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REVIEW

Have We Overlooked the Importance of Serine/Threonine Protein Phosphatases in Pancreatic Beta-Cells? Role Played by Protein Phosphatase 2A in Insulin Secretion

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Summary

Genetic predisposition and environmental influences insidiously converge to cause glucose intolerance and hyperglycemia. Beta-cell compensates by secreting more insulin and when it fails, overt diabetes mellitus ensues. The need to understand the mechanisms involved in insulin secretion cannot be stressed enough. Phosphorylation of proteins plays an important role in regulating insulin secretion. In order to understand how a particular cellular process is regulated by protein phosphorylation the nature of the protein kinases and protein phosphatases involved and the mechanisms that determine when and where these enzymes are active should be investigated. While the actions of protein kinases have been intensely studied within the beta-cell, less emphasis has been placed on protein phosphatases even though they play an important regulatory role. This review focuses on the importance of protein phosphatase 2A in insulin secretion. Most of the present knowledge on protein phosphatase 2A originates from protein phosphatase inhibitor studies on islets and beta-cell lines. The ability of protein phosphatase 2A to change its activity in the presence of glucose and inhibitors provides clues to its role in regulating insulin secretion. An aggressive approach to elucidate the substrates and mechanisms of action of protein phosphatases is crucial to the understanding of phosphorylation events within the beta-cell. Characterizing protein phosphatase 2A within the beta-cell will certainly help us in understanding the mechanisms involved in insulin secretion and provide valuable information for drug development.

INTRODUCTION

Diabetes mellitus is a multifactorial disease characterized primarily by absolute or relative deficiency of insulin that leads to hyperglycemia. There are two main forms of diabetes mellitus. In type 1 diabetes mellitus, there is an absolute insulin insufficiency caused by the immunological destruction of pancreatic beta-cells that produce and secrete insulin, and it accounts for approximately 10% of all cases of diabetes mellitus in the United States. However, type 2 diabetes mellitus, which constitutes approximately 90% of the cases, is characterized by insulin resistance in peripheral tissues and/or relative deficiency of insulin due to the failure of pancreatic beta-cells to secrete insulin [1]. Insulin resistance, frequently associated [1, 2] with but not exclusive to obesity [3], generates excessive stress on the beta-cells to hypersecrete insulin to compensate. Eventually when beta-cells are unable to cope

up with the sustained need for a state of hyperinsulinemia, overt diabetes mellitus ensues [4]. Although insulin resistance is critical in the development of type 2 diabetes mellitus, it is not always the first step in the cascade of events that leads to disease. Recent studies have shown that primary defect in insulin secretion can instigate hyperglycemia and ultimately lead to type 2 diabetes mellitus [5, 6, 7].

