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Signaling Underlying Pulsatile Insulin Secretion

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Regular oscillations of the circulating insulin concentrations were discovered in the monkey [28] and subsequently found in normal human subjects [50]. The characteristic insulin pattern is deteriorated in patients with type 2 diabetes [49] as well as in their close relatives [61]. Studies in non-diabetic subjects with suppressed endogenous insulin secretion and diabetic patients have indicated that less insulin is required to maintain normoglycaemia if the hormone is infused in a pulsatile manner compared to a constant rate [12, 57, 59, 63, 64]. This difference is probably explained by higher expression of insulin receptors, when insulin is delivered in pulses [27]. It is easy to envision a scenario for the development of type 2 diabetes in which deteriorated oscillations leads to insulin resistance with a compensating hypersecretion of the hormone. In susceptible individuals the increased insulin demand may eventually exhaust the pancreatic β-cells with resulting development of overt diabetes.

What is then the origin of the regular insulin oscillations? One possibility is that they result from a negative feedback loop between the liver and the pancreatic β-cell [50]. However, later studies have indicated that the oscillations occur independent of changes in plasma glucose, reflecting a pacemaker function in the pancreas [49, 58]. This conclusion is consistent with measurements of secretion from the isolated perfused dog pancreas [76]. Another fundamental aspect is the frequency of the insulin oscillations. Whereas the early studies on humans and monkeys indicated a periodicity of 10–15 min [28, 50], measurements in the dog showed 4–8 min. The latter estimate is similar to the periodicity observed from the perfused dog pancreas [75] and that based on blood sampling from the portal vein of dogs [66]. The portal insulin oscillations are very prominent, indicating that pulsatile secretion accounts for 70% of total secretion (Fig. 1). In the periphery the oscillations are less pronounced due to recirculation and the fact that the liver extracts almost 50% of the portal hormone [13]. The report of a lower frequency of the insulin oscillations in the peripheral blood may simply reflect difficulties in detecting insulin peaks due to a low signal-to-noise ratio [66]. Indeed, measurements on portal blood from patients with liver cirrhosis indicated a periodicity of 4.1–6.5 min [77]. Mechanisms underlying pulsatile insulin release will now be discussed at different levels of integration, starting with isolated pancreatic β-cells.

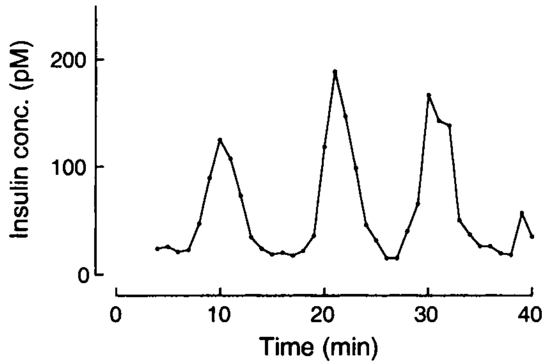

Figure 1. Concentration profile of portal vein insulin in a dog. Reproduced from Pørksen et al. [66] with permission.

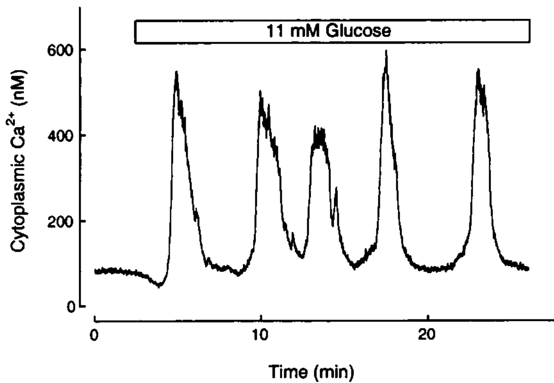

Figure 2. Effect of raising the glucose concentration from 3 to 11 mM on $[Ca^{2+}]i$ of a single mouse β-cell. $[Ca^{2+}]i$ was measured with the indicator fura-2.

ISOLATED PANCREATIC β-CELLS

Slow membrane oscillations of cytoplasmic Ca^{2+}

The cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) is probably the most important determinant of insulin secretion [41, 83]. Measuring $[Ca^{2+}]_i$ in individual mouse β-cells stimulated with glucose we discovered in 1988, that Ca^{2+} signalling is oscillatory with a periodicity of 2–6 min (Fig. 2) [30]. These $[Ca^{2+}]_i$ oscillations can be classified as membrane oscillations, being dependent on the presence of extracellular Ca^{2+} and inhibited by blockers of the voltage-dependent Ca^{2+} channels [32]. The initial rise of $[Ca^{2+}]i$ in response to glucose is preceded by a lowering, observed both in individual β-cells [14, 29, 31] and cell suspensions [36, 37]. In parallel studies of $[Ca^{2+}]_i$ in suspension of β-cells and insulin secretion from cells trapped in a Biogel column it became apparent that the initial lowering and subsequent increase of $[Ca^{2+}]_i$ are associated with inhibition and stimulation of secretion, respectively [54]. The early lowering of $[Ca^{2+}]_i$ is prevented by inhibitors of the sarco (endo)plasmic reticulum Ca^{2+} ATPase (SERCA), indicating that it is essentially due to sequestration of Ca^{2+} in the endoplasmic reticulum (ER) [14]. This conclusion was amply supported by our direct measurements of the effect of glucose on the ER Ca^{2+} content [78]. As discussed below, the ER may participate in the regulation of

