Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 69 (2005) 1681–1691
Commentary
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Novel regulatory roles for protein phosphatase-2A in the islet β cell

Anjaneyulu Kowluru *

Department of Pharmaceutical Sciences, Wayne State University and β Cell Biochemistry Research Laboratory, John D. Dingell VA Medical Center, Detroit, MI 48201, USA

Abstract

Protein phosphorylation constitutes one of the key signaling steps in physiological insulin secretion. The phosphorylation status of a given protein represents the balance of the activities of protein kinases and phosphatases, which induce the addition and removal of phosphate from that protein, respectively. Although several extant studies were focused on the identification and characterization of protein kinases in islets, relatively little information is available on the localization and regulation of protein phosphatases in β cells. Emerging evidence implicates protein phosphatase 2A (PP2A) in the phenomenon of insulin secretion. The three principal objectives of this commentary are to: (i) review the existing evidence, which suggests regulation, by glucose and other insulin secretagogues, of PP2A in the β cell; (ii) discuss the experimental evidence, which implicates PP2A-like enzymes in the dephosphorylation and inactivation of key β cell phosphoprotein substrates (e.g., Akt and Bcl-2), which may be necessary for β cell proliferation and survival, culminating in the loss of the β cell mass; and (iii) highlight potential avenues for future research, including the development of specific pharmacological and therapeutic interventional modalities for the inhibition of specific PP2A-like phosphatases for the prevention of loss of β cell mass leading to the onset of diabetes.
Published by Elsevier Inc.

Keywords: Protein phosphatase-2A; Pancreatic islet; Insulin secretion; Ceramide; Apoptosis; Diabetes mellitus

Glucose-induced insulin secretion from pancreatic β cells involves the generation of second messengers, such as, ions, cyclic nucleotides, and lipid hydrolytic products of phospholipases [1]. Some of the known actions of these modulators include regulation of various protein kinases indigenous to pancreatic β cells. Indeed, several extant studies have demonstrated localization of such kinases in normal rat islets as well as clonal β cells; these include Ca^{2+}-, Ca^{2+}/calmodulin-, cAMP- and phospholipid-dependent protein kinases [2].

The phosphorylation status of proteins is regulated by the balance of the activities of protein kinases and phosphatases, which induce the incorporation and removal of phosphate from these proteins, respectively [3]. Although several earlier studies were focused on the identification and characterization of protein kinases in islets, relatively little is known with regard to the localization and regulation of protein phosphatases (PPases) in β cells. In this context, previous studies [4–8] have implicated protein phosphatase 2A (PP2A) in the phenomenon of insulin secretion. The purpose of this article is, therefore, to put forth a discussion not only on the contributory roles of PP2A-like proteins in the overall well-being of the islet β cell, but also to review the existing experimental evidence, which implicates these proteins in the dephosphorylation and inactivation of specific β cell phosphoprotein substrates (e.g., Bcl-2 and Akt); functional inactivation of these proteins is expected to result in the loss of the β cell mass leading to the onset of diabetes.

1. Structural composition of PP2A

The PP2A family of enzymes represents a major class of serine–threonine PPases, which have been implicated in

Abbreviations: ACC, acetyl CoA carboxylase; CAPP, ceramide-activated protein phosphatase; CML, carboxylmethylation; GAPP, glutamate- and magnesium-activated protein phosphatase; IHP, inositol hexakiphosphate; mTOR, mammalian target of rapamycin; OKA, okadaic acid; PPases, protein phosphatases; PP2A, protein phosphatase 2A; PP2Ac, catalytic subunit of protein phosphatase 2A; PPM1, protein phosphatase methyl transferase 1

* Present address: Department of Pharmaceutical Sciences, 3601 Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, 259 Mack Avenue, Detroit, MI 48202, USA. Tel.: + 1 313 576 4478; fax: +1 313 576 1112.
E-mail address: akowluru@med.wayne.edu.

0006-2952/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.bcp.2005.03.018

the regulation of many cellular events [9]. Several holoenzyme complexes have been isolated and characterized from a variety of tissues. Typically, the PP2A heterodimer complex is comprised of a scaffolding A subunit with an apparent molecular-mass of 65 kDa and the 36 kDa catalytic subunit (PP2Ac). This A/C subunit heterodimer interacts with the regulatory B subunit yielding the PP2A holoenzyme. At least two different families of A (i.e., A_α and A_β) and C (C_α and C_β) have been identified. Several families of the B subunits (e.g., B, B′, B″, and B‴; each comprising of multiple members in these subfamilies) have been identified as well. The binding of B subunit to the A/C heterodimer is felt to provide further stability to the holoenzyme. It is also suggested that the variable B subunit[s] influences substrate specificity and/or subcellular localization of a given PP2A holoenzyme complex. It is estimated that the combination of all subunits (e.g., A–C) could produce >75 different trimeric holoenzymes, although the precise number of the possible holoenzyme complexes that actually exist in cells still needs to be determined [9,10]. While the A and C subunits are ubiquitously expressed, certain B subunits are expressed in a tissue-specific manner and at various stages of cellular development [9,10]. Recent years have witnessed significant progress in the area of functional regulation of PP2A, specifically via post-translational modification steps (see the following section).

2. Regulation of PP2A function by carboxylmethylation

Unlike the regulatory B subunits, the PP2Ac is highly conserved, with >70% sequence homology among different species. The six C-terminal amino acid residues (TPDYFL) are felt to be absolutely conserved in all known PP2Ac subunits, and the three C-terminal residues (YFL) are also conserved in protein serine/threonine PPases PP4 and PP6, implicating a role for these residues in the functional regulation of these enzymes [10]. Several previous studies demonstrated that the C-terminal leucine residue in PP2Ac undergoes reversible carboxylmethylation (CML), which is catalyzed by protein phosphatase methyltransferase 1 (PPM1), the structure of which has been deduced recently [10]. It may be pointed out that existing experimental evidence provides conflicting conclusions with regard to potential contributory roles for CML of PP2Ac on its phosphatase function. For example, using purified PP2Ac and carboxylmethyltransferase preparations, Favre et al. have demonstrated a 30–50% increase in the phosphatase activity following the CML of PP2Ac [11]. Compatible with these data, we also reported a 25% increase in phosphatase activity in INS cell and normal rat islet cytosolic fractions following the CML of PP2Ac [4]. Interestingly, in contrast to these stimulatory effects, De Baere et al. observed no effects of the CML on catalytic function of either the dimeric or trimeric forms of PP2A [12]. This discrepancy might be due to (but not limited to), differences in cell types and experimental conditions employed, including substrates used in in vitro phosphatase activity measurements, and purified versus crude preparations of phosphatase and carboxylmethyltransferases utilized in these studies. Further, it is important to note that the stimulatory effects of CML on the activity of PP2A are physiologically meaningful in the context of the β cell, since we reported marked inhibitory effects of glucose (in intact β cells) or its metabolites (in broken cell preparations) on the CML of PP2Ac [8]. In addition, earlier data from other laboratories on inhibition of phosphatase activity by glucose or its metabolites [6,7] are compatible with our original formulation that glucose-induced insulin secretion may, in part, be due to its ability to inhibit specific PPases (e.g., PP2A) thereby retaining putative exocytotic proteins in their phosphorylated (active) conformation [8]. However, additional studies are required to firmly establish potential relevance of the CML of PP2Ac in PP2A function, specifically in the context of physiological insulin secretion.