Protein Phosphorylation and Insulin Secretion

Irrespective of the cause of hyperglycemia, insulin secretion is without a doubt, a key cellular event that needs to be characterized in order to treat diabetes mellitus effectively. For example, the classic group of drugs, sulfonylureas, utilized routinely to treat hyperglycemia in type 2 diabetes mellitus act primarily by enhancing insulin secretion [8]. Therefore, understanding the complex mechanisms involved in insulin secretion is crucial for understanding this disease. Many decades of extensive research from all over the world have shown that there are multiple pathways involved in regulating this complex stimulus-secretion coupling in the beta-cells. One such mechanism is via activation of phospholipases and protein kinase C [9]. Hormones such as acetylcholine activate this pathway via phosphoinositide, which causes an increase in intracellular Ca^{2+} levels and diacylglycerol, which then activates protein kinase C that subsequently phosphorylates various substrates. Another mechanism is by stimulation of G protein coupled adenyl cyclase activity and activation of protein kinase A [10]. Hormones such as vasoactive intestinal peptide and glucagon like peptide-1 activate these pathways. Mechanistically, the most well studied pathway is the K_{ATP} channel-dependent pathway, whereby, increased concentrations of glucose and other nutrients cause depolarization of the beta-cells via closure of the K_{ATP} channel. Closure of this channel increases Ca^{2+} entry and this rise in intracellular calcium concentrations stimulates insulin release [11, 12]. In the K_{ATP} channel-independent pathway, a site distal to the elevation of intracellular calcium levels is involved. The exact mechanisms of action of this pathway have not been clearly defined, and several candidate mechanisms exist. Calcium-dependent kinases have been implicated to play a role in phosphorylation and subsequent signal transduction [13, 14]. It is apparent that multiple signaling pathways are able to modulate insulin secretion, but the molecular mechanisms may be differentially regulated by protein phosphorylation. This phosphorylation event involves several different protein kinases such as calmodulin-dependent protein kinase II (CaMKII), protein kinase A (PKA), protein kinase C (PKC) and mitogen-activated protein kinases (MAPK) acting alone or in concert on different substrates leading to an increase in insulin release [15, 16, 17, 18]. The activation of these kinases, in turn, will lead to phosphorylation of various substrates that will ultimately result in insulin exocytosis. Although kinases act on different substrates, phosphorylation seems to be an important orchestrator of insulin release. Hitherto, investigators have tried hard to resolve the importance of one kinase over another and to find out how kinases play specific roles in regulating this phosphorylation event. Beta-cell phosphatases, on the other hand, have not been extensively researched. Phosphorylation events can be regulated by two sets of opposing enzymes-kinases and phosphatases.

Protein Phosphatases

Reversible phosphorylation regulates almost all aspects of cell life, from metabolic pathways to cell death [19]. At least one-third of human proteins contain covalently bound phosphate [20]. More than 98% of protein phosphorylation occurs on serine and threonine residues. The degree of phosphorylation is modulated by changes in the activities of protein kinases and protein phosphatases [21]. Because a single phosphatase catalytic moiety associates with several different regulatory or targeting subunits, the total number of functional

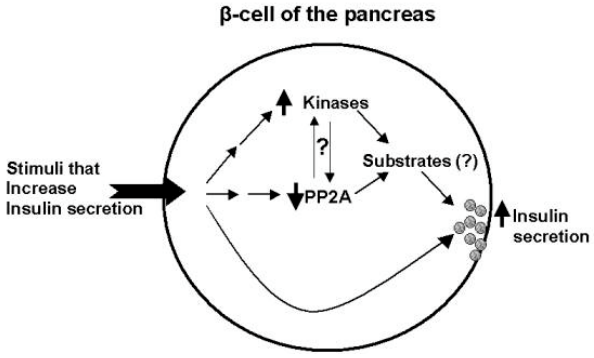

Figure 1. Model showing PP2A's role in insulin secretion from the beta-cells. Upon stimulation, multiple pathways converge to increase insulin secretion. One such pathway is via the kinases, which are activated by insulin secretagogues and thus increase the phosphorylation state within the beta-cells. A complimentary regulatory pathway is via PP2A, which dephosphorylates and maintains a dephosphorylated state. Certain insulin secretagogues inactivate PP2A thus inhibiting the dephosphorylation event. However, the complex mechanisms involved in the regulation of this inactivation of PP2A and the substrate(s) through which PP2A increases insulin secretion within the beta-cell is not clearly defined.

