive attribute of biological system is to do the right thing at the right time in the right place. In heart, the temporal entrainment sequence (SA-node to Purkinje fibers to the ventricles) ensures the correct spatial sequence. Failures in this entrainment process can result in some of the clinically observed cardiac arrhythmias. Thus, Durham[96] has speculated, "it is more plausible that every region (of the organism) can potentially oscillate at some frequency. Those parts with the highest inherent frequency will establish a phase lead and drive other regions. Waves will, therefore, move along membranes down gradients of inherent frequency". Another crucial biological process in which precise spatial control is of central importance is the development of multicellular organisms from a single fertilized egg cell[97]. The events are particularly ordered in time and in space. However, the spatial organization of Ca^{2+} oscillations in pancreatic β-cell is not yet convincing. With digital imaging of the Ca^{2+}-dependent fluorescence signal it has been demonstrated that $[Ca^{2+}]_i$ varies substantially within the cell[98a]. $[Ca^{2+}]_i$ first increases in a rim close to the plasma membrane. As the duration of the depolarization is increased, the $[Ca^{2+}]_i$-transient extends progressively deeper into the cell. However, at least during the first 350ms, $[Ca^{2+}]_i$ remains highest in the vicinity of the plasma membrane. It is of interest, that the increase in $[Ca^{2+}]_i$ is particularly rapid and pronounced in the upper right part of the cell whereas other parts of the cell remain relatively unaffected[98a]. The observation, that the $[Ca^{2+}]_i$-increase is more pronounced in certain parts of the cell may indicate an uneven distribution of Ca^{2+}-channels in the β-cell membrane. It is tempting to speculate that regions of the plasma membrane with a high Ca^{2+}-channel density correspond to 'hot spots' of exocytosis. Thus the spatial organization of oscillatory Ca^{2+} signal could lead to a provision of high Ca^{2+} concentration needed at the exocytotic sites while lower $[Ca^{2+}]_i$ may be sufficient to activate other essential processes, such as the movement of insulin granules from storage to release sites[98b].

Discrimination between signal and noise. Calcium signal is digitized in the form of oscillations[95,99] and a digitally encoded signal with the all or none property has favorable 'signal-to-noise' ratios[70,100]. By relying on large, discrete digital events, e.g. calcium oscillations, cells can readily distinguish an "intentional" calcium signal from potentially spurious wanderings of the steady-state, cytoplasmic calcium concentration. Indeed, in the brain, bursts of electrical activity are more readily perceived as signals than are action potentials that arrive singly.

Conclusion

The periodic changes of $[Ca^{2+}]_i$ is of great physiological and pathological importance since $[Ca^{2+}]_i$ oscillates in synchrony with electrical activity and oscillations in $[Ca^{2+}]_i$ correspond to pulsatile insulin release[13-17]. It has also been proposed that oscillatory insulin secretion is important in terms of insulin action on target organs, perhaps because of reducing down-regulation of receptors and thereby

enhancing hormone action[101]. Several studies have demonstrated a greater hypoglycemic effect of insulin infused in a pulsatile manner and an enhancement of glucose disposal[102-104]. The greater potency of pulsatile insulin administration has also been demonstrated in perifused liver and in humans with IDDM[105,106]. Whether the loss of oscillations during the development of type 2 diabetes contributes to insulin resistance has not yet been established. But it is a widely acknowledged fact that the regular pattern of oscillatory insulin release is altered or lost in both developing type 1 and type 2 diabetes and disappearance of $[Ca^{2+}]_i$ oscillations is a sensitive indicator of β-cell damage[107-112]. Thus, a detailed study of the mechanisms which underlay the presence of regular $[Ca^{2+}]_i$ oscillations may help to find out the molecular and physiological defects involved in the pathogenesis of diabetes.

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