Section 4: Oscillations in β-Cells

Control Mechanisms of the Oscillations of Insulin Secretion In Vitro and In Vivo

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The mechanisms driving the pulsatility of insulin secretion in vivo and in vitro are still unclear. Because glucose metabolism and changes in cytosolic free Ca^{2+} ($[Ca^{2+}]_c$) in β-cells play a key role in the control of insulin secretion, and because oscillations of these two factors have been observed in single isolated islets and β-cells, pulsatile insulin secretion could theoretically result from $[Ca^{2+}]_c$ or metabolism oscillations. We could not detect metabolic oscillations independent from $[Ca^{2+}]_c$ changes in β-cells, and imposed metabolic oscillations were poorly effective in inducing oscillations of secretion when $[Ca^{2+}]_c$ was kept stable, which suggests that metabolic oscillations are not the direct regulator of the oscillations of secretion. By contrast, tight temporal and quantitative correlations between the changes in $[Ca^{2+}]_c$ and insulin release strongly suggest that $[Ca^{2+}]_c$ oscillations are the direct drivers of insulin secretion oscillations. Metabolism may play a dual role, inducing $[Ca^{2+}]_c$ oscillations (via changes in ATP-sensitive K^+ channel activity and membrane potential) and amplifying the secretory response by increasing the efficiency of Ca^{2+} on exocytosis. The mechanisms underlying the oscillations of insulin secretion by the isolated pancreas and those observed in vivo remain elusive. It is not known how the functioning of distinct islets is synchronized, and the possible role of intrapancreatic ganglia in this synchronization requires confirmation. That pulsatile insulin secretion is beneficial in vivo, by preventing insulin resistance, is suggested by the greater hypoglycemic effect of exogenous insulin when it is infused in a pulsatile rather than continuous manner. The observation that type 2 diabetic patients have impaired pulsatile insulin secretion has prompted the suggestion that such dysregulation contributes to the disease and justifies the efforts toward understanding of the mechanism underlying the pulsatility of insulin secretion both in vitro and in vivo. *Diabetes* 51 (Suppl. 1):S144–S151, 2002

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5-HT, 5-hydroxytryptamine; $[Ca^{2+}]_c$, cytosolic free Ca^{2+} concentration; IP_3, inositol 1,4,5-trisphosphate; K_{ATP} channel, ATP-sensitive K^+ channel.

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Oscillations occurring at highly variable rhythms characterize many biological events such as contraction, neurotransmission, and secretion. At the cellular level, they may involve oscillations of signals as diverse as membrane potential, Ca^{2+} concentration, metabolism and activity of protein kinases, protein phosphatases, or pumps. Insulin secretion is also a pulsatile phenomenon. The mechanisms underlying this pulsatility have been widely studied but are still a matter of debate. They will be briefly reviewed in this article, which complements recent articles on the same subject (1,2) but presents different interpretations.

In vivo oscillations of insulin secretion. The plasma insulin concentration oscillates even during postabsorptive periods (3–6). Two major frequencies characterize these oscillations. Ultradian oscillations (period of ~120 min) probably result from a feedback loop between glucose production and insulin secretion and will not be further discussed here (7,8). There also exist faster oscillations that were initially reported to have a period of ~15 min in peripheral blood (3–5,9), and turned out later to have a period between 5 and 10 min in portal blood (10,11) or in peripheral blood using deconvolution algorithms (6,12). The finding that the C-peptide concentration oscillates nearly in phase with insulin indicates that cyclic secretion rather than cyclic clearance produces the oscillations of insulinemia (13–15). Pulsatile insulin secretion has been reported to account for ~70% of total insulin secretion in normal human subjects (6,10), and the major effect of glucose is to increase the amplitude of the oscillations (11,12,16,17).

In vitro oscillations of insulin secretion. The first convincing evidence for pulsatile insulin secretion in vitro was obtained with the isolated dog pancreas perfused with a constant concentration of glucose (18,19). The oscillations had a period of 5–10 min. Similar findings were made with the isolated pancreases of other species including monkeys (20) and humans (21). Oscillations of insulin secretion from groups of 12–200 islets have also been reported; their period was 5–10 min (22–24) or 15–30 min (25–28). Monitoring insulin secretion from single islets requires a sensitive radioimmunoassay (29) or enzyme-linked immunosorbent assay (30), or uses an indirect approach, based on the amperometric detection of 5-hydroxytryptamine (5-HT) that has accumulated in insulin granules during preincubation of the islet with the amine