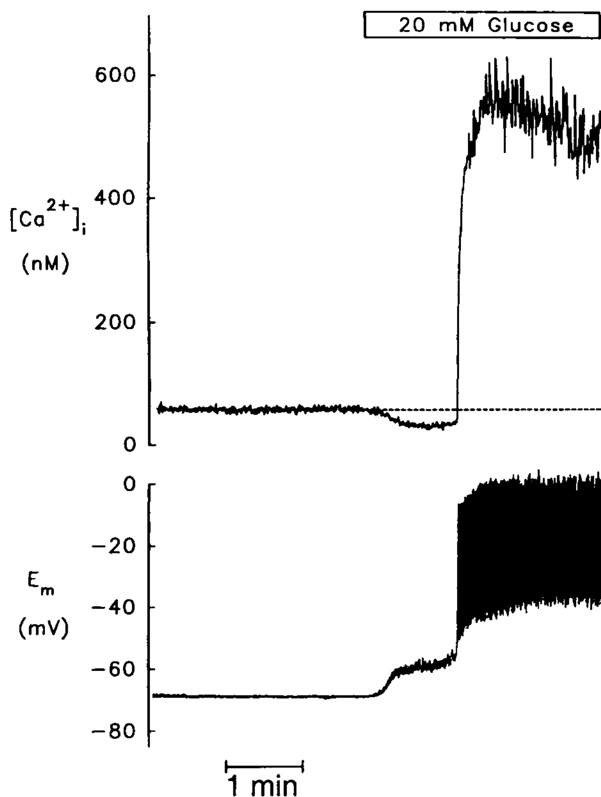

Figure 3. Changes in membrane potential (Em) and $[Ca^{2+}]_i$ in a single mouse β-cell elicited by raising glucose from 3 to 20 mM . $[Ca^{2+}]_i$ was measured with the indicator fura-2 and membrane potential with the perforated-patch whole-cell configuration of the patch clamp technique. Reproduced from Chow et al. [14] with permission.

insulin secretion also by mechanisms other than removal of Ca^{2+} from the cytoplasm.

The SERCA activation leading to initial lowering of $[Ca^{2+}]_i$ in glucose-stimulated β-cells results from an increased ATP formation during the metabolism of the sugar [78]. This lowering coincides with depolarisation [14], which is due to the closure of ATP-dependent K^+ (K_{ATP}) channels [3, 4]. When the membrane is depolarised to the threshold level for opening the voltage-dependent channels, there is a sudden increase in $[Ca^{2+}]_i$ paralleled by action potentials (Fig. 3). The subsequent slow oscillations of $[Ca^{2+}]_i$ do not require functional SERCA pumps [51]. The electrophysiological events resulting in the oscillations have been difficult to demonstrate. So far there is only one study reporting parallel measurements of slow $[Ca^{2+}]_i$ oscillations and electrical activity [17]. Fig. 4 shows that each peak of $[Ca^{2+}]_i$ is accompanied by a protracted burst of action currents. During an oscillation the magnitude of the Ca^{2+} elevation is proportional to the frequency of the action currents. Since the K_{ATP} channels are important determinants of the membrane potential, it is likely that oscillations in metabolism are causing those of $[Ca^{2+}]_i$. Using the activity of the K_{ATP} channels as indicator of the cytoplasmic concentration of ATP we found that this metabolite oscillates with a frequency similar to that of the slow $[Ca^{2+}]_i$ oscilla-