Recent evidence appears to further substantiate the formulation that the CML of PP2Ac promotes the holoenzyme assembly and catalytic function of the PP2A [9,10]. Furthermore, known inhibitors of PP2A (e.g., okadaic acid; OKA), attenuate the CML of PP2Ac by binding to its carboxy terminus [4], thereby preventing access of the PPM1 to its target site (i.e., Leu-309). These findings thus implicate the C-terminal motif of PP2Ac in the functional regulation of PP2A. Along these lines, we previously reported [4] the CML of PP2Ac in intact normal rat islets and clonal β cells. The CML of the PP2Ac increased its catalytic activity, suggesting a key role for CML in the functional regulation of PP2A. Moreover, OKA, but not 1-nor-okadaone, its inactive analog, inhibited the CML of PP2Ac and PPase activity in intact and broken β cell preparations. The methylated PP2Ac also underwent rapid demethylation catalyzed by a methyl esterase localized in islet homogenates. Ebelactone, a known inhibitor of methyl esterases, significantly delayed the demethylation of PP2Ac, and also reversibly inhibited glucose- and ketoisocaproate-induced insulin secretion from normal rat islets [4]. Together, these data identified, for the first time, a methylation–demethylation cycle for PP2Ac in the β-cell and suggested a key functional relationship between PP2A activity and the CML of PP2Ac. However, in this context, it may be emphasized that data derived from the use of various pharmacological agents (e.g., ebelactone) to elucidate potential contributory roles for post-translational modifications, such as the CML of PP2Ac, in islet stimulus-secretion coupling must be interpreted rather cautiously since such a pharmacological approach is bound to generate ambiguity relating to the specificity of the inhibitors used, which may vary between different cell systems and conditions employed. A more definitive

approach to further probe potential physiological relevance of these pathways would be to use isozyme-specific RNAi or antisense oligonucleotides, or genetically manipulated experimental animal models.

3. Regulation of PP2A function by phosphorylation

In addition to the CML, PP2Ac undergoes tyrosine phosphorylation (at Tyr-307). However, in contrast to the CML, which promotes the holoenzyme assembly and catalytic activation of PP2A (see above), tyrosine phosphorylation has been shown to inhibit the catalytic function of PP2A [13]. However, no such studies have been carried out in the islet β cell thus far. It may be speculated that glucose- (and other secretagogue-) induced insulin secretion involves rapid regulation of PP2A function via its methylation at the Leu-309 residue and/or phosphorylation at Tyr-307 residues. This hypothesis needs to be verified through additional investigations.

4. Potential mechanisms of regulation PP2A in islet β cell

Several earlier studies demonstrated direct stimulation, by insulin secretagogues (e.g., glucose), of phosphorylation of islet endogenous proteins, implying that such an event is critical for insulin secretion [2]. Some of these studies also demonstrated direct activation of specific protein kinases by nutrient secretagogues in the isolated β cell [2]. However, very little has been studied with regard to potential regulation, by various insulin secretagogues or the second messengers of insulin secretion, of PPases in the β cell. Following is a brief review of the current evidence on potential (direct) effects on PP2A function by various known modulators of β cell function.

4.1. Regulation by glucose and its metabolites

Using permeabilized β cell preparations Sjoholm et al. [6] reported significant inhibition of PPase activities and concomitant stimulation of insulin secretion by glycolytic and Krebs cycle intermediates. These observations indicated that specific intermediates of glucose metabolism might retain critical exocytotic proteins in their phosphorylated state, which may be necessary for insulin secretion. More recent studies from our laboratory [8] provided additional mechanistic details with regard to PPase inhibitory effects seen in the presence of glycolytic and Krebs cycle intermediates of glucose metabolism. Since the CML of PP2Ac is felt to promote the holoenzyme assembly and subsequent functional activation of PP2A, we investigated putative regulation by glucose (in intact cells) or it metabolites (in cell lysates) on the CML of PP2Ac in insulin-secreting INS cells. We noticed a marked inhibition by

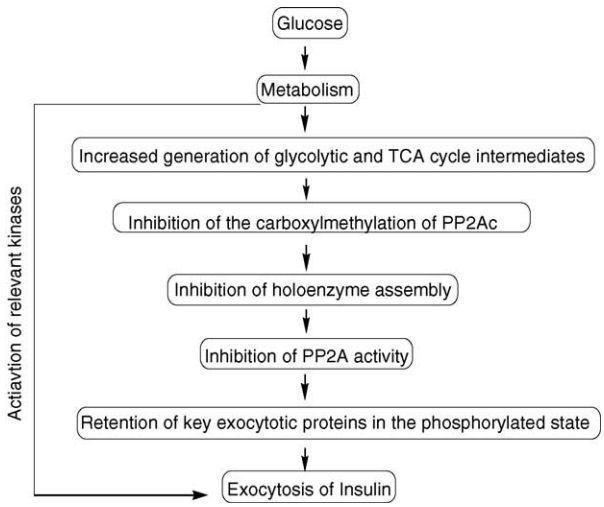

Fig. 1. Potential mechanisms underlying glucose-stimulated insulin secretion from the islet involving PP2A: A relative increase in intracellular levels of specific intermediates of glucose metabolism leads to inhibition of the CML of PP2Ac, which results in the inhibition of the holoenzyme assembly and the catalytic function of PP2A. This, in turn, retains putative exocytotic proteins in their phosphorylated state, which may be necessary for insulin secretion. Together, this model suggests dual regulatory effects of glucose on the phosphorylation status of candidate proteins, namely, stimulation and inhibition of protein kinases and PPases, respectively. CML: carboxylmethylation; PP2Ac: catalytic subunit of PP2A; PPases: protein phosphatase.

specific intermediates of glucose metabolism of the CML of PP2Ac in INS cell lysates [8]. Such inhibitory effects were also demonstrable in intact cells by glucose. Mannoheptulose, an inhibitor of glucose metabolism, completely prevented inhibitory effects of glucose on the CML of PP2Ac. Based on the available evidence, I propose (Fig. 1) that specific intermediates of glucose metabolism inhibit the CML of PP2Ac, resulting in functional inactivation of PP2A. This, in turn, might retain the key signaling proteins of the insulin exocytotic cascade in their phosphorylated state, leading to stimulated insulin secretion.

4.2. Regulation by calcium

Very little is known with regard to potential contributory roles of metal ions, specifically, calcium, on the catalytic function of PP2A in the isolated β cell. Previous studies demonstrated transient inhibition of PP2A activity in insulin-secreting cells by a membrane depolarizing concentration of KCl [7]. Compatible with these data, we recently reported that membrane-depolarization also induced inhibition of the CML of PP2Ac in intact INS cells [8]. It appears that the inhibitory effects of calcium on the CML of PP2Ac are specific since we observed no effects of other metal ions, such as manganese or magnesium on the CML of PP2Ac in β cell lysates [8]. Together, these findings support the formulation that calcium-induced secretion might also involve functional inactivation of PP2A, presumably via inhibition of the CML of PP2Ac. Thus, it may be reasonable to conclude that

calcium might exert dual effects on the net phosphorylation status of a given protein via activation and inhibition of specific kinases and PPases, respectively. In this context, it may be pointed out that both PP2A as well as PP1 are divalent cation-independent enzymes [3], and that earlier studies have demonstrated no significant effects by calcium [even in the presence of mM concentrations) on the function of purified PP2A preparations [7]. While these data do not directly support above described observations, they also do not exclude the possibility that calcium may still be of importance in a phosphorylation cascade in vivo (e.g. set in motion by potassium depolarisation), involving several kinases and phosphatases, in which PP2A may participate.

