Glucose and Insulin Synergistically Activate Phosphatidylinositol 3-Kinase to Trigger Oscillations of Phosphatidylinositol 3,4,5-Trisphosphate in β-Cells*

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In insulin-secreting β-cells, activation of phosphatidylinositol 3′-OH-kinase with resulting formation of phosphatidylinositol 3,4,5-trisphosphate (PIP₃) has been implicated in the regulation of ion channels, insulin secretion, and gene transcription as well as in cell growth and survival, but the kinetics of PIP₃ signals following physiological stimulation of insulin secretion is unknown. Using evanescent wave microscopy and a green fluorescent protein-tagged PIP₃-binding protein domain for real-time monitoring of plasma membrane PIP₃ concentration in single MIN6 β-cells, we now demonstrate that glucose stimulation of insulin secretion results in pronounced PIP₃ oscillations via autocrine stimulation of insulin receptors. Glucose lacked effect when insulin secretion was prevented with the hyperpolarizing agent diazoxide, but the sugar dose dependently enhanced the PIP₃ response to maximal insulin stimulation without affecting the rate of PIP₃ degradation. We conclude that glucose is an important co-activator of phosphatidylinositol-3′-OH-kinase and that the plasma membrane PIP₃ concentration in β-cells undergoes oscillations due to pulsatile release of insulin.

In addition to endocrine stimulation of glucose uptake and storage in liver, muscle, and adipose tissue, insulin has autocrine effects on β-cells, regulating proliferation, survival, insulin synthesis, and secretion. Insulin has been reported to have both stimulatory (3–7) and inhibitory (8–12) feedback effects on β-cells. Because some of the discordant observations may depend on different times allowed for insulin action, it is important to characterize the kinetics of insulin receptor-induced signaling events.

Binding of insulin to its receptor is associated with receptor autophosphorylation and tyrosine phosphorylation of insulin-receptor substrate (IRS) proteins and other adapter molecules (13–15), which in turn leads to activation of the phosphatidylinositol 3′-OH-kinase (PI3-kinase) (16) and Ras-mitogen-activated protein kinase (17) pathways. PI3-kinase catalyzes formation of the membrane phospholipid phosphatidylinositol 3,4,5-trisphosphate (PIP₃), which is an important regulator of ion channels (18), protein kinases, and guanine nucleotide exchange factors (19, 20).

Glucose is known to activate PI3-kinase in β-cells, an effect that has been attributed to autocrine activation of insulin receptors by secreted insulin (13, 14). However, little is known about the kinetics of this process, and it is unknown whether the pulsatile pattern of insulin release is reflected in the time course of PI3-kinase activation. In the present study, we used a PIP₃-binding pleckstrin homology (PH) domain tagged with GFP in combination with evanescent wave microscopy for real-time monitoring of the plasma membrane PIP₃ concentration in individual MIN6 β-cells. It is demonstrated that glucose directly potentiates PI3-kinase activity in the presence of insulin and that this synergism, together with pulsatile release of insulin, results in pronounced oscillations of the plasma membrane PIP₃ concentration in glucose-stimulated β-cells.

EXPERIMENTAL PROCEDURES

Materials—The PH domain from protein kinase B/Akt fused to the green fluorescent protein (GFP-PHₐkt) was used as a translocation biosensor for membrane PIP₃ concentration (21). This PH domain has a well documented binding preference for PIP₃ and phosphatidylinositol 3,4-bisphosphate (P13,4P₂) over other phosphoinositides and inositolpolyposphates (22), and it was verified that receptor-induced changes in phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate did not induce GFP-PHₐkt translocation. To confirm that the GFP-PHₐkt responses reflected changes of PIP₃ rather than P13,4P₂, experiments were also performed with GFP-tagged GRP1 (gen-