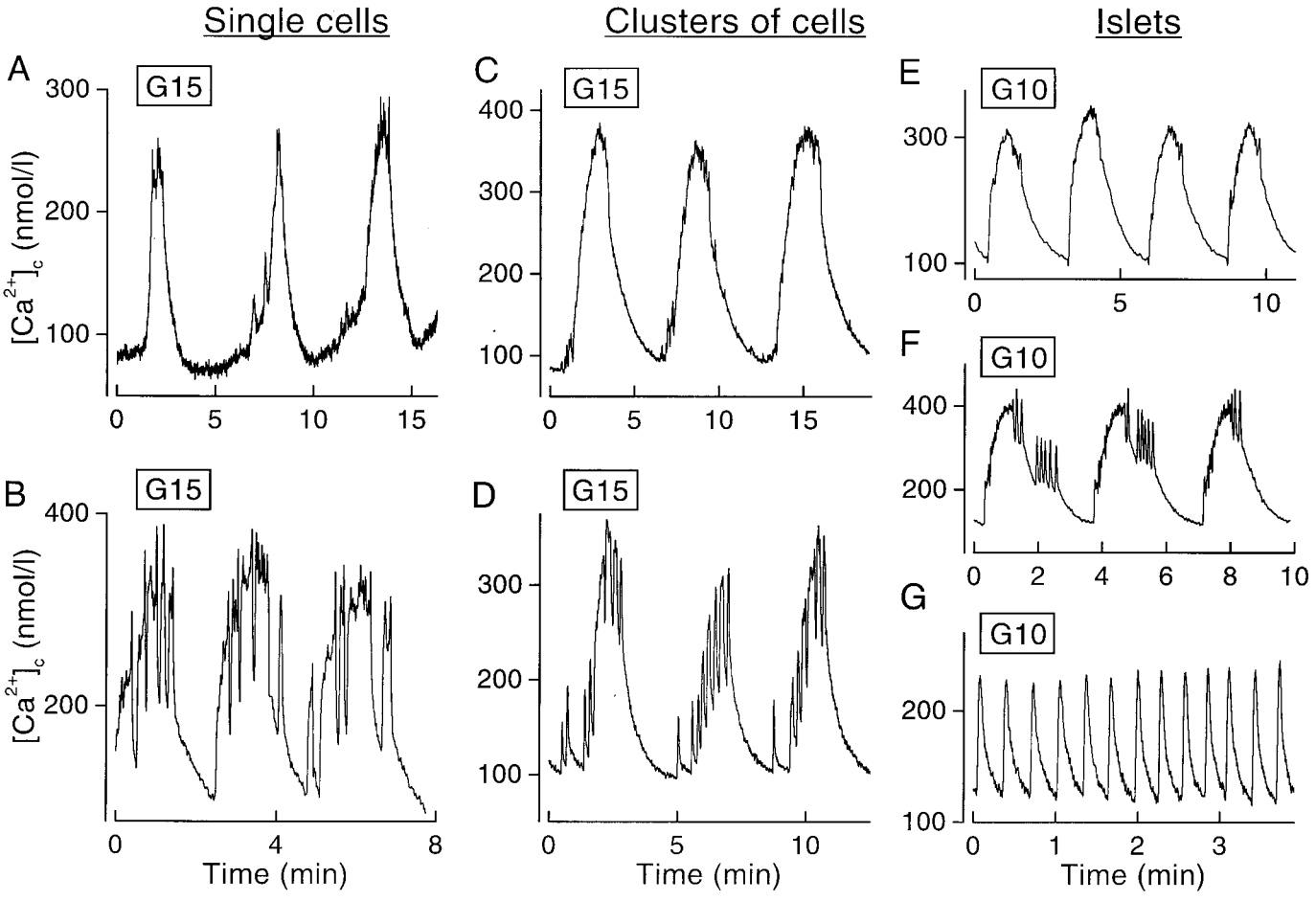

FIG. 1. Various patterns of $[Ca^{2+}]_c$ oscillations induced by 10 or 15 mmol/l glucose (G10 or G15) in single β-cells (*A*, *B*), clusters of 5–10 islet cells (*C*, *D*), and single islets (*E*– *G*) from normal mice. Slow oscillations were present in the three types of preparations. Mixed oscillations were rare in single cells, more common in clusters of islet cells, and frequent in islets. Regular and rapid $[Ca^{2+}]_c$ oscillations were seen only in islets.