4.3. Regulation by amino acids

Sjoholm et al. [7] reported inhibitory effects by specific amino acids (e.g., arginine and glutamine) on PPase activities in isolated β cells. However, potential mechanisms whereby these amino acids elicit their inhibitory effects on PPase function remain to be verified. It may be likely that L-arginine's effects on PP2A activity may be mediated via an increased biosynthesis of polyamines, which have been shown to elicit regulatory effects on PP2A [14]. It is likely that the effects of L-glutamine on PP2A activity may be mediated by the increased glutamate synthesis, since glutamine is the precursor for the biosynthesis of glutamate via deamidation. Glutamate, in turn, has been shown to exert regulatory effects on PP2A function in the islet β cells. For example, we reported localization of a PP2A-like PPase activity in isolated β cells, which was stimulated by a mixture of glutamate and magnesium (referred to as GAPP; [15]). We studied roles for GAPP in the islet β cell, specifically in the context of its activation of acetyl CoA carboxylase (ACC). ACC, which is regulated by phosphorylation (inactive)–dephosphorylation (active), catalyzes the formation of malonyl-CoA, a precursor in the biosynthesis of long-chain fatty acids, which have been implicated in physiological insulin secretion ([15] and references therein). We observed that ACC activity was stimulated by magnesium in a concentration-dependent manner in lysates from a variety of insulin-secreting cells. Of all the dicarboxylic acids tested, only glutamate, albeit ineffective by itself, significantly potentiated magnesium-activated ACC. Further, ACC stimulation by glutamate and magnesium was markedly reduced by OKA. Pretreatment of the cytosolic fraction with anti-PP2A serum attenuated the GAPP-mediated activation of ACC, thereby suggesting that ACC may be regulated by an OKA-sensitive PP2A-like enzyme. Streptavidin-agarose chromatography studies have indicated that glutamate- and magnesium-mediated effects on ACC are attributable to increased dephosphorylation of ACC mediated by GAPP. These findings suggest that the stimulatory effects of glutamate and magnesium on ACC might involve activation of an OKA-sensitive PP2A-like enzyme that dephosphorylates and activates ACC.

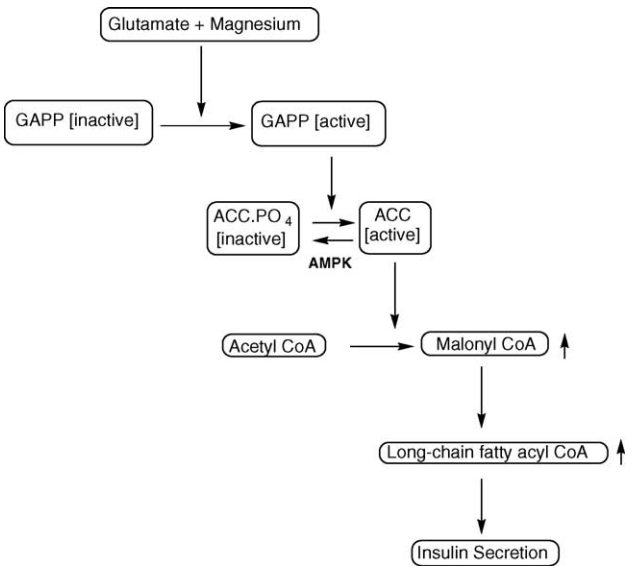

Fig. 2. Novel regulation by GAPP of ACC activity and insulin secretion in the islet: We propose that GAPP activation leads to dephosphorylation and activation of ACC, which catalyzes the conversion of acetyl CoA to malonyl CoA. Malonyl CoA is the precursor for the generation of long-chain fatty acyl CoA derivatives, which have been shown to be relevant for physiological insulin secretion. GAPP: glutamate- and magnesium-activated protein phosphatase; ACC: acetyl CoA carboxylase.

Further, 5-amino-imidazolecarboxamide (AICA) riboside, a stimulator of AMP-kinase, significantly inhibited glucose-mediated activation of ACC and insulin secretion from isolated β-cells [15]. Together, these findings suggest a unique regulatory mechanism for the activation of ACC in the pancreatic β-cell, leading to the generation of physiological signals that may be relevant for physiological insulin secretion (Fig. 2). While these findings identify a novel glutamate- and magnesium-sensitive PPases in the β cell, potential regulation of such proteins in the intact β cell, specifically in the context of physiological insulin secretion, remains to be examined further. Such studies are specifically important since considerable degree of controversy still exists with regard to a "second messenger" role for glutamate in physiological insulin secretion.

4.4. Regulation by adenine and guanine nucleotides

The net intracellular concentrations of adenine and guanine nucleotides (or intracellular ATP/ADP or GTP/GDP ratios) have been shown to control physiological insulin secretion [1]. Previous studies have suggested that adenine and guanine nucleotides also modulate PPase activity in the pancreatic β cell [7]. Our own experimental evidence indicated that micromolar concentrations of ATP, ADP, GTP or GDP each inhibited the CML of PP2Ac and catalytic activity of PP2A [5]. In these studies, we also observed that the nucleotide-mediated inhibition of CML of PP2Ac and the catalytic activity of PP2A were completely prevented by manganese or magnesium. Together, these findings suggest that divalent metal ions protect

against the inhibition by purine nucleotides of the CML of PP2Ac perhaps permitting PP2A to function under physiologic conditions. Additional studies are needed to verify potential significance of these observations in physiological insulin secretion.

4.5. Regulation by sulfonylureas

Using rat pancreatic islet lysates, Gagliardino et al. studied potential regulation by sulfonylureas of PPase activities [16]. They also reported a dose-dependent inhibition by sulfonylureas (e.g., glibenclamide) of PPase activity. Interestingly, kinetic analysis of these data indicated that the $K_{i\ 0.5}$ value for glibenclamide's action correlated well with its K_d for binding site, its EC_{50} on K_{ATP} channel and EC_{50} on insulin secretion. These findings implicate PPases as potential loci for sulfonylureas in the β cell. In contrast, recent investigations from Lehtihet et al [17] observed no clear effects of hypoglycemic sulfonylureas (e.g., glibenclamide) on PPase 1 and PP2A activities in RINm5F cell homogenates. Future studies will need to determine potential regulatory effects of sulfonylureas on islet endogenous PPases.

