Physiologic Implications of Phosphoinositides and Phospholipase C in the Regulation of Insulin Secretion

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Summary The secretion of insulin from the pancreatic β-cell must be commensurate to satisfy the insulin requirements of the organism. This cell has a great flexibility to meet these requirements which are increased not only by the ingestion of nutrients (increase of plasma glucose) but also by the sensitivity of target tissues to insulin as well. The insulin secretion is a complex biochemical event regulated by a host of potential second messenger molecules acting alone or in concert. These events include the cation calcium, which gains access to the β-cell via the opening of voltage-regulated channels, cAMP and phosphoinositide-derived second messenger molecules, generated as a consequence of phospholipase C (PLC) activation. In this review, we focused on phosphoinositides, PLC/Phosphokinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) cascade in the regulation of insulin secretion. We also described our studies on the mechanism of the β-cell desensitization using perfused islets. It is suggested that a failure of the signaling events contribute to the pathogenesis of diabetes in which the β-cell can no longer secrete the required amounts of insulin. It has been observed that chronic exposure to high glucose desensitizes the β-cells to subsequent stimulation. We suggested that the failure of PLC activation can be attributed in the impairment of insulin secretion by chronic sustained glucose exposure. It may contribute to the vicious circle of impaired insulin secretion leading up to diabetes.

Key Words insulin secretion, β-cell, phosphoinositides, phospholipase C

Insulin release from the pancreatic β-cell must respond acutely and correctly to meet the insulin requirements of the organism. These requirements are increased not only by the ingestion of nutrients but also by decreased sensitivity of target tissues to insulin. During the process of nutrient ingestion, digestion and absorption, insulin must be secreted to maintain fuel homeostasis. The adequacy of this response is usually assessed by measurements of plasma glucose. Additionally, if the target tissues exhibit resistance to its actions, more insulin should be secreted to surmount this cellular defect. Hence, the β-cell must be prepared for the degree of insulin sensitivity or insulin resistance of target tissues, such as liver, muscle and adipose tissues, as well as to anticipate and sense fuel availability. Indeed, it is remarkable that the β-cell has the ability to adapt and perform so effectively. It is essential that the flexibility of the β-cell be maintained. If the β-cell fails to adapt and secrete the necessary amounts of insulin, there are profound clinical consequences; the burgeoning population of Type 2 diabetes supports this conclusion. Currently, the therapy for Type 2 diabetes aims at maintaining or improving the secretory capacity of the β-cell and increasing sensitivity of the target organs to insulin. Elucidating the mechanisms that regulate insulin secretion and how they are altered in diabetes is expected to provide new strategies for restoring β-cell function. In this review, we primarily focus on signaling pathways of the β-cell that participate in the regulation of pancreatic insulin release.

The Regulation of Insulin Secretion from the β-Cell

Over the last several decades, numerous studies have been published concerning β-cell function and several well-established facts emerge. First, glucose is the primary regulator of insulin secretion. Islets containing 500–600 β-cells are exquisitely sensitive to changes in the level of glucose, a property conferred by the high Km glucose phosphorylating enzyme glucokinase (Fig. 1). Second, glucose activates secretion after it has been metabolized to generate metabolites or cofactors that are the actual initiators of exocytosis. ATP is a leading molecule that controls the ATP-sensitive potassium channel and the degree of membrane polarity. Third, mitochondrial signals appear to be essential in the cascade of events that culminate in insulin secretion. Fourth, many second messenger systems including phospholipase C and adenylate cyclase (1–3) are activated by glucose-derived signals. It has also been established that the β-cell possesses the essential elements of...
the insulin signaling cascade that have been well-studied in insulin sensitive tissues such as liver and muscle. Like them, the β-cell expresses the insulin receptor, insulin receptor substrate 1 (IRS-1) and 2 (IRS-2), and phosphatidylinositol 3-kinase (PI3K) (4–8). Their contribution to the regulation of insulin secretion has to be considered as well.