(31). Depending on the sampling frequency, variable periods were reported for pulsatile insulin secretion from the islet. Thus, oscillations with a period of 2–5 min were detected with a sampling interval of 10–20 s (29,30,32,33). These oscillations could be resolved into faster oscillations (period of 12–30 s) when the sampling interval was decreased to 3 s (32,34) or by using the amperometric real-time measurement of 5-HT release (35). Glucose increased the amplitude of the oscillations of the perfused pancreas (18) and of single islets (30,33,36) or groups of islets (24). At the single-cell level, several methods can monitor exocytosis of insulin granules with an excellent temporal resolution. They include the amperometric detection of 5-HT (37,38) or insulin itself (39), β-cell capacitance recording (38), and fluorimetric measurements of either quinacrine accumulated in secretory granules (40) or Zn^{2+} coreleased with insulin (41). Except in one study (37), no spontaneous oscillations of secretion were observed during glucose stimulation of single β-cells, probably because these techniques only detect large changes, cannot be used for long recordings, or put major constraints on the experimental protocols.

Mechanisms of control of insulin secretion. Glucose stimulates insulin secretion by activating two pathways that require metabolism of the sugar by β-cells (42). The triggering pathway, also referred to as the K_{ATP} channel-dependent pathway, depends on a rise in the ATP/ADP ratio that closes ATP-sensitive K^+ channels (K_{ATP} channels) in the plasma membrane. The resulting decrease in K^+ permeability leads to membrane depolarization, opening of voltage-dependent Ca^{2+} channels, Ca^{2+} influx, and eventual rise of the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) that triggers exocytosis. The amplifying pathway, also referred to as the K_{ATP} channel-independent pathway, depends on an already elevated $[Ca^{2+}]_c$ but does not involve a further change in $[Ca^{2+}]_c$. It increases the efficiency of Ca^{2+} on exocytosis. The pulsatility of insulin secretion might result from oscillations in either of these transduction pathways. Because metabolism and $[Ca^{2+}]_c$ play key roles in the control of insulin secretion and have been reported to oscillate, many efforts have been spent to investigate which of these two mechanisms is the primum movens of pulsatile insulin secretion.

$[Ca^{2+}]_c$ oscillations in β-cells. Isolated pancreatic islets have widely been used to study the effects of glucose on β-cell $[Ca^{2+}]_c$. Three types of $[Ca^{2+}]_c$ oscillations can be observed during stimulation by the sugar (Fig. 1*E–G*): regular and rapid (frequency of 2–3/min), regular and slow (frequency of ~0.2/min), or mixed, characterized by rapid oscillations superimposed on slow ones (35,43,44). Oscillations of $[Ca^{2+}]_c$ are also detected in islets in vivo (45). Image analysis has demonstrated that the changes in

[Ca^{2+}]$_c$ are synchronized between all β-cells within an islet (46,47). [Ca^{2+}]$_c$ also oscillates in glucose-stimulated single β-cells (2,48), but these oscillations are much less regular than those in islets and often occur at a low frequency only (Fig. 1*A*) (49). Mixed oscillations also occur in isolated cells (49) (Fig. 1*B*), suggesting that this peculiar pattern does not necessarily result from the sum of different signals produced in distinct β-cell populations of the islet (50). [Ca^{2+}]$_c$ oscillations are more regular in clusters of islet cells than in single β-cells, and this regularity (not the frequency) increases with the number of coupled cells (Fig. 1*C* and *D*) (49).

The essential role of Ca^{2+} influx in the generation of [Ca^{2+}]$_c$ oscillations by glucose, in either whole islets or single β-cells, is demonstrated by their abrogation upon omission of extracellular Ca^{2+} (2,47) or blockade of voltage-dependent Ca^{2+} channels (51). [Ca^{2+}]$_c$ oscillations are linked to oscillations of the membrane potential in β-cells (46,47), and it is assumed that mixed [Ca^{2+}]$_c$ oscillations result from an irregular (so-called "periodic") electrical activity (35,52,53). Synchronization of the β-cell electrical activity (54) by gap junctions is likely to underlie the synchronization of [Ca^{2+}]$_c$ oscillations between β-cells within the islet (47,49,55). Synchronization by extracellular signals has been proposed (56,57), but our experiments do not support this hypothesis (58).

The endoplasmic reticulum also contributes to glucose-induced [Ca^{2+}]$_c$ oscillations. We have recently shown that it dampens the amplitude of the oscillations by rapidly taking up Ca^{2+} during the upstroke phase and releasing Ca^{2+} during the descending phase (59). Our model contrasts with a more widely received opinion that the endoplasmic reticulum amplifies glucose-induced [Ca^{2+}]$_c$ oscillations by releasing Ca^{2+} during the periods of depolarization in β-cells (50,60–63). Such a release could be triggered by depolarization (depolarization-induced Ca^{2+} release, mediated by type 1 ryanodine receptors), by inositol 1,4,5-trisphosphate (IP_3-induced Ca^{2+} release), or by Ca^{2+} itself (Ca^{2+}-induced Ca^{2+} release, mediated by type 2 or type 3 ryanodine receptors or IP_3 receptors). Because amplification of the Ca^{2+} signal through some of these mechanisms usually requires the presence of caffeine or cAMP-producing agents (50,62,63), it is unclear whether it is operative during stimulation by glucose alone.