4.6. Regulation by inositol hexakiphosphate

In a recent study, Lehtihet et al. have reported [17] a dose-dependent inhibition of both PP1 and PP2A activities by inositol hexakiphosphate (IHP) in the β cell. These findings could have potential implications in further assigning a role for PPases in glucose-stimulated insulin secretion since earlier studies from this group demonstrated transient, but significant, increase in the intracellular concentrations of IHP in a glucose-stimulated β cell [18]. Therefore, it seems likely that inhibition of protein dephosphorylation by IHP might represent one of the mechanisms involved in physiological insulin secretion (see the following for additional discussion). It appears that the inhibitory effect of this signaling molecule on PP2A activity may not be due to its inhibition of the CML of PP2Ac, since we failed to observe any clear effects of IHP on the CML of PP2Ac under the conditions it inhibited the phosphatase activity (Kowluru et al., unpublished observations). Moreover, recent experimental evidence indicates that besides inhibiting PP2A activity, IHP also affects PKC activity and thus may be involved in orchestrating a phosphorylation cascade [19]. In summary, it seems that the islet PP2A is regulated, albeit differentially, by various modulators of insulin secretion. Moreover, based on the above discussion, it also appears that modulation of the CML of PP2Ac and catalytic function of PP2A may be dissociable under specific regulatory conditions by various modulators of islet function.

5. Potential regulatory roles for PP2A-like enzymes in the metabolic dysfunction and demise of the islet β cell

5.1. Regulation of PP2A by ceramides

Recent years have witnessed considerable progress in the area of pathophysiology of islet dysfunction, which tends to suggest that accumulation of intracellular ceramide, often demonstrable following long-term exposure of the β cell to fatty acids or cytokines, leads to metabolic dysfunction of the islet (see the following).

Ceramide and its metabolites are known to regulate cell proliferation and/or apoptotic demise in multiple cell types [20]. Intracellularly generated ceramide (either via de novo biosynthesis from fatty acids or via the hydrolysis of sphingomyelin) is proapototic in nature. Table 1 summarizes (in a chronological fashion) available data on potential roles of ceramide and its metabolites in β cell function. While there is general agreement that ceramide is

Table 1
Ceramides and their metabolites in the β cell function

Observations [reference]
C2- and C6 ceramides elicit inhibitory effects on insulin production and mitogenesis. Effects of ceramide mimicked those of IL-1β [21].
Neither IL-1β nor TNFα induce sphingomyelin hydrolysis in isolated islets. These studies concluded that sphingomyelin hydrolysis and ceramide generation are not critical for cytokine-induced NO release [22].
IL-1β-induced ceramide and DAG generation results in the activation of *c-Jun* kinase and ATF-2 in RINm5F cells [23].
A ceramide-activated protein phosphatase is present in HIT-T15 and INS-1 cells [24].
Ceramide content is significantly increased in prediabetic and diabetic ZDF rat islets. Fumonisin, a ceramide synthetase inhibitor, blocks fatty- acid induced DNA laddering in cultured ZDF islets [25].
IL-increases intracellular NO, ceramides, prostaglandins in insulin-secreting β cells [26].
Ceramides decrease β cell viability in a manner akin to cytokines. Cytokines fail to stimulate sphingomyelin hydrolysis and ceramide generation [27].
TNFα induces apoptosis in pancreatic β cells through TNFα receptor linked apoptotic factors and intracellular ceramide production [28].
The acid sphingomyelinase inhibitor SR33557 counteracts TNFα-mediated potentiation of IL-1β-induced NFkB activation in Rinm5F cells [29].
Sphingosine-1-phosphate stimulates insulin secretion from HIT-T15 cells and mouse islets [30].
Palmitate-induced effects on β cell dysfunction are mediated via intracellular accumulation of ceramide and the activation of mitochondrial apoptotic pathway [31].
Fatty acid-induced islet cell death is partially prevented by inhibitors of ceramide synthesis, and may be Bcl-2 regulated [32].
De novo synthesis of ceramide plays a regulatory role in lipoapoptosis of the β cell [33].
Endothelial differentiation gene receptors are localized in the β cell and may mediate sphingosine-1-phosphate mediated signaling steps [34].
Palmitate induces inhibition of glucose-induced insulin gene expression via ceramide synthesis [35].

generated in the β cell following exposure to fatty acids (e.g., palmitate), considerable debate still exists with regard to ceramide generation in models of β cell dysfunction elicited by cytokines (e.g., IL-1β; see Table 1). Salient features of those studies that addressed intra-islet accumulation of ceramide are described in Table 1. Studies from Unger's laboratory have reported significant increase in ceramide content in prediabetic and diabetic ZDF rat islets. Fumonisin, a ceramide synthetase inhibitor blocked fatty acid-induced DNA laddering in cultured ZDF islets [25]. Maedler et al. [31] reported that palmitate-induced effects on β cell dysfunction are mediated via intracellular accumulation of ceramide and the activation of mitochondrial apoptotic pathway. More recent studies from Poitout's laboratory described inhibition by palmitate of glucose-stimulated insulin gene expression via ceramide synthesis [35].

Several lines of recent evidence suggest that ceramides have direct stimulatory effects on specific kinases (see below) as well as phosphatases [36,37]. These phosphatases (referred to as ceramide-activated protein phosphatases; CAPPs) have been shown to be activated by both short chain as well as long-chain ceramides [38]. A series of elegant studies from the laboratory of Hannun have demonstrated that both protein phosphatase 1 (PP1) and PP2A are activated by ceramides [38]. In this context, original studies by Dubrowsky et al. [36] suggested significant structural similarities between CAPP and PP2A. Further studies from this laboratory also established structure-activity relationships between CAPP activation mediated by various analogs of ceramide. For example, using phenyl sepharose and MonoQ anion exchange chromatography, Hannun et al. were able to purify CAPP from rat brain [37]. Purified CAPP yielded three major bands on SDS-gels, which comigrated with the three subunits of PP2A. Western blot analysis of the purified CAPP indicated that it exists in heterotrimeric (i.e., AB′C and AbαC) as well as heterodimeric (A/C) forms.

Together, PP2A appears to be one of the phosphatases that is regulated by ceramide. In support of the formulation that ceramide plays a significant role in β cell dysfunction, we described the first evidence to suggest localization of a CAPP within the islet β cell [24]. We reported that cell-permeable ceramides stimulated OKA-sensitive (but not insensitive), PP2A-like phosphatase activity in a concentration-dependent manner. Further, OKA (in low nanomole concentrations) inhibited CAPP activity in low nM concentrations, suggesting that CAPP may be akin to PP2A. However, no stimulatory effects by ceramides were demonstrable on the CML of PP2Ac, suggesting that the CML step is not essential for CAPP activation. Together, our data identified CAPP as one of the possible loci at which ceramides might exert their effects on β cells leading to altered insulin secretion, and decreased cell viability followed by apoptotic cell demise [24]. However, it is not known whether the β cell PP1 is also regulated by ceramides; this needs to be verified further.

What then are potential phosphoprotein substrates, whose dephosphorylation is mediated by CAPP? Original studies by Ruvolo et al. provided convincing evidence to indicate ceramide-induced dephosphorylation of Bcl-2 via a mitochondrial PP2A-like enzyme [39]. Further, they implicated B56α subunit of PP2A in ceramide-induced dephosphorylation of mitochondrial Bcl-2. Along these lines, we recently identified and characterized an OKA-sensitive CAPP activity in the mitochondrial fraction purified from insulin-secreting cells, which catalyzed the dephosphorylation of Bcl-2 (unpublished work from this laboratory). Therefore, it is reasonable to speculate that activation of CAPP leads to mitochondrial abnormalities, including changes in the membrane potential, release of cytochrome C and subsequent activation of caspases, leading to metabolic dysfunction and demise of the β cell. It is also likely that CAPP might mediate dephosphorylation and inactivation of other key signaling proteins involved in β cell proliferation, such as Akt (protein kinase B). Akt is functionally activated in its phosphorylated form, which is necessary for its signaling role in cell proliferation [40]. Indeed, accumulating evidence suggests potential dephosphorylation and inactivation of Akt mediated by CAPP-like phosphatase [41,42]. While such data are lacking in the β cell, it may be reasonable to speculate that CAPP might mediate dephosphorylation and inactivation of Akt leading to inhibition of cellular proliferation.