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2 The abbreviations used are: [Ca²⁺], cytoplasmic Ca²⁺ concentration; PI3-kinase, phosphatidylinositol 3′-OH-kinase; PIP₃, phosphatidylinositol 3,4,5-trisphosphate; PH, pleckstrin homology; GFP, green fluorescent protein; GFP-PHₐkt, pleckstrin homology domain from Akt fused to GFP; GRP1, general receptor for phosphoinositides-isoform 1.
eral receptor for phosphoinositides-isofrom 1), the PH domain of which only binds PIP_3 (22). Because of its strong nuclear localization, however, GRP1-GFP was used only in the experiments shown in Fig. 5B. The plasmids encoding the biosensor constructs as well as GFP targeted to the membrane via covalent lipid modification (GFP-CAAX) was generously provided by Professor Tobias Meyer, Stanford University. Diazoxide was a kind gift from Schering-Plough Int. (Kenilworth, NJ). Adrenaline, insulin, and LY294002 were from Sigma. Dulbecco’s modified Eagle’s medium and Lipofectamine 2000 were obtained from Invitrogen, and Fura Red was provided by Molecular Probes Invitrogen (Portland, OR).

**Cell Culture and Transfection**—MIN6 cells (passage 17–30) (23) were maintained in Dulbecco’s modified Eagle’s medium containing 4500 mg/liter glucose and supplemented with 15% fetal calf serum, 2 mM L-glutamine, 50 μM 2-mercaptoethanol, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a 5% CO_2 humidified atmosphere. The cells were seeded onto poly-L-lysine-coated 25-mm glass coverslips to achieve 50% confluency on the day of transfection. Transient transfection was performed with 2 μg of plasmid DNA and Lipofectamine 2000 in a ratio of 1:2.5 in 1 ml of Dulbecco’s modified Eagle’s medium during 4 h followed by repeated washing and further culture in Dulbecco’s modified Eagle’s medium for 12–24 h. Prior to experiments, the cells were transferred to a buffer containing 125 mM NaCl, 5.9 mM KCl, 1.28 mM CaCl_2, 1.2 mM MgCl_2, and 25 mM HEPES with pH adjusted to 7.40 with NaOH and incubated for 45 min at 37 °C. In experiments with [Ca^{2+}], measurements, the cells were preincubated in the presence of 10 μM acetoxyethyl ester of the fluorescent Ca^{2+} indicator Fura Red.

**Fluorescence Microscopy**—The subcellular localization of GFP-PHAkt was analyzed using a confocal system (Yokogawa CSU-10 spinning disk head; Andor Technology, Belfast, Northern Ireland) attached to a Diaphot 200 microscope (Nikon, Kanagawa, Japan) equipped with a 60×1.40-NA objective. The 488-nm beam from an argon ion laser (Melles-Griot, Didam, The Netherlands) was coupled to the scanhead through an optical fiber (Point-Source, Southampton, UK). Fluorescence was detected at 520/35 nm using an Orca-AG camera (Hamamatsu Photonics, Hamamatsu City, Japan) under MetaFluor software control (Molecular Devices Corp., Downingtown, PA).

The plasma membrane concentration of GFP-PHAkt was recorded with an evanescent wave microscopy setup built around an Eclipse TE2000 microscope (Nikon) with a 60×1.45-NA objective as previously described (24). Selection of excitation and emission wavelengths was made with the following filters (center wavelength/half-bandwidth nm): GFP exc 488/10 nm, em 525/25 nm; Fura Red exc 488/10 nm and em 630 nm long pass. The fluorescence was registered by an Orca-ER camera (Hamamatsu) under MetaFluor software control (Molecular Devices). If not otherwise stated, images were acquired by 50–100-ms exposure every 5 s. To avoid the potentially harmful laser light, the beam was blocked by an electronic shutter (Sutter Instruments, Novato, CA) between image captures.