Mechanisms of the oscillations of insulin secretion by the islet

The Ca^{2+} hypothesis. Because a rise in β-cell [Ca^{2+}]$_c$ is required for glucose to stimulate insulin secretion (64), and because glucose-induced [Ca^{2+}]$_c$ oscillations occur synchronously within all β-cells of an islet, it has been proposed that oscillations of insulin secretion are driven by [Ca^{2+}]$_c$ oscillations. This proposal was directly supported by simultaneous measurements of insulin secretion and [Ca^{2+}]$_c$ in the same islet. They showed that each [Ca^{2+}]$_c$ oscillation induced by glucose is accompanied by a synchronous oscillation of insulin secretion even when the frequency of the oscillations is modified (Fig. 2) (29,31,35,36,65). A similar temporal correlation was observed at the single-cell level (37). It also holds when the mitochondrial substrate, α-ketoisocaproate, or the hypoglycemic sulfonylurea tolbutamide are used as stimuli (29,66).

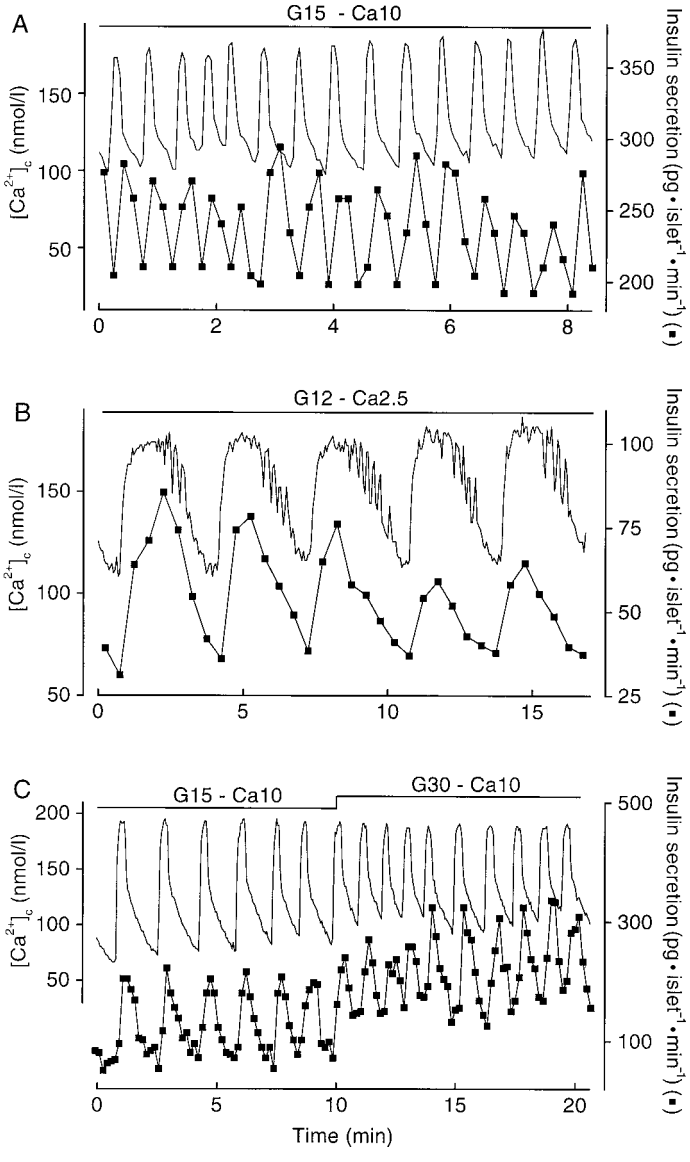

FIG. 2. Effect of glucose on the oscillations of [Ca^{2+}]$_c$ and insulin secretion measured simultaneously in single mouse islets. *A*: During perifusion with 15 mmol/l glucose and 10 mmol/l Ca^{2+} (G15–Ca10), the islet displayed regular oscillations of [Ca^{2+}]$_c$. Frequent sampling (every 10 s) of the effluent for insulin assay made it possible to follow the associated oscillations of insulin secretion. *B*: During perifusion with 12 mmol/l glucose and 2.5 mmol/l Ca^{2+} (G12–Ca2.5), the islet displayed mixed oscillations of [Ca^{2+}]$_c$. The slower sampling rate (every 30 s) only permitted detection of the slow insulin secretion oscillations. *C*: Increasing the glucose (G) concentration of the medium from 15 to 30 mmol/l did not affect the peak of [Ca^{2+}]$_c$ oscillations but increased that of insulin secretion oscillations, indicating that glucose increases the efficiency of [Ca^{2+}]$_c$ on secretion. The Ca^{2+} concentration of the perifusion medium was 10 mmol/l throughout. Insulin was measured in fractions of effluent collected every 10 s (adapted from Gilon and Henquin [36]).