Therefore, it is plausible that CAPP might play regulatory roles in the loss of β cell mass either via inducing apoptotic demise of the β cell via dephosphorylation and inactivation of Bcl-2 or via dephosphorylation and inactivation of key signaling proteins (e.g., Akt) involved in β cell proliferation (Fig. 3). This warrants an immediate need for additional experimentation not only to understand subunit structure and nature of regulation by ceramide of CAPP in the islet, but also to develop specific inhibitors (see the following), which might prove beneficial for the preservation of loss of β cell mass often demonstrable in models of β cell dysfunction (Fig. 3).

5.2. Other potential actions of ceramide

In addition to activating the CAPP, ceramide has been shown to activate specific protein kinases, such as the Raf-1 kinase. Yao et al. [43] reported that a ceramide-activated protein kinase phosphorylates Raf-1 at Thr-269 residue, which appears to be critical for its activation of MEK kinase cascade. Based on our current knowledge in the area of Raf-1/MEK kinase signaling cascade, it is reasonable then to assume that ceramide-mediated activation of this signaling step would provide a proliferative signal, instead of an apoptotic signal leading to loss of β cell mass. Interestingly, however, our recent findings appear to impli-

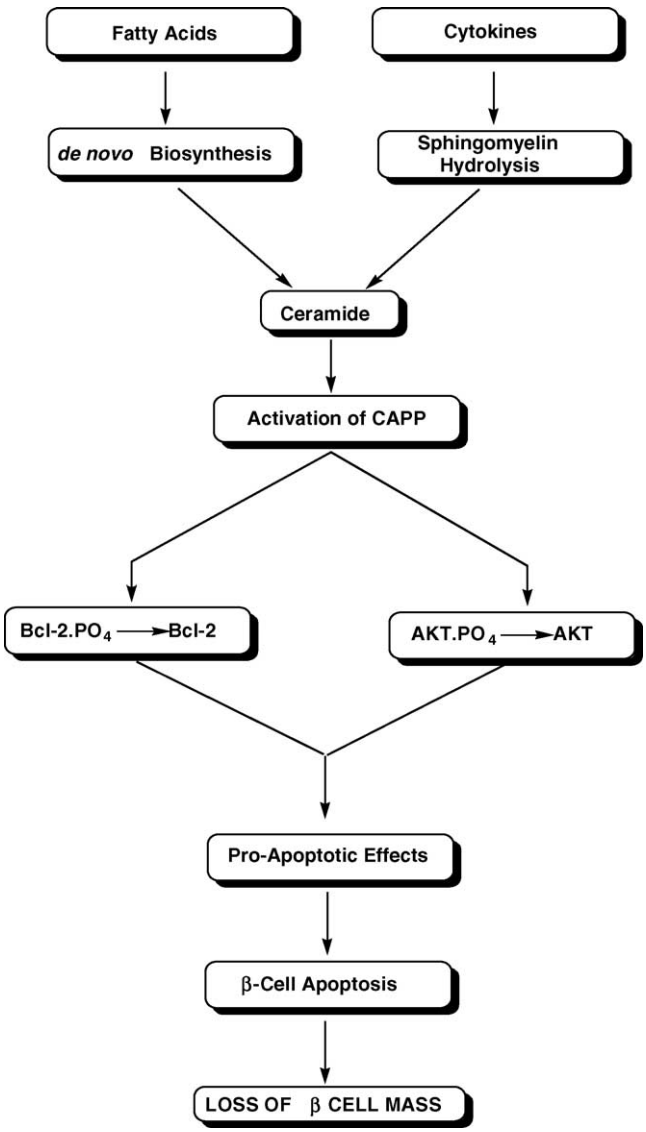

Fig. 3. Proposed roles for CAPP in the loss of islet β cell mass: Intracellular accumulation of ceramide takes place either via de novo biosynthesis from fatty acids or from the hydrolysis of sphingomyelin by sphingomyelinase. I propose that there may be at least two substrates for CAPP in the islet β cell. The first one is Bcl-2, which is pro-apoptotic in its dephosphorylated form. It also appears that CAPP mediates dephosphorylation and inactivation of Akt, rendering it proapoptotic as well. Together, it is likely that an increase in intracellular generation of ceramide could promote β cell apoptosis as well as inhibition of cellular proliferation culminating in the loss of β cell mass and the onset of diabetes. CAPP: ceramide-activated protein phosphatase.

cate the Ras/Raf-1 signaling pathway in cytokine-induced iNOS gene expression, NO release and subsequent metabolic dysfunction of the islet. For example, using selective inhibitors of requisite post-translational modifications (e.g., farnesylation and palmitoylation) of Ras as well as Clostridial toxins, which irreversibly glucosylate and inactivate Ras [44–46], we demonstrated a critical role for Ras in IL-induced iNOS gene expression and NO release in the β cell. Therefore, it is likely that cytokine-mediated effects on the β cell might include activation of CAPP (and dephosphorylation of mitochondrial Bcl-2) as well as ceramide-activated protein kinases (and activation of Raf-1 kinase) at different stages of signaling pathway leading to the metabolic dysfunction of the β cell.

It may also be pointed out that intracellularly generated NO appears to play positive as well as negative modulatory roles in β cell function. For example, recent studies by Rizzo and Piston [47] demonstrated an essential role for NO in the normal functioning of the islet β cell. They showed that functional regulation and association of glucokinase with the secretory granule fraction requires post-translational *S*-nitrosylation, which is mediated via the neuronal form of NOS-derived generation of NO. On the other hand, NO released following activation of the inducible form NOS elicits detrimental effects on β cell viability leading to apoptosis [44–46]. Together, this evidence appears to implicate positive as well as negative modulatory roles for NO in islet function.

5.3. Regulation of nuclear PP2A by cytokines

Proinflammatory cytokines (e.g., IL-1β) also appear to modulate activation of PP2A-like phosphatases in β cells. For example, unpublished evidence from our laboratory indicates localization of an OKA-sensitive PP2A-like protein phosphatase in the nuclear fraction purified from insulin-secreting INS-1 cells. A protein with an M_r of 36 kDa (similar to the size of PP2Ac) also underwent post-translational CML in an OKA-sensitive manner. Exposure of INS-1 cells to IL-1β resulted in a marked increase in nitric oxide release with a concomitant reduction in the CML and phosphatase activity. We propose that cytokine-mediated inhibition of nuclear PP2A might retain key nuclear proteins (e.g., lamin B) in their phosphorylated state leading to nuclear changes and apoptotic demise of the β cell [46].