**Measurements of Insulin Secretion**—MIN6 cells were seeded into 24-well plates and grown to ~70% confluency. Before measurements of insulin secretion the cells were preincubated for 90 min at 37 °C and 5% CO_2 in Krebs Ringer Bicarbonate Heps (KRBBH) buffer (130 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO_4, 1.2 mM KH_2PO_4, 2.5 mM CaCl_2, 10 mM HEPES, and 5 mM NaHCO_3) supplemented with 0.1% bovine serum albumin and 3 mM glucose and with pH adjusted to 7.40 with NaOH. Insulin secretion was then determined by further incubation for 30 min at 37 °C and 5% CO_2 in KRBBH buffer supplemented with various combinations of secretagogues and test substances. Insulin concentrations in the medium were determined using a mouse insulin enzyme-linked immunosorbent assay kit (Merckodia, Uppsala, Sweden). Total DNA was extracted from the cells and the concentration determined by spectrophotometry. Insulin release was normalized for total cellular DNA content and expressed in relation to the secretion obtained with 3 mM glucose.

**Data and Statistical Analysis**—Image analysis was performed using MetaFluor, MetaMorph (Universal Imaging), and ImageJ (W. S. Rasband, National Institutes of Health) software. Fluorescence intensities are expressed as changes relative to initial fluorescence after subtraction of background (ΔF/ΔF_0). The Fura Red traces have been inverted to show increases of [Ca^{2+}], as upward deflections. The SigmaStat software (SPSS Inc., Chicago, IL) was used for regression analysis and Igor Pro (WaveMetrics, Lake Oswego, OR) and Illustrator (Adobe Systems Inc., San José, CA) software for curve fitting and illustrations. All data are presented as mean values ± S.E. Differences were statistically evaluated by two-tailed Student’s t test.

**RESULTS**

**Insulin Stimulates Dose-dependent PIP_3 Formation in Insulin-secreting MIN6 β-Cells via Activation of PI3-Kinase**—Confocal microscopy imaging of GFP-PHAkt-expressing MIN6 β-cells maintained in basal medium containing 3 mM glucose demonstrated that the fluorescence was homogenously distributed in the cytoplasm with a slight enrichment in the nucleus (Fig. 1A). Stimulation of the cells with 100 nM insulin resulted in a pronounced translocation of the biosensor to the plasma membrane accompanied by a reduction of fluorescence intensity in the cytoplasm. To selectively image plasma membrane fluorescence we applied evanescent wave microscopy, which provides excitation restricted to a volume within ~100 nm of the plasma membrane. With this technique the insulin-induced GFP-PHAkt translocation is detected as an increase of fluorescence throughout the membrane adhering to the coverslip (Fig. 1B). The membrane fluorescence increased by 30 ± 3% with half-maximal translocation at 45 ± 5 s (n = 43). After removal of the stimulus the fluorescence returned to base line with t_{1/2} = 90 ± 7 s (n = 23; Fig. 1B). Consistent with the GFP-PHAkt translocation reflecting PI3-kinase-mediated formation of PIP_3, there was no response when insulin was added in the presence of 100 μM PI3-kinase inhibitor LY294002 but pronounced GFP-PHAkt translocation when the inhibitor was removed (Fig. 1C). The insulin-induced GFP-PHAkt translocation was dose dependent with half-maximal and maximal effects obtained at 454 pm and ~10 mM insulin, respectively (Fig. 1D). This dose-dependence is more pronounced at shorter exposure time and higher number of experiments.
Glucose Regulation of PI3-Kinase in β-Cells

**FIGURE 1. Insulin dose dependently stimulates PIP_3 formation in insulin-secreting MIN6 β-cells via activation of P13-kinase.** A, confocal microscopy images of a GFP-PHAkt-expressing MIN6 β-cell before and after stimulation with 100 nM insulin (n = 20). The graphs show fluorescence intensity along the lines indicated in the images. B, evanescent wave microscopy recording of GFP-PHAkt translocation induced by 100 nM insulin (n = 43). The images are from the time points indicated with arrowheads. Scale bars, 5 μm. C, reversible inhibition of insulin-induced GFP-PHAkt translocation by 100 μM LY294002 (n = 10). D, dose dependence of insulin-triggered translocation of GFP-PHAkt (filled circles) and plasma membrane-targeted GFP (GFP-CAAX, filled triangles). Means ± S.E. for 6–20 observations at each concentration. The GFP-PHAkt data are fitted to a Hill equation.