phosphatase holoenzymes is expected to be similar to the number of protein kinases [22]. Protein phosphatases are divided into three families namely: phospho protein phosphatase (PPP), protein phosphatase magnesium dependent (PPM), and protein tyrosine phosphatase (PTP) according to amino acid sequence homology, protein structure, and sensitivity to inhibitors [23]. PPP and PPM comprise phosphoserine and phospho-threonine specific enzymes, whereas, the PTP includes phosphotyrosine specific and dual specificity phosphatases that can de-phosphorylate all three phosphoresidues [24]. The PPP class includes PP1, PP2A, PP2B, PP4, PP5, PP6 and PP7 as well as their various isoforms. The PPM includes PP2C and related isoforms [25, 26]. Even within the same PPP family significant structural diversity is present. The catalytic domains of these phosphatases have a high degree of identity. However, their ability to form heteromeric complexes with a variety of regulatory subunits makes them unique. These regulatory domains or subunits localize the protein complexes to a specific subcellular compartment, modulate the substrate specificity, or alter catalytic activity [27, 28]. The recent heightened interest in phosphatases has led to the discovery of a large number of isoforms and targeting subunits. Limited evidence exists as to where the various subunits are expressed and what substrates are involved in the beta-cells. There is a pressing need for understanding the mechanisms involved in the regulation of protein phosphorylation in the beta-cells through a phosphatase perspective. Insulin secretagogues that increase the phosphorylation state within the beta-cell by activating kinases have been shown to suppress beta-cell phosphatase activities. Certain secretagogues such as glucose that increase L-glutamate concentrations within the beta-cell and subsequent insulin secretion [29], have been shown to act by regulating protein phosphatase activity [30]. Inositol hexakisphosphate, the dominant inositol phosphate in beta-cells, has been shown to inhibit serine threonine phosphatases in a concentration dependent manner and increase insulin secretion [31]. Another insulin secretagogue, L-arginine, a metabolic precursor to polyamines, has been shown to cause a rapid and transient decrease in protein phosphatase activity in beta-cells [32]. Also, sulfonylureas, used routinely to reduce hyperglycemia in type 2 diabetes mellitus subjects, inhibits these phosphatases [33] although some studies have argued that sulfonylureas do not affect phosphatases [34]. The main function of the beta-cell is to synthesize and secrete insulin. This paper focuses on the role played by phosphatases, particularly PP2A, in regulating the secretion aspect of beta-cells (Figure 1).

Protein Phosphatase 2A (PP2A)

PP2A holoenzyme consists of a constant dimeric core, i.e. the catalytic subunit (PP2AC) and the A subunit (PP2AA), associated with one of the family of the B (PP2AB), subunit. PP2AC is the enzymatically active component and it has two isoforms: alpha and beta. The expression of the PP2AC is tightly controlled resulting in

a constant level of PP2A [35]. The PP2AA appears to function primarily as a scaffolding protein that serves to assemble the holoenzyme complex. Two distinct PP2AA isoforms are present which share 86% homology [36]. The PP2AB is thought to act as a targeting module that directs the enzyme to various intracellular locations and also provides distinct substrate specificity. All of these subunits exist in various isoforms within the body, so that the ABC holoenzyme is a structurally diverse enzyme enabling a single catalytic subunit to associate with a wide array of regulatory subunits. The PP2AB is made up of four unrelated families named B, B', B" and B"' with several different members, all of which are able to bind to the PP2AA in a mutually exclusive manner to form a distinct ABC holoenzyme complex. While the PP2AA and PP2AC are present in all cells, some of the PP2AB are expressed in a tissue-specific fashion and at distinct developmental stages [37]. The B family of the regulatory PP2AB consists of four members with differential expression in the brain [38]. The structural feature of this family is the presence of WD-40 repeats (tryptophan-aspartate (WD)), and they mediate various protein-protein interactions [39]. The B' family contains at least five members and each member has more than one isoform with different localizations within the cell. These members are unique because of their ability to be phosphorylated [37]. The B" family has at least four members and is present in many organs within the body. The B"' family consists of at least two members and they all contain the WD-40 repeats. They are predominantly nuclear in localization and may play a role in Ca^{2+} dependent signaling [40].

PP2A has been implicated in the regulation of a multitude of cellular functions, such as metabolism, transcription and translation, RNA splicing and DNA replication, development and morphogenesis, as well as cell cycle progression and transformation [41]. Within the beta-cell PP2A has been suggested, via a circuitous route, to play a role in protein synthesis [42], and perhaps it is involved in a multitude of other cellular functions. For purposes of simplicity, this paper focuses on the secretion aspect in the beta cell.