5.4. Is PP2A also activated by nutrient secretagogues?

In the above sections, an argument was made to indicate that insulin secretagogues exert inhibitory effects on PP2A to retain key exocytotic proteins in their phosphorylated state, which may be required for insulin secretion. However, evidence is also available to suggest that glucose promotes activation of PP2A in the islet β cell. Yan et al. [48] recently reported regulatory roles for PP2A in glucose-mediated effects dephosphorylation of the elongation factor-2 (EF-2) and subsequent protein translation in INS-1-derived 832/13 cells. Further, Sato et al. reported OKA-induced inhibition of glucose-stimulated insulin secretion from mouse pancreatic islets [49], suggesting that phosphatase activation is necessary for glucose-stimulated insulin secretion. These investigators provided evidence to suggest that OKA-mediated inhibition of insulin secretion is due to an attenuated calcium signal in the β cell and its efficacy on insulin exocytosis [49]. Together, the above

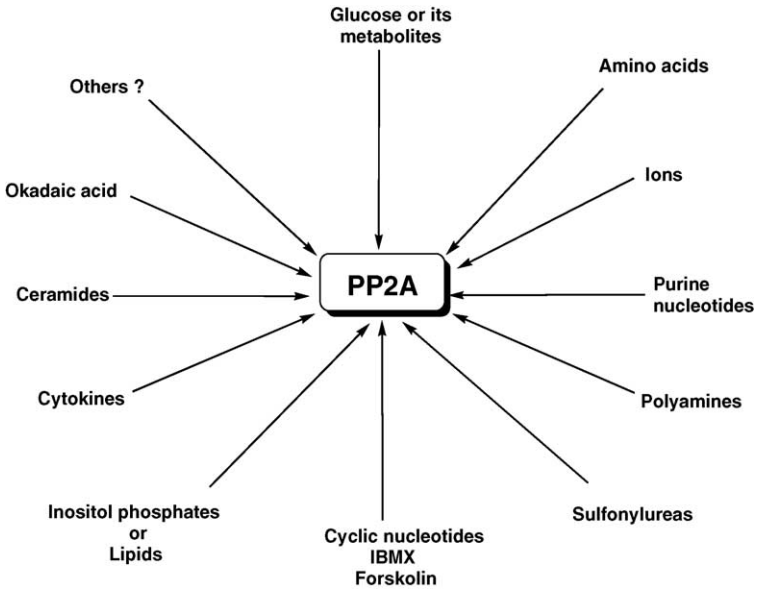

Fig. 4. Multifactorial regulation of PP2A-like enzymes in the islet β cell: PP2A plays a central role in the islet cell survival and demise. Note that most, but not all, of these modulators are known to regulate PP2A function via their effects on the CML of PP2Ac. It remains to be determined whether the modulators, which do not act via regulation of the CML of PP2Ac, have any regulatory roles in the holoenzyme assembly or their effects are due to direct regulation of the phosphatase function. CML: carboxylmethylation; PP2Ac: the catalytic subunit of protein phosphatase 2A.

observations provide additional insights into the complex regulatory mechanisms of protein phosphorylation–dephosphorylation that take place within the β cell, which are mediated by glucose to facilitate insulin secretion from the islet β cell.

6. Conclusions and future directions

It is apparent from the foregoing discussion that PP2A - like enzymes play significant regulatory roles in the normal health and disease of the islet β cell. It is apparent that this enzyme[s] is subjected to multifactorial regulation in the islet (Fig. 4). While potential regulatory effects of some of these agents, including insulin secretagogues, are understood to a large degree, very little is known with regard to regulatory mechanisms underlying individual holoenzyme assemblies that appear to dictate the substrate specificity, subcellular localization of PP2A and its sensitivity to various modulators. It may be reasonable to speculate that different holoenzyme assemblies might dictate the sensitivity of PP2A to various modulators such as glucose (or its metabolites), amino acids, ceramide, etc. Furthermore, the post-translational CML of PP2Ac appears to be required for the regulation by these modulators, which further warrants additional investigations.

Based on this discussion, there seem to be significant potential for at least three possible avenues for future research in this area. The first one is to examine modulatory roles of PP2A as a regulator of protein kinases, which remains an unexplored frontier in the islet. For example, PP2A appears to regulate dephosphorylation and inactivation of several important kinases, which are transiently phosphorylated and activated following stimulation (see [50] for a review). While considerable effort has been invested into identification of various kinases that phosphorylate and activate their kinase substrates, very little is known, specifically in the islet, on phosphatase-mediated regulation of these kinases. Again, PP2A appears to be at the center since >30 protein kinases are modulated by PP2A under in vitro conditions and that several of these kinases appear to form complexes with PP2A [50]. Some of these kinases, which are of potential significance to the islet include: p70 S6 kinase, protein kinase C, AMP-kinase, calcium-calmodulin-dependent protein kinase, MEK and ERK kinases. Another important kinase that is involved in the regulation of islet function, specifically at the level of translation, is the mammalian target of rapamycin (mTOR), which is a serine/threonine protein kinase. Experimental evidence in multiple cell types clearly suggests that PP2A plays significant role in rapamycin-sensitive pathway in the induction of translation via interaction with Tap42/α4, the regulatory component of TOR signaling cascade [51]. However, very little is known with regard to potential roles of PP2A in the mTOR signaling pathway in the islet. This is a promising area for future investigation.

A second area that requires significant attention is about the functional modulation of ion channel activity via phosphorylation–dephosphorylation reactions (see [52] for a review). For example, recent studies by Berggren et al. have demonstrated that ion channels in hippocampal neurons (and potentially β cells) are modulated via phosphorylation–dephosphorylation cascade [53]. Based on additional experimental evidence, they proposed a model which states that functional status of calcium channel proteins depends up on a fine balance between the protein

kinase A-mediated phosphorylation (to retain the active conformation) and a serine/threonine-sensitive protein phosphatase (e.g., PP2A)-mediated dephosphorylation (resulting in an inactive conformation). These observations become physiologically relevant since IHP has been shown to directly inhibit PP2A-like phosphatase (see above) while indirectly stimulating intracelluar accumulation (and thereby protein kinase A activation) of cAMP [53]. Therefore, it is conceivable that disruption of basal phosphorylation of channel proteins via activation of putative phosphatases, such as PP2A, could result in metabolic dysfunction of the islet β cell. Additional studies are required, however, to determine not only the nature and identity of these phosphatases, but also to develop structure-specific inhibitors for these PPases, which could be employed to prevent the loss ion channel function due to dephosphorylation.

A third area with significant potential for further research is the development of novel inhibitors for these phosphatases for the prevention of loss of β cell mass and onset of diabetes. This could be a daunting task since the PP2A holoenzyme is heterotrimeic in its composition with numerous variants of the regulatory, structural and catalytic subunits. Furthermore, not much is known with regard to potential subcellular distribution of these phosphatases, and targeting of these inhibitors to specific subcelluar compartment may prove to be an additional challenge. As discussed above, our own findings appear to suggest localization of PP2A-like phosphatases in the cytosolic [4,5,14,24], nuclear (Veluthakal, Wadzinski, Kowluru, unpublished) and mitochondrial [54] fractions. It should also be kept in mind that individual subunits may be subjected to translocation from one subcellular compartment to the other in the presence of an agonist to facilitate the formation of an ''appropriate'' holoenzyme [39]. Despite these inherent complexities, it is likely that potentially new information will emerge in the coming years with regard to novel pharmacological probes to modulate PP2A function.