When [Ca^{2+}]$_c$ was forced to oscillate by imposed, repetitive depolarizations of β-cells with 30 mmol/l K^+ (in the presence of diazoxide and 15 mmol/l glucose), insulin secretion also oscillated. In contrast, when [Ca^{2+}]$_c$ was stably elevated by 30 mmol/l K^+, no oscillations of insulin secretion were detected (Fig. 3) (67). Although these data suggest that Ca^{2+} is the oscillophore and the moment-to-moment driver of insulin secretion, the hypothesis was challenged by reports of dissociations between [Ca^{2+}]$_c$

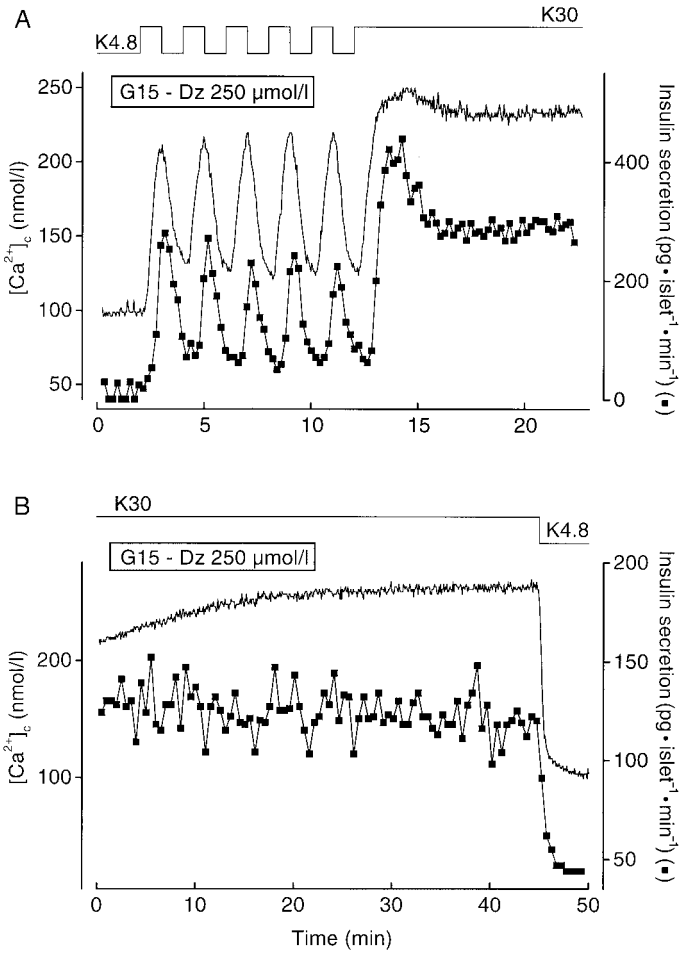

FIG. 3. Insulin secretion does not oscillate at high and stable $[Ca^{2+}]_c$. Single mouse islets were perifused with a medium containing 15 mmol/l glucose (G) and 250 µmol/l diazoxide (Dz) throughout. $[Ca^{2+}]_c$ was varied by changing intermittently the K^+ concentration of the medium between 4.8 and 30 mmol/l as indicated on the top of each panel. $[Ca^{2+}]_c$ and insulin secretion were measured simultaneously. Rapid (every 12 s, *A*) or slow (every 30 s, *B*) sampling of the effluent for insulin assay did not reveal oscillations of insulin secretion at high and sustained $[Ca^{2+}]_c$. (Adapted from Jonas et al. [67].)

changes and insulin secretion. In *ob/ob* mouse islets, insulin secretion was found to oscillate at basal and stable $[Ca^{2+}]_c$ when glucose was omitted from the medium, in the absence or presence of clonidine or diazoxide (68). Oscillations of insulin secretion were also observed during sustained and stable elevation of $[Ca^{2+}]_c$ with high tolbutamide, K^+, or glucose concentrations in *ob/ob* and rat islets (24,33,69). Because many of the experiments reporting dissociations between $[Ca^{2+}]_c$ and insulin secretion were performed with islets from *ob/ob* mice, we recently investigated the possibility that the islets from hypoleptinemic, hyperglycemic, and hyperinsulinemic *ob/ob* mice differ from those of normal mice (70). We indeed found that *ob/ob* mouse islets are more prone than islets from young normal mice to display fluctuations of insulin secretion at stably elevated $[Ca^{2+}]_c$. However, even in these islets, the fraction of total insulin secretion that occurred in a pulsatile manner did not exceed 10–13%, which is far less than the proportion of pulsatile secretion when $[Ca^{2+}]_c$ oscillates in β-cells (70).