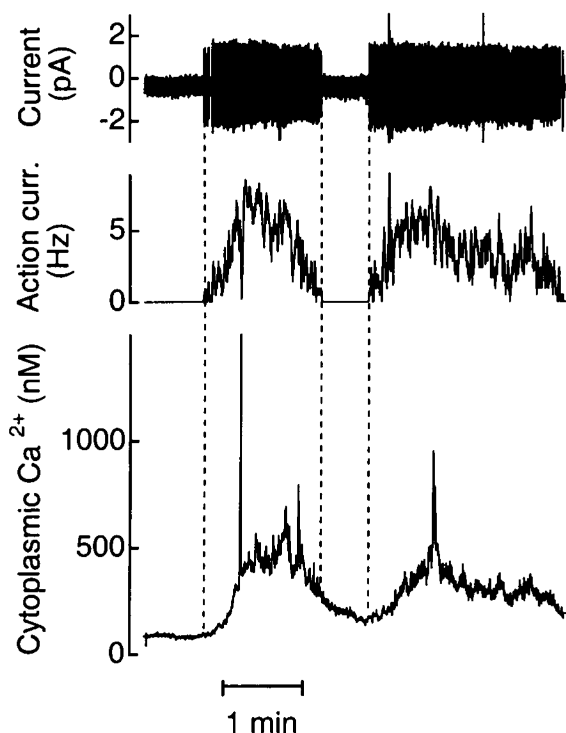

Figure 4. Slow oscillations of $[Ca^{2+}]_i$ coincide with bursts of action currents in a single mouse β-cell exposed to 11 mM glucose. $[Ca^{2+}]_i$ was measured with the indicator fura-2 and action currents recorded with the cell-attached configuration of the patch clamp technique. The middle trace shows the frequency of the action currents. Reproduced from Dryselius et al. [17] with permission.

tions in the presence of sub-stimulatory glucose concentrations [18]. These data indicate that metabolic oscillations occur also in the resting β-cell.

Intracellular Ca^{2+} transients

Our studies of 45Ca fluxes have shown that glucose is a potent stimulus of Ca^{2+} sequestration in an intracellular pool mobilised by inositol 1,4,5-trisphosphate (IP3) [40, 42, 43]. Later measurements of $[Ca^{2+}]_i$ [37, 38] and direct estimates of free Ca^{2+} in the ER [78] indicated that sequestration is more sensitive to glucose than secretion, being half-maximally stimulated at the threshold concentration for glucose-stimulated insulin release (5–6 mM). IP3-mediated mobilisation of Ca^{2+} from the ER results in transients of $[Ca^{2+}]_i$, which are sufficiently pronounced to activate a hyperpolarising K^+ current [1, 55]. Similar transients, occurring spontaneously in glucose-stimulated β-cells, cause temporary arrest of the electrical activity generating the slow $[Ca^{2+}]_i$ oscillations [17]. The transients, which are prevented by SERCA inhibition, are promoted not only by glucose but also by glucagon or other agents raising cAMP as well as by depolarisation [51]. Whereas glucose acts by filling the ER with Ca^{2+}, depolarisation stimulates the IP3 formation and cAMP probably sensitises the IP3 receptors. Due to the absence of glucagon-producing α-cells the transients are less frequent in isolated β-cells than in those located in pancreatic islets. We have proposed that the hyperpolarising action of the transients changes

the glucose-induced bursts of electrical activity underlying the slow $[Ca^{2+}]_i$ oscillations into the much faster pattern characterising β-cells located in islets (see below) [53]. Interestingly, the $[Ca^{2+}]_i$ transients show synchronisation between β-cells lacking physical contact, indicating that a diffusible factor is involved in their generation [34]. Such a factor may participate in the co-ordination of β-cell activity, which is a prerequisite for pulsatile insulin release from the pancreas (see below).

Insulin secretion

After trapping dissociated β-cells among Biogel polyacrylamide beads, it became possible to study the kinetics of insulin secretion in a perfusion system [54]. However, pulsatile secretion cannot be studied, since the cells are not well co-ordinated when lacking physical contact. The sensitivity of available immunological techniques for measuring insulin is not sufficient to measure the kinetics of secretion from single β-cells. An indirect approach is based on the observation that serotonin accumulates in the secretory granules and is co-secreted with insulin [35]. Using carbon microelectrodes for amperometric detection of serotonin, even individual secretory events can be detected with millisecond time resolution from the part of the β-cell in close apposition to the electrode [73]. However, so far it has not been possible to measure the total secretion from an individual β-cell with this approach, since serotonin released on the side opposite to the electrode is unlikely to be detected. Insulin is stored in crystalline form as a 2-zink-insulin hexamer, and free Zn^{2+} is co-secreted with insulin [20]. Using the fluorescent indicator zinquine, such release has been monitored with confocal microscopy from single β-cells [69]. The time resolution of the approach is lower than for electrochemical detection of serotonin but sufficient for detecting pulsatile release. In this case only the secretory events from a part of the cell surface is detected, limited to the optical section under study. Improved sensitivity of the immunological methods for measuring insulin and/or procedures to capture all of the released serotonin or Zn^{2+} can be expected to allow studies of pulsatile secretion from single β-cells in the near future.