In the body, PP1, PP2A and PP2B constitute the majority of the phosphatases [43]. Also, in the beta-cell other serine threonine protein phosphatases have not been identified. So, most beta-cell studies performed on protein phosphatases have grouped PP1 and PP2A since they are inhibited by a group of inhibitors [44, 45]. Understanding their functional roles is predominantly based on studies that have utilized a number of naturally occurring inhibitors. However, due to the differences in the effects of these inhibitors *in vitro* and *in vivo*, concentrations of inhibitors far in excess of the $[IC]_{50}$ for most enzymes must be used, and so interpretation of results is difficult. In addition, due to differences in subcellular targeting the local concentrations of individual phosphatases in distinct regions of the beta cell would be quite different from one another. Differential accessibility of the inhibitors to different regions of the cell also makes interpretation of *in vivo* effects difficult.

The majority of these inhibitors have a degree of specificity for different members of the PPP family. Okadaic acid inhibits all members of the PPP family with a degree of selectivity for PP2A and the closely related PP4. PPM phosphatases are unaffected by okadaic acid or any other natural inhibitor. Calyculin A is also a potent inhibitor of the PPP family, but in contrast with okadaic acid, shows little specificity for individual family members. Tautomycin, on the other hand, has a higher affinity for PP1 over other family members and may therefore be useful in conjunction with other inhibitors to identify roles for PP1 inside cells [46]. Fostriecin is unique among inhibitors in that it has strong selectivity for PP2A and PP4 over other PPP family members and is therefore a particularly useful tool in delineating functional roles for these phosphatases [47, 48]. To differentiate the action of PP1 and PP2A, other PP2A inhibitors such as endothall can be used [49].

However, tissue or species variations of inhibition constants can profoundly hamper interpretation of data. The development of compounds that alter the activity of specific phosphatases is rapidly emerging as an important area in drug discovery.

Beta-cell studies using phosphatase inhibitors have shown that inhibition of PP1 and PP2A enhances insulin secretion from islets and insulin secreting cell lines (INS-1, RIN-5, MIN-6 and HIT-T15). Although using the inhibitors alone may not be sufficient to elicit insulin response; but, in the presence of glucose the inhibitors enhance insulin response. However, some studies have demonstrated that inhibiting protein phosphatases alone is sufficient for insulin secretion [50]. Nevertheless, there is a general consensus that upon glucose stimulation or Ca^{2+} influx [51], inhibition of protein phosphatases enhances insulin secretion. Some studies have shown that inhibition of phosphatases reduce insulin secretion [52, 53]. This inconsistency in the studies could be because long-term exposure to these inhibitors or using high concentration of inhibitors reduces insulin secretion by being toxic to beta-cells. Surprisingly, PP2A specific inhibitors have not been used within the beta-cell to demonstrate its involvement in insulin secretion. Okadaic acid and nodularin, for example, respectively have over 100-fold and 50-fold higher potency of inhibition towards PP2A than towards PP1 [54]. Due to lack of specificities, and in order to gain insight as to which phosphatase is involved, the subcellular co-localization of PP1 and PP2A needs to be addressed.

Besides phosphatase inhibitors, stimulation of beta-cells with the insulin secretagogues such as L-arginine, L-glutamine, as well as KCl and ATP decreases PP1 and PP2A activities. Interestingly, ATP and ADP inhibit PP2A more than PP1 suggesting that PP2A is primarily involved in insulin secretion [55]. In the beta-cell, ATP is known to increase upon uptake and metabolism of stimulatory concentrations of glucose as well as amino acids. In fact, increased ATP is an indicator of high-energy state which physiologically leads to an increase in insulin secretion to signal target tissues to store excess energy into fats. However, addition of cAMP, cGMP, or prostaglandins E2 and F1 alpha at widely different concentrations failed to affect protein phosphatase activities although insulin secretion was enhanced [55]. This suggests that protein dephosphorylation is not necessarily the sole mechanism through which insulin secretion occurs. This could explain the discrepancies between data where one group shows an increase in insulin secretion upon inhibiting phosphatases and another group shows the exact opposite. Nevertheless, during an event where protein phosphatases are involved in insulin secretion, PP2A appears to be playing the primary role. Perhaps, upon very strong stimuli, other phosphatases may be involved in regulating the phosphorylation state.