The metabolic hypothesis. Oscillations of different metabolic variables, such as O_2 consumption, ATP/ADP ratio, NAD(P)H, dihydroxyacetone-phosphate, glucose 6-phosphate, and lactate release, have been observed in intact or permeabilized normal or clonal insulin-secreting cells (22,27,71–77). These oscillations have frequencies compatible with those of insulin secretion. The identification of these metabolic oscillations and the observation of pulsatile insulin secretion independent from $[Ca^{2+}]_c$ changes (24,33,68,69) prompted the suggestion of metabolism-driven oscillations of insulin secretion (1,78).

Oscillations of a metabolic signal in β-cells may result from intrinsic properties of various pathways (for example glycolysis, see [78]) or be secondary to oscillations of $[Ca^{2+}]_c$ (79–81). In the first case only can the metabolic oscillations be regarded as the true regulators of the oscillations of secretion. This metabolic regulation can be achieved in two ways. Through an action on K_{ATP} channels, intrinsic metabolic oscillations may induce oscillations of the membrane potential and Ca^{2+} influx, but $[Ca^{2+}]_c$ oscillations remain the ultimate effector of the insulin pulsatility. Alternatively, intrinsic metabolic oscillations may modulate the action of Ca^{2+} on insulin secretion through the amplifying pathway. The proof of this second possibility requires the demonstration that oscillations of a metabolic signal do occur and drive large oscillations of insulin secretion in the absence of $[Ca^{2+}]_c$ changes. However, only few experiments performed with intact cells support the existence of metabolic oscillations at stable $[Ca^{2+}]_c$. Thus, oscillations of the K_{ATP} channel activity were observed in intact single β-cells perifused with 3 mmol/l glucose, when $[Ca^{2+}]_c$ was presumably low and stable. Oscillations of O_2 consumption nearly in phase with oscillations of insulin secretion were detected in the presence of 3 mmol/l glucose or of a combination of 11 mmol/l glucose and a high concentration of tolbutamide (1 mmol/l) (77). Strangely, increasing the glucose concentration in the medium did not change the amplitude of the oscillations of O_2 consumption but markedly increased that of the oscillations of insulin secretion. In single clonal β-cells (HIT), oscillations of O_2 consumption were detected in the presence, not in the absence, of glucose. They had a period of ~3 min, similar to that of insulin secretion oscillations, and persisted at the same frequency in a Ca^{2+}-free medium (76). It is important to keep in mind that clonal cells might behave differently from normal β-cells, because their glycolysis is preferentially anaerobic whereas glycolysis in normal β-cells is preferentially aerobic (82). Different results were obtained by simultaneous measurements of $[Ca^{2+}]_c$ and metabolic parameters in single intact mouse islets. Oscillations of glucose and O_2 consumption were detected only in the presence of stimulating concentrations of glucose (74). These oscillations were synchronous to those of $[Ca^{2+}]_c$ and abolished by omission of Ca^{2+} or damped by addition of nifedipine (74,75). They thus seem to correspond to Ca^{2+}-induced metabolic oscillations.

Other observations also cast doubts on the existence of metabolic oscillations independent from $[Ca^{2+}]_c$ changes. We did not detect oscillations of the K_{ATP} channel activity in single metabolically intact β-cells under conditions of low and stable $[Ca^{2+}]_c$ (83). No oscillations of ATP concentration were detected in the mitochondrial and cytoso-