CELL CLUSTERS AND PANCREATIC ISLETS

Slow membrane oscillations of $[Ca^{2+}]_i$

Individual β-cells respond to glucose in a heterogeneous manner, the slow membrane oscillations of $[Ca^{2+}]_i$ being triggered at concentrations of the sugar characteristic for each cell. Moreover, there are considerable differences between cells with regard to amplitude and frequency of the oscillations [39], existing frequencies remaining unaffected when raising the glucose concentration [33]. When a cluster of β-cells is exposed to an intermediary glucose concentration, some of the cells go from the resting into the oscillatory state. Due to gap junctions the $[Ca^{2+}]_i$ oscillations become almost perfectly synchronised. Further increase of the glucose concentration recruits more β-cells into the active phase and their oscillations will be entrained with those of the previously active cells. Fig. 5 illustrates the principle of recruitment with increasing numbers of β-cells being active with elevation of the

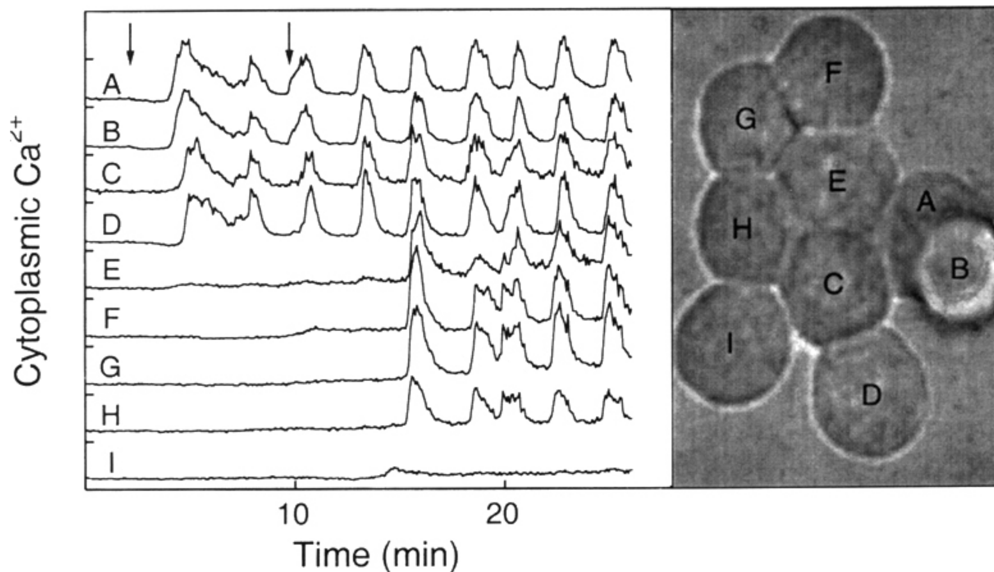

Figure 5. Effects of raising the glucose concentration from 3 to 11 (first arrow) and 20 mM (second arrow) on $[Ca^{2+}]_i$ in mouse β-cells within a small cluster. $[Ca^{2+}]_i$ was measured with the indicator fura-2. Traces A-I correspond to the cells with the same labels in the drawing in the right panel. Each mark on the ordinate indicates the zero nM level for $[Ca^{2+}]_i$ for the trace above and/or the 200 nM level for the trace below. Reproduced from Gylfe et al. [39] with permission.

glucose concentration. The rapid entrainment of the oscillatory activity is probably due to gap junctional coupling increasing in parallel with β-cell activity [2].

Slow oscillations of $[Ca^{2+}]_i$ are seen also when measuring the entire Ca^{2+} signal from glucose-stimulated islets of Langerhans [7, 8, 24, 53, 56], indicating that cell co-ordination is operating efficiently among thousands of cells. The mechanisms underlying the slow $[Ca^{2+}]_i$ oscillations in the islets can therefore be expected to be the same as in single β-cells. Measurements of the native NAD(P)H fluorescence from islets have provided evidence for oscillations in metabolism in one study [67] but not in others [22, 62]. Recording the oxygen tension in individual pancreatic islets it was possible to obtain additional evidence for primary oscillations in metabolism with a frequency similar to that of $[Ca^{2+}]_i$ [46, 47]. Both the rate of metabolism [68] and consumption of ATP [16] are affected by $[Ca^{2+}]_i$. It is therefore important that the slow oscillations in oxygen consumption are still observed in glucose-stimulated islets when maintaining resting levels of $[Ca^{2+}]_i$ by blocking the voltage-dependent channels [46, 47].

The electrophysiological correlate to the slow $[Ca^{2+}]_i$ oscillations in islets have been difficult to capture. Using intracellular electrodes there are no observations of slow bursts of action potentials like those in single β-cells (see above). However, the electrical activity (see below) exhibits rhythmic fluctuations in time with a frequency characteristic for the slow oscillations of $[Ca^{2+}]_i$ [15, 44].