In this context, at the outset, it may be worthwhile for the islet biologists to initiate research efforts for the development of novel inhibitors for the CAPP, since considerable amount of recent research is directed toward understanding the holoenzyme composition of CAPP [20,36–39]. It is important to note that some of the known inhibitors of PP2A, such as OKA, calyculin A, tautomycin, microcystin, nodularin, and cantharidin also inhibit other PPase activities (albeit with different degrees of specificity) and hence can not be used as specific inhibitors for CAPP. Recent reports by Sakoff and McCluskey provided potential clues for the development of phosphatase inhibitors via crystal structure and molecular modeling approaches [55]. Investigations from Hannun's group provided fresh insights into the structural requirements for ceramide activation of PP2A; they also yielded excellent clues on potential transformation of these novel ceramide analogs into inhibitors of CAPP function [56]. Given the importance of ceramide in modulating the phosphatase activities, development of specific inhibitors for CAPP in particular, would be beneficial in preserving the β cell mass and the onset of diabetes. Further, identification and synthesis of specific inhibitors of various PPases might prove useful in the development of therapeutic intervention modalities for disease states, such as cancer and Alzheimer's disease, since PPases have been implicated in these pathophysiology of these disease states as well [57].

Acknowledgement

I thank the Department of VA Medical Research Service, the National Institutes of Health (DK 56005) and the American Diabetes Association for financial support. I also thank the Department of VA for the Research Career Scientist award. I would like to acknowledge the able assistance and support from my former colleagues at the University of Wisconsin—Madison and my present colleagues at Wayne State University and the John D Dingell VA Medical Center-Detroit.

References

[1] Prentki M, Matschinsky FM. Calcium, cAMP, and phospholipid-derived messengers in coupling mechanisms of insulin secretion. Physiol Rev 1987;67:1185–248.
[2] Jones PM, Persaud SJ. Protein kinases, protein phosphorylation, and the regulation of insulin secretion from pancreatic beta-cells. Endocr Rev 1998;19:429–61.
[3] Cohen P. The structure and regulation of protein phosphatases. Annu Rev Biochem 1989;58:453–508.
[4] Kowluru A, Seavey SE, Rabaglia ME, Nesher R, Metz SA. Carboxylmethylation of the catalytic subunit of protein phosphatase 2A in insulin-secreting cells: evidence for functional consequences on enzyme activity and insulin secretion. Endocrinology 1996;137:2315–23.
[5] Kowluru A, Metz SA. Purine nucleotide- and sugar phosphate-induced inhibition of the carboxyl methylation and catalysis of protein phosphatase-2A in insulin-secreting cells: protection by divalent cations. Biosci Rep 1998;18:171–86.
[6] Sjoholm A, Lehtihet M, Efanov AM, Zaitsev SV, Berggren PO, Honkanen RE. Glucose metabolites inhibit protein phosphatases and directly promote insulin exocytosis in pancreatic beta-cells. Endocrinology 2002;143:4592–8.
[7] Sjoholm A, Honkanen RE, Berggren PO. Inhibition of serine/threonine protein phosphatases by secretagogues in insulin-secreting cells. Endocrinology 1995;136:3391–7.
[8] Palanivel R, Veluthakal R, Kowluru A. Regulation by glucose and calcium of the carboxylmethylation of the catalytic subunit of protein phosphatase 2A in insulin-secreting INS-1 cells. Am J Physiol Endocrinol Metab 2004;286:1032–41.
[9] Janssens V, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem J 2001;353:417–39.
[10] Leulliot N, Quevillon-Cheruel S, Sorel I, de La Sierra-Gallay IL, Collinet B, Graille M, et al. Structure of protein phosphatase methyl-

transferase 1 (PPM1), a leucine carboxyl methyltransferase involved in the regulation of protein phosphatase 2A activity. J Biol Chem 2004;279:8351–8.

[11] Favre B, Zolnierowicz S, Turowski P, Hemmings BA. The catalytic subunit of protein phosphatase 2A is carboxyl-methylated in vivo. J Biol Chem 1994;269:16311–7.

[12] De Baere I, Derua R, Janssens V, Van Hoof C, Waelkens E, Merlevede W, et al. Purification of porcine brain protein phosphatase 2A leucine carboxylmethyltransferase and cloning of the human homologue. Biochemistry 1999;38:16539–47.

[13] Chen J, Martin BL, Brautigan DL. Regulation of protein serine–threonine phosphatase type-2A by tyrosine phosphorylation. Science 1992;257:1261–4.

[14] Sjoholm A, Honkanen RE. Polyamines regulate serine/threonine protein phosphatases in insulin-secreting cells. Pancreas 2000;20: 32–7.

[15] Kowluru A, Chen HQ, Modrick LM, Stefanelli C. Activation of acetyl-CoA carboxylase by a glutamate- and magnesium-sensitive protein phosphatase in the islet beta-cell. Diabetes 2001;50:1580–7.

[16] Gagliardino JJ, Rossi PF, Garcia ME. Inhibitory effect of sulfonylureas on protein phosphatase activity in rat pancreatic islets. Acta Diabetol 1997;34:6–9.

[17] Lehtihet M, Honkanen RE, Sjoholm A. Inositol hexakisphosphate and sulfonylureas regulate beta-cell protein phosphatases. Biochem Biophys Res Commun 2004;316:893–7.

[18] Larsson O, Barker CJ, Sjoholm A, Carlqvist H, Michell RH, Bertorello A, et al. Inhibition of phosphatases and increased calcium channel activity by inositol hexakiphosphate. Science 1997;278:471–7.

[19] Efanov AM, Zaitsev SV, Berggren PO. Inositol hexakisphosphate stimulates non-Ca^{2+}-mediated and primes Ca^{2+}-mediated exocytosis of insulin by activation of protein kinase C. Proc Natl Acad Sci USA 1997;94:4435–9.

[20] Ruvolo PP. Intracellular signal transduction pathways activated by ceramide and its metabolites. Pharmacol Res 2003;47:383–92.

[21] Sjoholm A. Ceramide inhibits pancreatic beta-cell insulin production and mitogenesis and mimics the actions of interleukin-1 beta. FEBS Lett 1995;367:283–6.

[22] Kwon G, Bohrer A, Han X, Corbett JA, Ma Z, Gross RW, et al. Characterization of the sphingomyelin content of isolated pancreatic islets. Evaluation of the role of sphingomyelin hydrolysis in the action of interleukin-1 to induce islet overproduction of nitric oxide. Biochim Biophys Acta 1996;1300:63–72.

[23] Welsh N. Interleukin-1 beta-induced ceramide and diacylglycerol generation may lead to activation of the c-Jun NH2-terminal kinase and the transcription factor ATF2 in the insulin-producing cell line RINm5F. J Biol Chem 1996;271:8307–12.

[24] Kowluru A, Metz SA. Ceramide-activated protein phosphatase-2A activity in insulin-secreting cells. FEBS Lett 1997;418:179–82.

[25] Shimabukuro M, Higa M, Zhou YT, Wang MY, Newgard CB, Unger RH. Lipoapoptosis in beta-cells of obese prediabetic fa/fa rats. Role of serine palmitoyltransferase overexpression. J Biol Chem 1998;273: 32487–90.