After several introductory comments, this review will focus on those cellular signaling pathways that appear essential for proper β-cell function. Figure 1 shows the flow scheme of insulin secretion. A configuration of tightly regulated metabolic events coincides with an activation of the β-cell by glucose. The β-cell shows a remarkably sensitive response to glucose in a dose-dependent manner (Fig. 2). After glucose is transported into the β-cell, the phosphorylation of glucose by a high Km hexokinase (glucokinase) informs the β-cell of any change in the glucose level. It has been well-studied that this enzyme plays an important role as the metabolic pacemaker as described in detail by others (9). Suffice it to say that the β-cell is sensitive to any small increase in the glycemic level via alterations in metabolic flux controlled by glucokinase. Glucokinase is most sensitive to glucose within the physiologic range of 5–15 mM, representing a virtually linear response with the increase in the glucose level. It is still unclear which of the many metabolites and/or cofactors generated by an increase of metabolic flux is the actual trigger for secretion. The level of ATP is certainly crucial, but other cofactors such as NADH or NAD(P)H have also been considered as possible coupling candidates as well (10–12). Many different compounds may work concurrently and their combined effect is the 20–40-fold increase in secretion that occurs during maximal glucose stimulation.

The increase in the ATP level or the changes in the
The protein kinase C activator diacylglycerol. Depending on a calcium-mobilizing second messenger, IP$_3$, one of a number of phospholipase C isozymes to generate the information harnessed within them released in the plasma membrane, the phosphoinositides (PIs), phosphatidylinositol, phosphatidylinositol-3,4,5-triphosphate (PIP$_3$), and the protein kinase C activator diacylglycerol. Depending on the isozyme of PLC activated, the calcium dependency of this process varies. The nutrient isozyme activated by glucose is tightly controlled by calcium availability and a limited supply of the cation abolishes both PLC activation and the insulin secretory response to glucose. In the case of neurohumoral agonists, the presence of the cation is less demanding and a significant albeit somewhat reduced activation of PLC can be accomplished by these agonists (15).

How does the glucose make an impact on the PLC activation? What is the evidence that suggests events in the PLC/protein kinase C (PKC) signaling system play an important role in the regulation of insulin secretion? Before the involvement of PLC in insulin secretion, studies supporting a role for PKC in the secretion of insulin should be described. Years of research have been conducted on a role for PKC. In the early 1980s, Malaisse, Rasmussen and others (16–18) showed that the pharmacologic activator of PKC, the phorbol ester phorbol 12-myristate 13-acetate (PMA) resulted in a slowly rising phase of secretion, a response somewhat reminiscent of second phase release. As more persuasive experimental evidence, it was reported that PKC translocated to the membrane during glucose-induced insulin secretion and that the phosphorylation state of an established PKC substrate was increased as well (19–22).

Because PLC provides activating intermediates for PKC, it is logical to propose that PLC is also involved as well. It has been reported by a number of investigators that PLC plays an important role in the cholinergic regulation of insulin secretion (23–26). The concept that emerged from these studies was that acetylcholine or its nonhydrolyzable analogue carbachol were potent activators of PLC, and also potently activated secretion in the presence of a suitable glucose level. It was also demonstrated that the activation of PLC primed or sensitized the islets to a subsequent glucose challenge even if not accompanied by acute secretion (27). Some experimental observations made not only with cholinergic agonists but also with cholecystokinin and phorbol esters (28, 29) led us to propose that priming, among other events, was regulated to a large extent by the PLC/PKC signaling cascade (30–32). These studies suggested that in anticipation of an imminent glucose challenge, vagally-released acetylcholine sensitizes the β-cell to the glucose stimulus. This allowed for a more rapid and robust secretory response than would have occurred had this priming event not taken place. Priming thus limited the duration and extent of β-cell exposure to glucose but amplified its secretory impact on the β-cell. This is significant since extended exposure to glucose has a phenomenon often referred to a glucose toxicity but one that is more appropriately described as glucose-induced desensitization (33–36).

It has also been established the β-cells exhibit a dose-dependent increase in PLC activation in response to physiologically relevant increases in the glycemic level bathing them. This response parallels the metabolic and secretory response to glucose (37–40) and plays an important role in the subsequent insulin secretory response.
response. How does glucose influence PLC? It has been reported that a metabolic signal, in all likelihood derived from mitochondrial metabolism, is involved (30, 41). In addition, calcium is essential for nutrient-induced PLC activation since the accumulation of inositol phosphates or the efflux of labeled \(^{3}^{H}\)-inositol from prelabelled cells is effectively antagonized by the omission of calcium or by calcium channel blockers (14, 23, 42). The tight calcium dependency of PLC activation by glucose differs from that noted when PLC is activated by cholinergic stimulation (14, 15, 23). This and the fact that maximally effective glucose and carbachol are at least additive in their effects and at times synergistic has led us to propose that each agonist activated a distinct isozyme of PLC. Based on what has been established in other systems, it is likely that a PLC \(\beta\) isozyme is activated by carbachol and that PLC \(\delta 1\) is regulated by nutrients including glucose and \(\alpha\)-ketoisocaproate (30, 43–46).