Studies have shown direct inhibition of PP2A by glucose and its metabolites by membrane-depolarizing concentrations of KCl in beta-cells [56]. Fructose-2,6-bisphosphate and glucose-1,6-bisphosphate, which are known to allosterically activate phosphofructokinase, one of the rate-limiting enzymes in the glycolytic pathway, have been reported to have inhibitory effects on porcine heart PP2A [56, 57]. It has also been shown that 3-phosphoglycerate and phosphoenolpyruvate increase protein phosphorylation in permeabilized beta-cells [58] to support this model. Inositol hexakisphosphate, which increases insulin secretion by inhibiting protein phosphatases, has been shown to preferentially inhibit PP2A at lower concentrations [34], once again supporting the model that PP2A primarily regulates the phosphorylation state that regulates insulin secretion. As shown in Figure 1, upon stimulation, multiple pathways converge to increase insulin secretion from the beta-cells. One such pathway is via the kinases, which are activated by certain insulin secretagogues and thus increase the phosphorylation state within the beta-cells. Certain stimuli that increase insulin secretion lead to an inactivation of PP2A probably in conjunction with the activation of kinases and presumably

regulate this phosphorylation state. The complex mechanisms involved in the regulation of this inactivation of PP2A and the substrate(s) through which PP2A exerts its role is not clearly defined.

One mechanism through which the activity of PP2A is regulated is by covalent modifications. PP2AC undergoes modifications such as carboxymethylation at its C-terminal leucine (Leu-309) [59], which leads to an increase in insulin secretion. Ebelactone, an inhibitor of PP2AC demethylation, markedly reduced nutrient-induced insulin secretion from normal rat islets. Taken together, these data seem to suggest a key modulatory role for PP2A in insulin secretion [60]. Carboxymethylation not only dictates its interaction with other subunits of the PP2A but also its substrate specificity, subunit assembly, its subcellular localization and association with regulatory proteins [61]. Consistent with the PP2A regulating insulin secretion model is the finding in beta-cells that the PP2AC undergoes carboxymethylation, an effect accompanied by increased PP2A activity and suppressed insulin secretion [60, 62]. Some studies have shown that carboxymethylation has either no effect [63] or decreased effect on the activity of PP2A [64] suggesting that modifications of PP2AC may affect other characteristics of PP2A. However, these effects were observed in non-insulin secreting cells. The presence of numerous PP2AB subunits and the lack of studies on these within the beta-cell illustrates the importance of characterizing the role played by PP2A in the beta-cell.

Furthermore, heat stable inhibitors [65], several other proteins [66], as well as certain lipid second messengers such as ceramide [67], have been implicated in the regulation of PP2A function. Ceramides are formed from the hydrolysis of sphingomyelin by membrane-bound sphingomyelinase. Localization of such an enzyme activity was reported in isolated rat islets, mouse islets and clonal beta-cells [68]. Ceramides either delivered exogenously or generated endogenously, inhibit insulin secretion by activating PP2A. This is not mediated via activation of the carboxyl methylation of the catalytic subunit of PP2A, suggesting yet another PP2A regulatory locus in the beta-cells [69]. This inhibition of insulin secretion was rescued by the addition of okadaic acid in the beta-cell. Saturated fats like palmitate and stearate if given in excess lead to the accumulation of ceramides [70]. Also, long-term exposure of isolated beta-cells to ceramides significantly reduced glucose and carbachol induced insulin secretion from these cells [71]. Some studies have demonstrated beta-cell necrosis in the presence of ceramides [72]. This could be the reason why obesity and lipotoxicity leads to decrease in insulin secretion although fatty acids have been shown to increase insulin secretion [73]. Another method of regulating PP2A is by phosphorylation of its tyrosine and serine threonine residues in the PP2AC [74, 75]. Kinases could be regulating this pathway (Figure 1). Alternatively, the action of kinases can be regulated by PP2A and thus regulate the overall phosphorylation state within the beta-cells. However, this has not been shown to be the case in the beta-cells.