1D). No further effect was observed with insulin concentrations as high as 10 μM (data not shown). Insulin failed to alter the fluorescence recorded from cells expressing GFP alone in the cytoplasm (n = 9, data not shown) or targeted to the plasma membrane (n = 11, Fig. 1D), providing further support for the specificity of the GFP-PHAkt response. Together, these data demonstrate that MIN6 cells respond to insulin with dose-dependent activation of PI3-kinase and formation of PIP_3 in the plasma membrane.

**PIP_3 Formation Triggered by Endogenous Insulin Secretion in MIN6 β-Cells**—It was next tested whether stimulation of endogenous insulin secretion resulted in activation of PI3-kinase. MIN6 cells expressing GFP-PHAkt, and loaded with the fluorescent Ca^{2+} indicator Fura Red were stimulated by raising the extracellular KCl concentration to 30 mM. The resulting depolarization triggered an almost instantaneous rise of the cytoplasmic Ca^{2+} concentration ([Ca^{2+}]_c) due to influx of the ion through voltage-dependent channels (Fig. 2A), followed by a pronounced increase of GFP-PHAkt fluorescence. The time course of the GFP-PHAkt translocation was biphasic with an initial peak reaching 28 ± 3% above base line (t_1/2 = 27 ± 2 s, n = 21), followed within 2 min by a sustained plateau 20 ± 2% above the initial intensity (n = 13; Fig. 2A). Both [Ca^{2+}]_c and GFP-PHAkt fluorescence returned to the prestimulatory levels after normalization of the KCl concentration (t_1/2 = 47 ± 5 s for GFP-PHAkt, n = 8). There was no effect of KCl depolarization in control cells expressing cytoplasmic (n = 9) or membrane-targeted GFP (n = 7; data not shown). When GFP-PHAkt-expressing cells were depolarized with 30 mM KCl in a Ca^{2+}-deficient medium containing 2 mM EGTA, there was neither rise of [Ca^{2+}]_c nor translocation of GFP-PHAkt (Fig. 2B). However, reintroduction of 1.3 mM Ca^{2+} immediately induced elevation.
Glucose Regulation of PI3-Kinase in β-Cells

of [Ca^{2+}]_i and GFP-PHAkt translocation (peak 28 ± 5%; plateau 18 ± 4%, n = 9), supporting the conclusion that Ca^{2+}-triggered exocytosis of insulin secretory granules results in pronounced autocrine activation of PI3-kinase. The lack of GFP-PHAkt response in cells stimulated in the nominal absence of extracellular Ca^{2+} was not due to an inhibition of the GFP-PHAkt translocation per se, because the cells readily responded to 100 nM exogenous insulin (18 ± 2%, n = 14; Fig. 2C).

The secretory responses of the cells were verified by conventional enzyme-linked immunosorbent assay detection of insulin release. Elevation of KCl to 30 mM induced an ~3.5-fold stimulation of insulin secretion, which was abolished (n = 3; p < 0.001) by removal of Ca^{2+} from the external medium (Fig. 2G).