A Signaling Role for PIP\(_2\) in the Regulation of Insulin Secretion

Several years ago, two reports suggested the involvement of PIP\(_2\) itself as an important regulator of insulin release (47, 48). These experiments demonstrated that the activity of the ATP-sensitive K channel was altered by the phospholipid content of the cell membrane and that PIP\(_2\) was particularly important in this regard. It is commonly assumed that the ATP-sensitive K channel is a major determinant of the membrane potential of the \(\beta\)-cell. When ATP levels increase, this channel closes and the opening of voltage-gated calcium channels follows. The influx of the divalent cation provides the impetus for the activation of exocytosis of insulin, a distal event regulated by a number of proximal second messenger systems including PLC and PKC. Thus, the activity, or more precisely the closing, of the ATP-sensitive K channel exerts a powerful regulatory influence on insulin secretion. This concept is also supported by additional experiments using diazoxide, a compound that completely abolishes glucose-induced insulin secretion (13, 49). Diazoxide maintains the patency of the ATP-sensitive K channel, and prevents depolarization of the subsequent cellular events that are dependent on calcium availability. How then does PIP\(_2\) influence the channel?

Shyng, Baukrowitz and coworkers (47, 48) reported that the membrane content of phospholipids, in particular PIP\(_2\), determined the sensitivity of the ATP-sensitive K channel to the nucleotide. As the membrane content of this phospholipid was increased, the channel became more resistant to ATP-induced closure and presumably less sensitive to activation. As the content was lowered, its sensitivity to ATP-induced closure increased. At this point, it must be mentioned again that the closure of the ATP-sensitive K channel is an essential condition for glucose-induced insulin release. In other words, these studies led to the concept that the availability of PIP\(_2\) plays an important role for secretory events and events that influence the membrane content of PIP\(_2\) can regulate secretion. Since the activation of PLC results in a subsequent decrease in the membrane content of PIP\(_2\), this membrane phospholipid may play as important a regulatory role in secretion as do the inositol phosphates and diacylglycerol derived from PIP\(_2\).

Involvement of PI3K in the Regulation of Insulin Secretion

More recent work has provided the evidence that PI3K also plays an important signaling role in \(\beta\)-cells. A number of investigators observed the potential involvement of PI3K in the control of insulin secretion. We also performed many studies using the PI3K inhibitor, wortmannin. By inhibition of the PI3K activity with 10–50 nM of wortmannin, glucose-induced insulin secretion was amplified dramatically (Fig. 3). This amplified secretion was dependent on both glucose metabolism and calcium influx but did not appear to involve PLC activation (7). A number of investigators reported that islets possess the requisite signaling molecules that are important in insulin signaling in other tissues, such as liver, muscle and adipose tissues (5, 6, 8, 50). In addition, the cogent studies of Eto, Kodawaki and their coworkers using knockout mice (5, 6), make the conclusion that PI3K plays a role in the regulation of insulin secretion seem most reasonable (5, 51, 52). This implies that insulin signaling, an area of intense clinical interest in the pathogenesis of diabetes, is operative in the cells that secrete the hormone and might play an important physiologic role.

It has been demonstrated that insulin exerts a negative feedback effect on its own release (53–58). These studies suggested that secreted insulin interacts with its own receptor to limit the further secretion of the hor-

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**Fig. 3.** Effect of wortmannin on 8 mM glucose-induced insulin secretion. Groups of rat islets were perfused for 30 min with 3 mM glucose. For an additional 50 min each group was stimulated with 8 mM glucose or with the combination of 8 mM glucose plus 50 nM wortmannin. The asterisks indicate a significant difference at these time points (72).
mone. It is reasonable to assume that this autocrine effect is mediated by insulin signaling components such as IRS-1, IRS-2 and PI3K, known to exist in β-cells as well as liver, muscle and adipose tissues. Because of the constitutive secretion of insulin even at low glucose levels and because the concentration of insulin at the interface of the β-cell membrane can be calculated to be about a million-fold greater than in the plasma, it might be argued that insulin exerts a tonic inhibitory and restraining effect on its own release. Much like the tonic secretion of acetylcholine at the sino atrial node exerts a strong inhibitory action on cardiac excitability via muscarinic type 2 receptors, the tonic secretion of insulin exerts a similar restraining effect on the secretion of insulin from the β-cell. This would explain why wortmannin has such a profound amplifying effect on glucose-induced release. By inhibiting PI3K, wortmannin removes the tonic, negative feedback effect that insulin exerts on its own secretion.