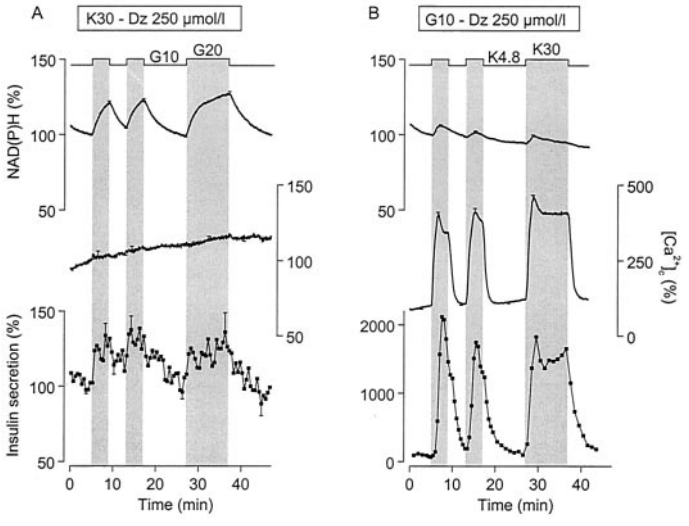

FIG. 4. Imposed metabolic oscillations are much less effective than imposed [Ca^{2+}]$_c$ oscillations on insulin secretion. *A*: Insulin secretion was measured (every 30 s) simultaneously either with NAD(P)H or [Ca^{2+}]$_c$ in single islets. The perifusion medium contained 30 mmol/l K^+ and 250 µmol/l diazoxide (Dz) throughout. The glucose (G) concentration was changed between 10 and 20 mmol/l as indicated on the top of the panel. Values are means ± SE for eight experiments of insulin secretion, of which four were combined with [Ca^{2+}]$_c$ and four with NAD(P)H measurements. *B*: NAD(P)H, [Ca^{2+}]$_c$, and insulin secretion were measured separately. The islets were perifused with 10 mmol/l glucose (G) and 250 µmol/l diazoxide (Dz) throughout. The K^+ concentration was changed between 4.8 and 30 mmol/l as indicated on the top of the panel. All values in *A* and *B* are expressed as a percentage of the values measured within each experiment during the last minute preceding the first application of 20 mmol/l glucose (*A*) or the first depolarization with 30 mmol/l K^+ (*B*). [Ca^{2+}]$_c$ corresponding to 100% were 222 ± 11 and 135 ± 33 nmol/l in *A* and *B*, respectively. Insulin secretion corresponding to 100% were 257 ± 32 and 12 ± 2 pg/min/islet in *A* and *B*, respectively. (Adapted from Ravier et al. [124].)

lic compartments of glucose-stimulated rat islets transfected with adenoviruses and expressing targeted luciferase (84). With the exception of one group that reported oscillations of NAD(P)H fluorescence in single islet cells (40), most authors do not detect NAD(P)H oscillations in islets that display [Ca^{2+}]$_c$ oscillations under the same conditions (47,85).

If Ca^{2+}-independent metabolic oscillations really exist in β-cells and play a role in the control of insulin secretion, models other than glycolytic oscillations will be required to explain the observation that the mitochondrial substrate α-ketoisocaproate can also induce oscillations of insulin secretion (66).

[Ca^{2+}]$_c$ is the main driver of the oscillations of insulin secretion. To establish whether metabolic or [Ca^{2+}]$_c$ oscillations are the main driver of the oscillations of insulin secretion, each parameter was forced to oscillate while the other was stabilized as much as possible. In the presence of diazoxide, to prevent the effect of glucose on the membrane potential, alternating between 10 and 20 mmol/l glucose in the continuous presence of 30 mmol/l K^+ was without effect on [Ca^{2+}]$_c$ but induced oscillations of metabolism [NAD(P)H fluorescence]. This resulted in small oscillations of insulin secretion (Fig. 4*A*). Conversely, when the islets were alternatively perifused with 4.8 and 30 mmol/l K^+ in the continuous presence of 10 mmol/l glucose and diazoxide, large [Ca^{2+}]$_c$ oscillations occurred, accompanied by only minor oscillations of NAD(P)H fluorescence. However, the resulting oscillations of insulin secretion had an amplitude ~100 times larger than that of the oscillations of secretion induced by imposed metabolic oscillations (Fig. 4*B*). In a model of permeabilized cells, oscillations of the Ca^{2+} concentration in the medium (hence within the cells) triggered oscillations of insulin secretion in the presence of a stable concentration of ATP and without possible glucose metabolism (86). Altogether, these data demonstrate that metabolic oscillations are poor effectors compared with [Ca^{2+}]$_c$ oscillations and strongly suggest that [Ca^{2+}]$_c$ oscillations are the direct drivers of the oscillations of insulin secretion at the islet level.