Fast membrane oscillations of $[Ca^{2+}]_i$

The Ca^{2+} signalling in islets is more complex than in individual β-cells. Apart from the slow oscillations of $[Ca^{2+}]_i$ there are also those with a tenfold higher frequency [7, 8, 24, 53, 72, 80] occurring in parallel with a bursting electrical activity [72]. Both types of oscillations are due to bursts of action potentials, reflecting openings of voltage-dependent Ca^{2+} channels. Apart from these similarities the slow and fast oscillations of $[Ca^{2+}]_i$ exhibit several differences. Whereas the slow oscillations are not affected by glucose in oscillating islets [8], increasing concentrations of the sugar enhances the duty cycle of the fast ones with longer periods of elevated and shorter periods of low $[Ca^{2+}]_i$ [72] until there is eventually a sustained elevation [7]. As shown in Fig. 6 the fast oscillations are sometimes superimposed on the slow ones [7, 8, 80]. We have recently demonstrated that this phenomenon reflects separate cell populations exhibiting either the fast or slow response [53]. Like the slow oscillations of $[Ca^{2+}]_i$ in single cells, those in islets remain essentially unaffected by SERCA inhibition. In this respect they are different from the fast ones, which disappear completely [21, 84, 85]. Indeed, SERCA inhibition immediately transforms the fast islet oscillations into slow ones [53]. These data indicate that the fast oscillations depend not only on Ca^{2+} influx through voltage-dependent channels but also on Ca^{2+} sequestration and/or release of intracellular Ca^{2+}.

Glucagon, which promotes intracellular $[Ca^{2+}]_i$ transients in individual β-cells by raising cAMP [51], transforms the slow islet oscillations into fast ones [53]. The protein kinase A inhibitor Rp-cyclic adenosine 3', 5'-monophosphorothioate (Rp-cAMPS) has the opposite effect (Fig. 6). We have therefore proposed that when occurring close in time in a sufficient number of tightly coupled β-cells the $[Ca^{2+}]_i$ transients trigger a dominating hyperpolarising K^+ current. This hyperpolarisation chops the slow oscillations into shorter events, thereby explaining the occurrence of the fast $[Ca^{2+}]_i$ oscillations [51, 53].

Depletion of the ER Ca^{2+} rapidly activates a voltage-independent entry of Ca^{2+} into the β-cell [38] by a store-operated (capacitative) mechanism [52]. There have been reports that a nonselective cation current, principally carried by Na^+, is activated by depletion of the Ca^{2+} stores [84, 85], but this idea is not generally accepted [60]. In some models for the generation of the fast $[Ca^{2+}]_i$ oscillations, the store-operated pathway helps to depolarise the β-cells and activate the potential-dependent influx of Ca^{2+} [21, 85]. The filling of the ER during the resulting elevation of $[Ca^{2+}]_i$ terminates the store-operated current and consequently blocks the voltage-dependent entry of the ion. There are different opinions about the reactivation of the store-operated current required for a new cycle. It is either due to active depletion [85] or to passive leakage of Ca^{2+} from the ER [21]. We have recently found that glucose stimulates ER sequestration of Ca^{2+} in the β-cell independent of its depolarising action [78]. Considering the very high Ca^{2+} affinity of this sequestration, it is unlikely that there is passive leakage of Ca^{2+} from the ER during glucose stimulation as proposed by Gilon et al. [21]. However, we do not exclude that the IP3-mediated depletion of ER Ca^{2+} related to the spontaneously occurring

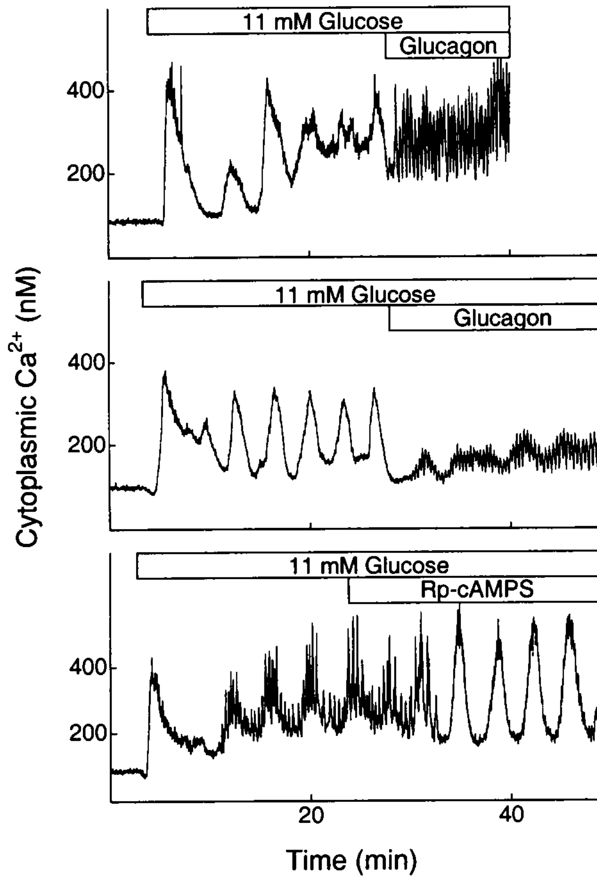

Figure 6. Effects of glucagon and Rp-cAMPS on the oscillatory $[Ca^{2+}]_i$ signalling in individual mouse pancreatic islets exposed to 11 mM glucose. $[Ca^{2+}]_i$ was measured with the indicator fura-2. Reproduced from Liu et al. [53] with permission.