Adrenaline Inhibits the Insulin Response Independent of [Ca^{2+}]_i—To verify that the depolarization-induced PIP_3 formation was due to secreted insulin and not a direct effect of the voltage-dependent Ca^{2+} influx, we stimulated the cells with 30 mM KCl in the presence of 10 μM adrenaline, a known inhibitor of insulin secretion (25). Addition of 10 μM adrenaline caused a small (6 ± 1%, n = 17) increase of plasma membrane GFP-PHAkt fluorescence, which probably did not reflect activation of PI3-kinase, as a similar increase was observed with membrane-targeted GFP (data not shown). The presence of adrenaline prevented the KCl-induced translocation of GFP-PHAkt (Fig. 2, D and E). In contrast, adrenaline had no effect on the depolarization-induced elevation of [Ca^{2+}]_i. Thus, KCl induced a 20 ± 1% (n = 6) change in Fura Red fluorescence compared with 19 ± 3% (n = 6) in the absence of adrenaline (Fig. 2, D and E). Moreover, adrenaline did not prevent the GFP-PHAkt response to 100 nM exogenous insulin (22 ± 2% fluorescence increase; n = 13, Fig. 2F). Conventional enzyme-linked immunosorbent assay measurements of insulin secretion verified that 10 μM adrenaline completely inhibited KCl-stimulated insulin release without effect on basal secretion (Fig. 2G). These data support the conclusion that the depolarization- and Ca^{2+}-induced PIP_3 formation in insulin-secreting cells is due to secreted insulin.

Glucose Stimulates PIP_3 Formation in Insulin-secreting Cells—It was next determined how the plasma membrane PIP_3 concentration was affected by glucose. Elevation of the glucose concentration from 3 to 20 mM resulted in an initial lowering of [Ca^{2+}]_i, known to reflect fueling of β-cell Ca^{2+}-ATPases responsible for sequestration of the ion into the endoplasmic reticulum (26), followed after 2–3 min by a pronounced increase reflecting voltage-dependent Ca^{2+} influx. The rise of [Ca^{2+}]_i was followed after 23 ± 2 s (n = 18) by a pronounced increase of plasma membrane GFP-PHAkt fluorescence (Fig. 3A). The glucose-induced GFP-PHAkt translocation was biphasic with an initial peak reaching 48 ± 3% (n = 59) above base line, followed by a sustained increase at 31 ± 2% (n = 35) above initial fluorescence (Fig. 3A). After lowering of the glucose concentration to 3 mM the GFP-PHAkt fluorescence returned to base line with t½ = 102 ± 12 s (n = 18).

Synergistic Effect of Glucose and Insulin on PIP_3 Formation—We also examined whether the glucose-induced formation of PIP_3 was entirely due to secreted insulin or whether glucose had an independent stimulatory effect. To this end MIN6 cells were stimulated with glucose in the presence of 250 μM of the hyperpolarizing agent diazoxide, which prevents elevation of [Ca^{2+}]_i by clamping the β-cell membrane potential close to the equilibrium potential for K+ . Elevation of the glucose concentration to 20 mM resulted in a small rise of GFP-PHAkt (Fig. 3B) with a magnitude equal to that seen in control cells expressing membrane-targeted GFP (7 ± 1%, n = 14). Removal of diazoxide resulted in additional (38 ± 4%; n = 40) increase of GFP-PHAkt fluorescence (Fig. 3B), not seen with membrane-GFP. The effects of glucose and diazoxide on plasma membrane PIP_3 concentration correlated well with their effects on insulin release (Fig. 3C). These experiments indicate that glucose has little effect on PIP_3 formation in the absence of secreted insulin.

To test whether glucose affected insulin-induced GFP-PHAkt translocation, MIN6 cells were stimulated with a maximally activating concentration of insulin (100 nM) in the presence of 250 μM diazoxide. This resulted in a 32 ± 1% (n = 44) increase of GFP-PHAkt fluorescence (Fig. 4, A and B). Under these conditions the response to exogenous insulin is saturated and diazoxide should prevent depolarization and endogenous release
of insulin. Nevertheless, elevation of the glucose concentration from 3 to 20 mM induced a rapid and sustained increase in 3-isobutylmethylxanthine (not shown). Moreover, the rate of PIP₃ degradation after inhibition of PI3-kinase with 100 μM LY294002 was unaffected by glucose (t₁/₂ = 42 ± 4 s, n = 15 at 3 mM versus 40 ± 5 s, n = 18 at 20 mM, Fig. 4F), indicating that glucose acts synergistically with insulin to accelerate PI3-kinase-dependent PIP₃ formation.