Mechanisms of the oscillations of insulin secretion in vivo. In vivo, insulinemia, glucagonemia, and glycemia oscillate at the same frequency. These oscillations are unaffected by agents modifying the function of the central nervous system (87). Initially, plasma insulin was reported to cycle nearly in and glucagon nearly out of phase with glucose (3,9,88,89), suggesting that pulses of insulin/glucagon secretion might induce pulses of glucose production by the liver; the resulting pulses of glycemia might in turn induce pulses of hormone release by the pancreas. Although very attractive, this hypothesis was challenged by subsequent studies that failed to find a consistent relationship between the periodic fluctuations in insulin, glucagon, and glucose (4,13,90). Moreover, suppression of glucose oscillations by a glucose clamp did not prevent the oscillations of insulinemia (91), and pulses of glucose or insulin failed to reset the cycles (17).

The observation that insulin and glucagon are secreted in a pulsatile manner by the perfused pancreas (18–21) has led to the proposal that a pacemaker system is present in the gland itself. An intrapancreatic network was suggested to pace and synchronize insulin secretion between all islets within the pancreas (92). Several observations are consistent with this proposal. Intrapancreatic neurons of the cat pancreas showed oscillations of their electrical activity with a period similar to that of the oscillations of insulin release (93). Pulsatile insulin secretion from the perfused dog (94) and rat (2) pancreas was altered by blockade of neurotransmission with tetrodotoxin or inhibition of postsynaptic nicotinic receptors (92). However, further identification of the nature of the neurotransmitter controlling the pulsatility of insulin secretion proved impossible because pharmacological blockade of endorphin, muscarinic, and α- or β-adrenergic receptors was without effect on the frequency of the oscillations in vivo (17,95) and in vitro (92). Several studies have reported oscillations of insulinemia after intrasplenic (96) or intrahepatic grafts (97,98) of isolated islets, or transplantation of a denervated pancreas (99,100). But the opinion that pulsatility of insulin secretion only reappears upon reinnervation of the grafted islets (98) is not shared by most groups (96, 97,99,100). The role of intrapancreatic ganglia in synchronizing the signals between islets is also challenged by the observation of pulses of insulin secretion by pieces of pancreas containing several islets and electrically silent ganglia (101). Despite all these studies, it remains unclear whether intrapancreatic ganglia are really the pacemaker of the pulsatility of insulin secretion in vivo. The alternative proposal, that the in vivo pulsatility of secretion reflects an intrinsic property of each islet, also has weaknesses. Thus, the electrical activity that controls [Ca^{2+}]$_c$

has been reported not to be synchronized between islets in situ (102). Whether insulin secretion was pulsatile under these conditions is, however, unknown.

Functional advantages of $[Ca^{2+}]_c$ and insulin secretion oscillations. What is the reason for the oscillatory behavior of β-cells? $[Ca^{2+}]_c$ oscillations might be energetically less costly than a sustained $[Ca^{2+}]_c$ elevation and, by limiting the time during which $[Ca^{2+}]_c$ is elevated, might decrease the risk of Ca^{2+}-cytotoxicity (103). This has never been documented for β-cells. $[Ca^{2+}]_c$ oscillations might be important for the activation of intracellular signals that control functions other than insulin secretion, such as gene expression (104). Changes in the frequency or duration of $[Ca^{2+}]_c$ oscillations might control insulin secretion more accurately than changes in the amplitude of a sustained $[Ca^{2+}]_c$ rise, and avoid inactivation or downregulation of transduction or effector pathways of stimulus-secretion coupling. However, our experiments have shown that, regardless of the oscillatory or steady pattern of the $[Ca^{2+}]_c$ elevation, insulin secretion at constant glucose is determined by the product mean $[Ca^{2+}]_c$ × time (67,105). No functional advantages of $[Ca^{2+}]_c$ oscillations have yet been identified in β-cells, but it should be emphasized that the long-term impact of a loss of oscillations has not been evaluated.

Oscillations of insulinemia might help in preventing insulin receptor downregulation (106) and development of insulin resistance (107). Indeed, insulin infused in a pulsatile fashion had a greater hypoglycemic effect than continuously infused insulin (108–111), being more potent in reducing hepatic glucose production and stimulating glucose utilization (112–115). This superior efficacy of pulsatile insulin is not unanimously accepted (116–118), however, perhaps because its disclosure critically depends on various in vivo factors, such as glucagonemia or insulinemia or the frequency of the pulsatility (5,109,119).

Some studies (16,17,120), but not all (12,121,122), have reported disorganized oscillations of insulinemia in patients with type 2 diabetes and their near relatives with mild glucose intolerance. It is unknown, however, whether these alterations are secondary to the prolonged hyperglycemia. Insulin resistance of obese patients also decreases the regularity of pulsatile insulin secretion (123). Because oscillations of insulinemia favor optimal glucose homeostasis, one may speculate that a disturbance of plasma insulin oscillations in type 2 diabetes contributes to the disease.

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