$[Ca^{2+}]_i$ transients in glucose-stimulated pancreatic islets may be important by activating the store-operated pathway.

Measurements of oxygen tension and glucose in islets have indicated that also the fast $[Ca^{2+}]_i$ oscillations are paralleled by those in metabolism [47]. This observation does not necessarily imply that the fast metabolic oscillations are triggering those in $[Ca^{2+}]_i$. The fast oscillations in metabolism may well result from a stimulation of mitochondrial metabolism in response to elevation of $[Ca^{2+}]_i$ in combination with increased consumption of ATP due to the energy-requiring removal of the ion from the cytoplasm [16, 47, 68].

Insulin secretion

Whereas the sensitivity of most immunoassays is sufficient for measurements of insulin secretion in batch type incubations, it is more difficult to study the kinetics of the release process. Since β-cell co-ordination is required for pulsatile insulin secretion, such studies should be performed on single pancreatic islets. The small amounts of insulin released to the superfusion medium are often close to the de-

tection limit, particularly when a high time resolution is required. To ensure adequate resolution of the insulin rhythmicity in the radioimmunoassay, excessive concentrations of extracellular Ca^{2+} (10 mM) have been used to retard the electrophysiological events underlying the fast oscillations of $[Ca^{2+}]_i$ [23, 25]. In this way the frequency of the fast $[Ca^{2+}]_i$ oscillations approaches that of the slow ones. Using ultra-sensitive ELISA, we have been able to study secretion kinetics at physiological Ca^{2+} concentrations with a time resolution of 3 sec [9, 10]. Amperometric detection of serotonin with a carbon fibre microelectrode impaled into an islet permits real time measurements of this amine co-secreted with insulin from preloaded islets [5, 6]. This approach monitors the concentration in the extracellular space rather than total secretion and is better for detecting rapid than slow oscillations.

Combination of the above-mentioned techniques for monitoring secretion with measurements of $[Ca^{2+}]_i$ have indicated that both the slow and fast oscillations of $[Ca^{2+}]_i$ are always paralleled by pulsatile insulin secretion [5–8, 10, 23, 25]. Elevation of the glucose concentration in the 10–20 mM range failed to affect the amplitude of the $[Ca^{2+}]_i$ oscillations but increased that of the insulin pulses, reinforcing the idea that more β-cells are recruited into the secretory state [6, 8, 9, 23]. In analogy to the oscillations of $[Ca^{2+}]_i$, the duty cycle of the fast insulin pulses increases with broader peaks and narrower nadirs when the glucose concentration is raised [6, 23], whereas the slow pulsatile secretion remains unaffected [8]. Another similarity with the $[Ca^{2+}]_i$ measurements is that mixed patterns of fast and slow pulses of insulin release are sometimes observed [6, 10].

Although there is general agreement that oscillations in $[Ca^{2+}]_i$ cause pulsatile release of insulin, it is controversial whether this secretory pattern can also be generated in the absence of $[Ca^{2+}]_i$ oscillations. Using ELISA we found that basal insulin secretion, which is associated with low and stable $[Ca^{2+}]_i$ levels, is pulsatile [81, 82]. The frequency of the pulses was identical but the amplitude much lower than that obtained in parallel with the slow $[Ca^{2+}]_i$ oscillations after glucose stimulation. Moreover, stimulation of secretion under conditions leading to stable elevation of $[Ca^{2+}]_i$ was associated with pulsatile secretion with the same frequency. The fact that stimulated secretion can be pulsatile irrespective of whether $[Ca^{2+}]_i$ oscillates is illustrated in Fig. 7. To explain this phenomenon we postulated that oscillations in metabolism cause pulsatile secretion at stable $[Ca^{2+}]_i$ levels by providing the ATP required for the release process [81]. Since oscillations in ATP/ADP ratio also underlie those in $[Ca^{2+}]_i$, it was also proposed that secretion is better stimulated when both of these factors oscillate in synchrony than when one is stable. Gilon et al. have reported that pulsatile secretion disappears when $[Ca^{2+}]_i$ is stable [45] but these authors have later confirmed our suggestion that pulsatile secretion can be accounted for by oscillations either in $[Ca^{2+}]_i$ or metabolism [70]. Some of the confusion regarding the role of oscillatory metabolism for the generation of pulsatile insulin release is due to the fact that different mechanisms explain the fast and slow oscillatory patterns (see discussion about fast and slow $[Ca^{2+}]_i$ oscillations).