Glucose-induced Oscillations of PIP₃—When the MIN6 cells were exposed to elevated glucose concentrations during extended periods of time (>10 min), it became apparent that the initial peak of GFP-PHₐkₙ translocation was followed by oscillations from an elevated level (Fig. 5A) with amplitudes and frequencies averaging 20 ± 1% change of membrane fluorescence and 0.224 ± 0.015 min⁻¹ (n = 69), respectively. A similar response pattern was seen with GRP1-GFP as PIP₂ biosensor (Fig. 5B). Simultaneous recording of [Ca²⁺]ᵢ, and GFP-PHₐkₙ, demonstrated that each rise of PIP₂ was preceded by an increase of [Ca²⁺]ᵢ, (Fig. 5C). Occasionally, rapid transients of [Ca²⁺]ᵢ, were observed in the presence of elevated glucose concentrations, and these were also associated with transient increases of GFP-PHₐkₙ fluorescence (Fig. 5D). More consistent rapid oscillations of both [Ca²⁺]ᵢ, and GFP-PHₐkₙ were observed after stimulation of the cells with 20 mM of the K⁺ channel blocker tetraethylammonium⁺ (Fig. 5E). The delay observed between the peak of [Ca²⁺]ᵢ, and that of GFP-PHₐkₙ translocation (20 ± 2 s, n = 18) reflects, at least in part, the time required for insulin receptor signal transduction. The lag between application of insulin and the first detectable GFP-PHₐkₙ translocation averaged 14 ± 2 s (n = 34) (Fig. 5F). These data show that glucose stimulation of β-cells is associated with oscillations of PIP₃ in the plasma membrane.

**DISCUSSION**

Two important conclusions can be inferred from the present data. First, the autocrine activation of insulin receptors in β-cells with PI3-kinase-dependent formation of PIP₃ is markedly amplified by glucose. Direct control of PI3-kinase by a nutrient stimulus constitutes a novel regulatory principle for this ubiquitous signaling enzyme. The synergism between glucose and insulin enables PI3-kinase to serve as a coincidence detector, which adds a layer of complexity to the regulation of...
Glucose Regulation of PI3-Kinase in β-Cells

β-cell function by nutrients and receptor agonists. Second, our study provides the first demonstration that the plasma membrane PIP3 concentration can undergo oscillations in response to physiological cell stimulation. The PIP3 oscillations reflect pulsatile release of insulin from individual β-cells combined with the glucose-induced amplification of PI3-kinase activity and rely on a short half-life of the lipid messenger. Oscillations should expand the information content of the PIP3 signal and contribute to the specificity and diversity of signaling downstream of PI3-kinase. In particular, the PIP3 oscillations may partake in the generation of oscillatory electrical activity and pulsatile insulin release.

Glucose and Insulin Synergistically Activate PI3-Kinase—Insulin receptors were first demonstrated in β-cells in the early 1980s (27, 28). Glucose-induced insulin secretion was later shown to activate insulin receptors as well as the downstream signaling proteins IRS-1 and PI3-kinase (13, 14). Consistent with PI3-kinase being activated via secreted insulin, knock down of insulin receptors with small interfering RNA prevented glucose activation of PI3-kinase (3). We now demonstrate that glucose has a direct effect on PI3-kinase activity in the presence of insulin. Although insulin stimulation alone resulted in pronounced activation of the enzyme, PIP3 formation was markedly enhanced after elevation of the glucose concentration. The larger effect by glucose cannot be explained by the insulin receptors being exposed to higher concentrations of the ligand, because the sugar also amplified the response to maximally activating concentrations of exogenous insulin and when insulin secretion was prevented with the ATP-sensitive K+ channel opener diazoxide. The effect of glucose is apparently due to accelerated PIP3 production, because the sugar was without significant effect on PIP3 degradation. Glucose stimulation of PI3-kinase activity required that the enzyme was already activated, because there was no significant stimulation of PIP3 formation in the absence of exogenous insulin under conditions when endogenous secretion was prevented with diazoxide. Earlier studies have demonstrated that glucose is necessary for insulin-like growth factor I-mediated β-cell growth (29), although it is not excluded that the amplifying effect of glucose was mediated via glucose-induced insulin secretion.