β-Cell Desensitization

It is well known that hyperinsulinemia is a consequence of obesity. This is caused by the excessive consumption of nutrients loaded with fuels capable of stimulating the β-cell. In western countries, most individuals remain obese while about 20% of these individuals will inevitably develop diabetes. Concerning genetic background, Asian people have less of a capacity to secrete insulin than Caucasians. This means that Asians more easily develop diabetes when obese. Some individuals’ β-cells, in the face of the continued demand for more and more insulin and insulin resistance, continue to adapt and function well, while in others their failure or decompensation inevitably leads to diabetes. How does it occur? It is suggested that a failure of the signaling events contributes to the pathogenesis of diabetes in which the β-cell can no longer secrete the required amounts of insulin, which is considered β-cell desensitization. It may contribute to the vicious circle of impaired insulin secretion leading up to diabetes.

The impact of sustained glucose stimulation on the pattern of insulin release from islets has been well-studied. By using the perfused pancreas, continued glucose exposure reduced rates of insulin release after the characteristic secretion of insulin in a biphasic pattern. This could not be attributed to any significant reduction in insulin content of the pancreas, suggesting a lesion not in biosynthesis but in signal transduction. Additionally, Grodsky and Bolaffi probed the evolution of the secretory effect in more detail and the term “third phase,” which is after the biphasic pattern, was applied to the reduced secretion that accompanied sustained glycemic stimulation. Their results suggest that signal generation plays an important role in β-cell desensitization.

We also have explored the effects of chronic glucose stimulation on information flow in the PLC/PKC signaling cascade. A series of experiments was conducted with the hypothesis that the impaired activation of PLC occurred simultaneously with the evolution of impaired secretion. This has been verified. Interestingly, with sustained 3-h exposure to a physiological glucose level (7 mM), both insulin secretion and PLC activation were impaired. In addition, sustained exposure in carbachol, a potent activator of PLC, induced a similar impairment. The untoward effects of sustained carbachol exposure could be abolished by the muscarinic antagonist atropine. In contrast, the potassium channel activator diazoxide impaired the ability of glucose to activate PLC, stimulate insulin secretion and to induce desensitization, but it did not affect carbachol-induced desensitization. These and other studies suggest that sustained activation of PLC launches a chain of events that impairs secretion while acute exposure to glucose stimulates both PLC and secretion. To avoid the subsequent impairment of insulin secretion by sustained glucose-induced stimulation of PLC, a sensitizing system to limit the duration of the exposure to glucose in the β-cell would be beneficial. It is assumed that the other signaling molecules such as acetylcholine, glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), by increasing the β-cell’s sensitivity to glucose, improve the secretory response to glucose and reduce the duration of exposure to glucose. This sensitizing action of other agonists protects the β-cell from glucose “toxicity,” third phase secretion or desensitization. However, chronic sustained elevation of the glucose level bathing them puts β-cells at risk for failure by impairing PLC performance. Desensitization of the β-cell may be intimately involved in the pathogenesis of diabetes.

Conclusions

This review focused on phosphoinositides, PLC/PKC and PI3K cascade in the regulation of insulin secretion. Our studies and others showed that these are essential elements in the insulin secretory system. There are three important lines of experimental investigation supporting this concept. First, glucose stimulation induced the calcium-dependent activation of PLC and insulin secretion. Both responses occur in parallel. Second, the failure of desensitized islets to respond appropriately to glucose can be attributed to the failure of PLC activation by this fuel. Third, because of the robustness of its response to glucose, tight constraints to glucose stimulation are also placed upon β-cells. The tonic activation of insulin signaling in β-cells, and in particular the downstream activation of PI3K, serves to temper their responsiveness to stimulation. Removal or interference of this inhibitory signaling pathway results in a more robust secretory response, a necessary adaptation to overcome the insulin resistance of peripheral tissues such as that noted in obesity. Much still remains to be learned about β-cell physiology. Most important will be the extrapolation of these findings into meaningful therapeutic maneuvers designed to alter the destructive course of obesity and diabetes.
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