Clearly, multiple signaling pathways can modulate the phosphorylation aspect of insulin secretion, but little is known, however, about how these various regulatory forces are coordinated and integrated to direct the function of PP2A towards its multifaceted tasks. The different isoforms of the A, B or C subunit of PP2A have not been identified within the beta-cell. The subcellular localization is another important piece of information that is missing. This information will provide valuable insight on its function and resolve discrepancies.

Protein Phosphatase 1 (PP1)

PP1 consists of a constant catalytic subunit and one or two variable regulatory subunits that target the phosphatase to a particular cellular compartment and/or act as substrate specifiers [76, 77]. Dozens of different regulatory subunits of PP1 have been described and for some of them a function has

been delineated [78]. Weaker interactions may subsequently enhance binding and modulate PP1 activity/specificity in a variety of ways. In addition, several 'modulator' proteins bind to the catalytic subunit but do not possess a domain targeting them to a specific location [79]. Although the role for a phosphatase in insulin secretion is evident, there has been no clear data to strongly suggest PP1 as a regulator of insulin secretion. Indirect evidence implicates a role for PP1 in insulin exocytosis [80]. A more recent study [81] showed that inhibitor-1 and dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32), the endogenous inhibitors of protein phosphatase 1, are present in beta-cell implying a role for PP1. They were found in the subcellular fractions containing plasma membrane-associated proteins, suggesting that this PP1 inhibitor is targeted to sites of exocytosis. In the brain these inhibitors have to be phosphorylated to act as inhibitors of PP1. However, in the beta-cell, upon glucose stimulation their phosphorylation status was not altered [81], suggesting that PP1 may not be involved primarily in insulin secretion. It is possible that stronger stimuli will recruit PP1 to assist PP2A to maintain a dephosphorylated state but more studies need to be performed to determine that.

Protein Phosphatase 2B (PP2B)

PP2B, also known as calcineurin, consists of a catalytic (calcineurin A) and a regulatory (calcineurin B) subunit and is a major mediator of Ca^{2+} signaling in different cell systems. Both calcineurin A and B subunits comprise several isoforms coded by different genes or generated by alternative splicing [82]. When activated by Ca^{2+}-calmodulin binding, PP2B affects gene expression by dephosphorylating specific substrates, including nuclear factor of activated T cells (NFAT). Following dephosphorylation, NFAT translocates from the cytoplasm to the nucleus and activates target genes in cooperation with other transcription factors [83]. PP2B activity can be blocked *in vitro* or *in vivo* by the immunosuppressive drugs cyclosporin A and FK506, which forms complexes with endogenous cyclophilin and FK506 binding protein 12 (FKBP12), respectively, and these complexes bind the catalytic subunit of calcineurin to inactivate it. In the beta-cells of the pancreas PP2B has been shown to play a role in insulin gene transcription [84] and this may be a reason why there is an increase in the incidence of diabetes mellitus among post transplant patients who are on PP2B modulating drugs such as cyclosporin A [85]. Some neurotransmitters such as somatostatin, galanin, and adrenaline that reduce insulin secretion have been shown to do so by regulating PP2B [86]. Although PP2B may play a role in insulin secretory granule transport via the dephosphorylation of kinesin [87], more studies need to be performed to fully understand its role in the beta-cell.