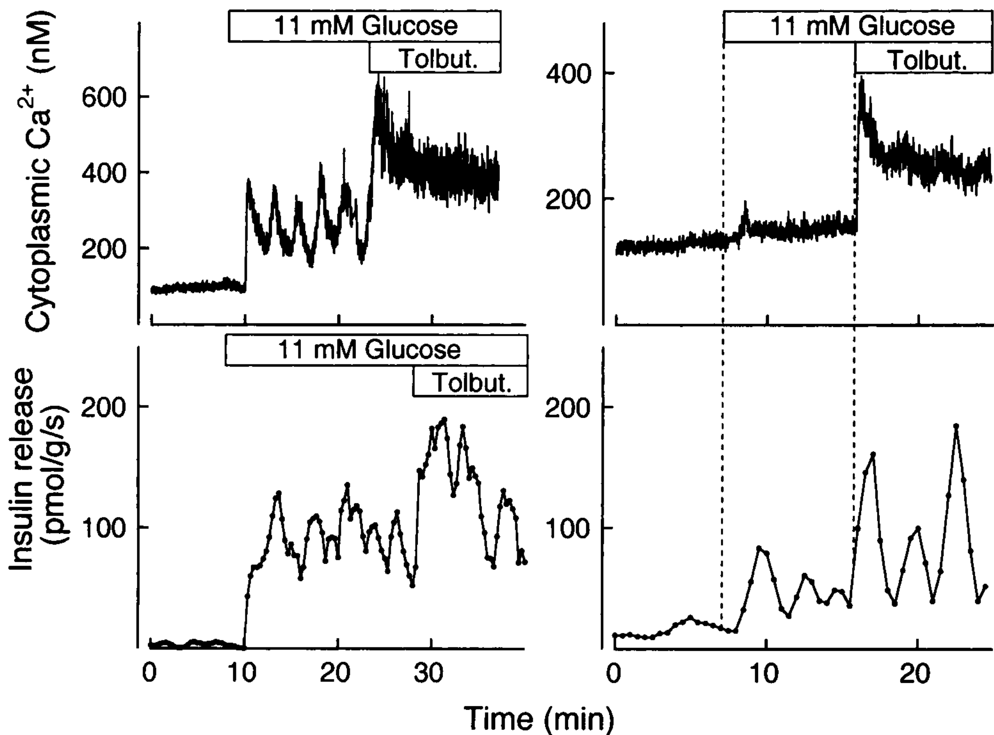

Figure 7. Effects of glucose and tolbutamide on $[Ca^{2+}]_i$ (upper panels) and insulin release (lower panels) of individual mouse pancreatic islets. The glucose concentration was raised from 3 to 11 mM and 1 mM tolbutamide added as indicated. $[Ca^{2+}]_i$ was measured with the indicator fura-2 in parallel with secretion with ELISA in the right panels and in separate experiments in the left panels. Reproduced from Westerlund et al. [81] with permission.

The pancreas

Apart from measurements of insulin secretion from the perfused pancreas there are few studies on β-cell physiology at the level of the entire pancreatic organ. Electrophysiologal in vivo studies have shown that the events underlying the glucose-induced fast $[Ca^{2+}]_i$ oscillations are identical to those in isolated islets [26, 71, 79]. Increasing concentrations of glucose consequently prolongs the phase with bursts of action potentials and shortens the intervening silent periods until there is eventually a continuous active phase. Interestingly, simultaneous *in vivo* recordings from two separate islets of Langerhans indicated that the fast oscillatory pattern is not synchronised [79]. This observation probably excludes a direct role for the fast $[Ca^{2+}]_i$ oscillations in the pulsatile release of insulin from the pancreas. Even if the fast oscillations of $[Ca^{2+}]_i$ were synchronised between islets it is not obvious that blood circulation allows the maintenance of distinct fast insulin oscillations in the portal vein. The major oscillations of portal insulin have a frequency [66] close to that characterising slow pulsatile secretion from isolated islets [8]. Like in isolated islets