[26] Sjoholm A. Aspects of the involvement of interleukin-1 and nitric oxide in the pathogenesis of insulin-dependent diabetes mellitus. Cell Death Differ 1998;5:461–8.

[27] Major CD, Gao ZY, Wolf BA. Activation of the sphingomyelinase/ceramide signal transduction pathway in insulin-secreting beta-cells: role in cytokine-induced beta-cell death. Diabetes 1999;48: 1372–80.

[28] Ishizuka N, Yagui K, Tokuyama Y, Yamada K, Suzuki Y, Miyazaki J, et al. Tumor necrosis factor alpha signaling pathway and apoptosis in pancreatic beta cells. Metabolism 1999;48:1485–92.

[29] Saldeen J, Jaffrezou JP, Welsh N. The acid sphingomyelinase inhibitor SR33557 counteracts TNF-alpha-mediated potentiation of IL-1beta-induced NF-kappaB activation in the insulin-producing cell line Rinm5F. Autoimmunity 2000;32:241–54.

[30] Shimizu H, Okajima F, Kimura T, Ohtani K, Tsuchiya T, Takahashi H, et al. Sphingosine 1-phosphate stimulates insulin secretion in HIT-T 15 cells and mouse islets. Endocrine J 2000;47:261–9.

[31] Maedler K, Spinas GA, Dyntar D, Moritz W, Kaiser N, Donath MY. Distinct effects of saturated and monounsaturated fatty acids on beta-cell turnover and function. Diabetes 2001;50:69–76.

[32] Lupi R, Dotta F, Marselli L, Del Guerra S, Masini M, Santangelo C, et al. Prolonged exposure to free fatty acids has cytostatic and pro-apoptotic effects on human pancreatic islets: evidence that beta-cell death is caspase mediated, partially dependent on ceramide pathway, and Bcl-2 regulated. Diabetes 2002;51:1437–42.

[33] Unger RH, Orci L. Lipoapoptosis: its mechanism and its diseases. Biochim Biophys Acta 2002;1585:202–12.

[34] Laychock SG, Tian Y, Sessanna SM. Endothelial differentiation gene receptors in pancreatic islets and INS-1 cells. Diabetes 2003;52:1986–93.

[35] Kelpe CL, Moore PC, Parazzoli SD, Wicksteed B, Rhodes CJ, Poitout V. Palmitate inhibition of insulin gene expression is mediated at the transcriptional level via ceramide synthesis. J Biol Chem 2003;278: 30015–21.

[36] Dobrowsky RT, Kamibayashi C, Mumby MC, Hannun YA. Ceramide activates heterotrimeric protein phosphatase 2A. J Biol Chem 1993; 268:15523–30.

[37] Galadari S, Kishikawa K, Kamibayashi C, Mumby MC, Hannun YA. Purification and characterization of ceramide-activated protein phosphatases. Biochemistry 1998;37:11232–8.

[38] Chalfant CE, Kishikawa K, Mumby MC, Kamibayashi C, Bielawska A, Hannun YA. Long chain ceramides activate protein phosphatase-1 and protein phosphatase-2A. J Biol Chem 1999;274:20313–7.

[39] Ruvolo PP, Deng X, Ito T, Carr BK, May WS. Ceramide induces Bcl2 dephosphorylation via a mechanism involving mitochondrial PP2A. J Biol Chem 1999;274:20296–300.

[40] Downward J. PI 3-kinase Akt and cell survival. Semin Cell Dev Biol 2004;15:177–82.

[41] Schmitz-Peiffer C, Craig DL, Biden TJ. Ceramide generation is suffiecient to account for the inhibition of insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palimitate. J Biol Chem 1999;274:24202–10.

[42] Salinas M, Lopez-Valdaliso R, Martin D, Alvarez A, Cuadrado A. Inhibition of PKB/Akt1 by C2-ceramide involves activation of ceramide-activated protein phosphatase in PC12 cells. Mol Cell Neurosci 2000;15:156–69.

[43] Yao B, Zhang Y, Delikat S, Mathias S, Basu S, Kolesnick R. Phosphorylation of Raf by ceramide-activated protein kinase. Nature 1995;378:307–10.

[44] Tannous M, Amin R, Popoff MR, Fiorentini C, Kowluru A. Positive modulation by Ras of interleukin 1β-mediated nitric oxide generation in insulin-secreting clonal β (HIT-T15) cells. Biochem Pharmacol 2001;62:1459–68.

[45] Chen HQ, Tannous M, Veluthakal R, Amin R, Kowluru A. Novel roles for palmitoylation of Ras in IL-1β-induced nitric oxide release and caspase 3 activation in insulin-secreting β cells. Biochem Pharmacol 2003;66:1681–94.

[46] Veluthakal R, Amin R, Kowluru A. Interleukin 1beta induces post-translational carboxylmethylation and alterations in subnuclear distribution of lamin B in insulin-secreting RINm5F cells. Am J Physiol Cell Physiol 2004;287:1152–62.

[47] Rizzo MA, Pison DW. Regulation of β cell glucokinase by S-nitrosylation and association with nitric oxide synthase. J Cell Biol 2003;161:243–8.

[48] Yan L, Nairn AC, Palfrey HC, Brady MJ. Glucose regulates EF-2 phosphorylation and protein translation by a protein phosphatase-2A-dependent mechanism in INS-1-derived 832/13 cells. J Biol Chem 2003;278:18177–83.

[49] Sato Y, Mariot P, Detimary P, Gilon P, Henquin JC. Okadaic acid-induced decrease in the magnitude and efficacy of the Ca2+ signal in

pancreatic beta cells and inhibition of insulin secretion. Br J Pharmacol 1998;123:97–105.

[50] Millward TA, Zolnierowicz S, Hemmings BA. Regulation of protein kinase cascades by protein phosphatase 2A. Trends Biochem Sci 1999;24:186–91.

[51] Hartley D, Cooper GM. Role of mTOR in the degradation of IRS-1: regulation of PP2A activity. J Cell Biochem 2002;85:304–14.

[52] Yang S-N, Berggren P-O. β-cell Ca_v channel regulation in physiology and pathophysiology. Am J Physiol Endocrinol Metab 2005;288: 16–28.

[53] Yang S-N, Yu J, Mayr GW, Hofman F, Larsson O, Berggren P-O. Inositol hexakiphosphate increases L-type Ca^{2+} channel activity by stimulation of adenylate cyclase. FASEB J 2001;15:1753–63.

[54] Veluthakal R, Palanivel R, Zha Y, McDonald P, Gruber S, Kowluru A. Ceramide induces mitochondrial abnormalities in insulin-secreting INS-1 cells: potential mechanisms underlying ceramide-mediated metabolic dysfunction of the β cell. Apoptosis (in press).

[55] Sakoff JA, McCluskey A. Protein phosphatase inhibition: structure based design. Towards new therapeutic agents. Annu Rev Pharmacol Toxicol 2005;54:725–50.

[56] Chalfant CE, Szulc Z, Roddy P, Bielawska A, Hannun YA. The structural requirements for ceramide activation of serine–threonine protein phosphatases. J Lipid Res 2004;45:496–506.

[57] Ducruet AP, Vogt A, Wipf P, Lazo JS. Dual specificity protein phosphatases: therapeutic targets for cancer and Alzheimer's disease. Annu Rev pharmacol Toxicol 2004. Epub ahead of print.