Other Serine Threonine Protein Phosphatases

Other serine threonine protein phosphatases are now being extensively studied. However, their presence or their roles in beta-cells are unknown. Phosphatases show a great structural diversity, and hormones, growth factors and metabolites tightly regulate their activities. PP4 is a predominantly nuclear phosphatase, but is also associated with the centrosomes and is involved in the initiation of microtubule growth regulation of the nucleation and/or stabilization of microtubules [88]. PP4 sequences share 94% identity with PP2A. Some of the inhibitors that inhibit PP2A can also inhibit PP4 [89] and it is possible that some of the effects that are attributed to PP2A within the beta-cells could be due to PP4. However, there have been no studies implicating the same. PP5 is less abundant *in vivo* and has a low basal activity [90]. It possesses four tetratricopeptide repeat (TPR) domains in its N-terminus that are implicated in protein-protein interactions [91], cell proliferation, differentiation and programmed cell death. It has been shown to interact with PP2A [92],

but, its presence or role in beta-cells is not known. PP6, with a sequence identity of about 56% to PP2A, [93] is a component of a signaling pathway regulating cell cycle progression in response to IL 2 receptor stimulation. It is stimulated by ceramides and has been implicated in cell cycle regulation [94]. PP7 is predominantly a nuclear phosphatase in plants and directly interacts with calmodulin, indicating that Ca^{2+} levels *in vivo* might play a regulatory role [95]. Its presence in the beta-cell is not known. PP2C belongs to the PPM family along with the pyruvate dehydrogenase phosphatase. PP2C are Mg^{2+} dependent protein phosphatases and they have been implicated in regulating MAPK [96].

Tyrosine kinases and phosphatases play critical roles in insulin secretion [97]. The protein tyrosine phosphatase alpha modifies insulin secretion in beta-cells [98]. However, review on those phosphatases is beyond the scope of this paper.

Conclusions

Type 2 diabetes mellitus is a devastating disease and one of the critical defects is impaired insulin secretion from the beta-cell. The principal function of the beta-cell is to provide insulin to the body. Therefore, most patients with type 2 diabetes mellitus have a relative or absolute deficiency of insulin [99]. To find other effective cures for type 2 diabetes mellitus, especially in its early stages, there is a need to understand the mechanisms involved in insulin secretion. Towards that effort, various investigators have studied the phosphorylation events that are essential for insulin secretion. Two key players, kinases and phosphatases regulate this phosphorylation event. This paper has focused on the phosphatases, particularly PP2A, which by its virtue to dephosphorylate modulates insulin secretion.

Of all the known PPP and PPM family of phosphatases, multiple evidences imply that PP2A is involved in insulin secretion. The presence of other phosphatases and the roles played by them needs to be studied. Further studies should focus on identifying the potential targets of PP2A within the beta-cell. Beta-cell is like a neuron in many aspects. Neurotransmitter release from the neuron is comparable to insulin release from the beta cell. PP2A is known to regulate neurotransmitter release. Using that model, PP2A-targets in the brain such as kinases themselves, neurofilaments, neurotubules and others should be looked for in the beta-cell.

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Abbreviations CaMKII: calmodulin-dependent protein kinase II; DARPP-32: dopamine and cAMP-regulated phosphoprotein of 32 kDa; FKBP12: FK506 binding protein 12; MAPK: mitogen-activated protein kinases; NFAT: nuclear factor of activated T cells; PKA: protein kinase A; PKC: protein kinase C; PP1: protein phosphatase 1; PP2A: protein phosphatase 2A; PP2AA: protein phosphatase 2A A subunit; PP2AB: protein phosphatase 2A B subunit; PP2AC: protein phosphatase 2A catalytic subunit; PP2B: protein phosphatase 2B; PP4: protein phosphatase 4; PP5: protein phosphatase 5; PP6: protein phosphatase 6; PP7: protein phosphatase 7; PPM: protein phosphatase magnesium dependent; PPP: phospho protein phosphatase; PTP: protein tyrosine phosphatase; WD: tyrosine aspartate; TPR: tetratricopeptide repeat

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