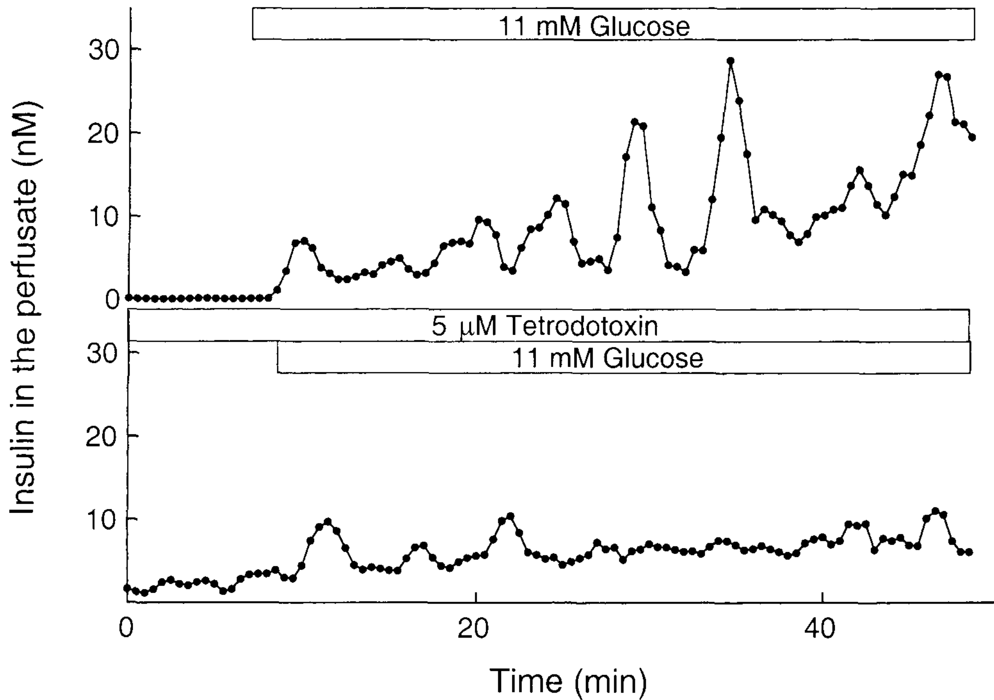

Figure 8. Effect of tetrodotoxin on glucose-induced pulsatile release of insulin from the perfused rat pancreas. The glucose concentration was raised from 3 to 11 mM as indicated in the absence (upper panel) or presence (lower panel) of 5 mM tetrodotoxin. Insulin in the perfusate was measured with ELISA.

there are slow fluctuations of the *in vivo* electrical activity [26] with a similar frequency as the slow oscillations of $[Ca^{2+}]_i$ and pulsatile insulin release from isolated islets [8].

Although the fast electrophysiological events are not synchronised between islets [79] pulsatile insulin secretion from the pancreas requires islet co-ordination. Occurring in the isolated pancreas and being temporarily disrupted by neurotransmitters such co-ordination was early attributed to intrapancreatic neurons [76]. This assumption was further corroborated by the observation that nerve blockade perturbs pulsatile insulin secretion [74]. The latter effect is illustrated in Fig. 8, showing that the glucose-induced pulsatile secretion from the perfused rat pancreas is prevented in the presence of tetrodotoxin although this neurotoxin does not affect pulsatile secretion from isolated pancreatic islets (data not shown). An elegant demonstration of the importance of neurons was obtained when studying the kinetics of insulin secretion from the perfused liver after intraportal transplantation of isolated islets [65]. The results indicated that pulsatile secretion does not correlate with revascularisation of the grafted islets but occurs in parallel with their reinnervation. A pertinent question is whether the co-ordination of the islets involves

neuronal sensing of the inherent islet rhythmicity or if nerves simply entrain the oscillatory activity by a phase-locking signal. The periodicity of such a signal is not necessarily the same as that of the slow islet rhythmicity. Nevertheless, it is of interest that intrapancreatic ganglia with a spontaneous slow electrical activity with 6-8 min intervals have been observed in the cat [48].

What is then the nature of the synchronising neural activity? It has been reported that nicotinic, muscarinic and adrenergic antagonists lack effects on pulsatile insulin secretion from the pancreas [75]. Among the non-adrenergic, non-cholinergic neurons attention should be paid to the nitrergic nerves, which are present in both the exocrine and endocrine parts of the pancreas [19]. We recently observed that fast transients of $[Ca^{2+}]_i$, due to intracellular mobilisation of the ion, are synchronised among pancreatic β-cell by diffusible factor(s) [34]. There was evidence that NO is such a Ca^{2+}-mobilising factor. Also ATP deserves attention when looking for a synchronising neural factor mobilising intracellular Ca^{2+} by acting on purinergic receptors [40]. Indeed, ATP was recently proposed to co-ordinate Ca^{2+} signalling among pancreatic islet cells lacking contact [11]. Irrespective of the nature of the synchronising agent, it can be envisioned that factor(s), inducing a cascade of $[Ca^{2+}]_i$ transients propagating through the islet, entrain the slow islet activity into the common pancreatic rhythm required for pulsatile insulin secretion.

As mentioned in the introduction disturbed oscillations in circulating insulin is a possible cause of diabetes in susceptible subjects. Based on the present discussion it is apparent that disturbed pulsatile insulin secretion can have many causes. Some may be due to defects in the generation of $[Ca^{2+}]_i$ oscillations in the β-cell. Others involve disturbed coupling between β-cells in the pancreatic islet. It is also possible that the synchronisation of the islets is not operating because of neural dysfunction. At each level of integration a number of potential errors may cause loss of pulsatile insulin release.

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