Control of PI3-kinase activity by glucose may be an important regulatory mechanism, particularly in the β-cells. With the high sensitivity of β-cells to insulin, the concentration of insulin reached even at low glucose concentrations in the narrow intercellular space within the islet should result in significant constitutive PI3-kinase activity. Glucose regulation of PI3-kinase would thus extend the dynamic range of enzyme activity beyond that achieved by receptor stimulation alone. A similar mechanism may be important for enhancing the response to growth factors and other PI3-kinase activators selectively under glucose-stimulated conditions. Further studies are warranted to clarify the mechanisms underlying direct glucose acti-
vation of PI3-kinase. It is possible that ATP generated by glucose metabolism directly stimulates PI3-kinase activity. Another possibility is that glucose-induced generation of reactive oxygen species (30) leads to PI3-kinase activation via receptor tyrosine kinases (31, 32).

Glucose-induced PIP3 Oscillations—The glucose-induced oscillations of PIP3 concentration in the plasma membrane are probably the result of pulsatile insulin release combined with glucose amplification of PI3-kinase activity and the observed short half-life (~40 s) of the lipid in the membrane. Insulin released from the pancreas (33) and from individual islets is pulsatile (2). Measurements of [Ca2+]i (34) and of Zn2+ co-released from the insulin secretory granules (35) indicate that isolated β-cells also show pulsatile secretory activity. The present observations provide further evidence that insulin is released in pulses from individual cells. Pulsatile insulin secretion is considered important to prevent desensitization or down-regulation of the receptors in the target tissues (36), and deterioration of the regular plasma insulin oscillations is observed in patients with type-2 diabetes (37) as well as in their close relatives (38).

The significance of the PIP3 oscillations is unclear. However, the lipid is a putative mediator of PI3-kinase activation of the ATP-sensitive K+ channel (10, 18). Oscillations of PIP3 may therefore not only be a consequence of pulsatile insulin secretion but via feedback effects on glucose-induced oscillations of the membrane potential and [Ca2+]i, also take part in the generation of pulsatile hormone release (10). The time course of PIP3 oscillations with the rise of PIP3 coinciding with decrease of [Ca2+]i, is consistent with PIP3 being involved in such a regulatory circuit.

It is well established that different time courses of messenger signals, including PIP3, may induce different downstream cellular responses. For example, in adipose cells the efficiency of insulin and platelet-derived growth factor to induce membrane insertion of the GLUT4 glucose transporter depends on the amplitude and duration of the PIP3 signals induced by the different receptors (21). Oscillatory signaling has not previously been described for PIP3 but is well established for Ca2+ and cAMP. Recent observations in our laboratory have shown that cAMP oscillations restrict the activity of protein kinase A to the cytoplasm (39). Other studies indicate that the frequency and amplitude of Ca2+ oscillations affect the specificity and efficiency of gene transcription (40). Future studies will clarify whether PIP3 oscillations contribute to selective activation of downstream processes in an analogous manner.

In conclusion, the present study provides new insights into the signaling downstream of the insulin receptor. Glucose is identified as an important co-activator of PI3-kinase, and glucose-induced insulin secretion is found to be associated with PIP3 oscillations in individual insulin-secreting cells. Dysregulation of the delicate balance between PIP3 production and degradation may contribute to the disturbed insulin secretory pattern in type-2 diabetes.

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