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Upregulation of phagocyte-like NADPH oxidase by cytokines in pancreatic beta-cells: Attenuation of oxidative and nitrosative stress by 2-bromopalmitate

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Abstract

Phagocyte-like NADPH oxidase (Nox2) has been shown to play regulatory roles in the metabolic dysfunction of the islet β -cell under the duress of glucolipotoxic conditions and exposure to proinflammatory cytokines. However, the precise mechanisms underlying Nox2 activation by these stimuli remain less understood. To this end, we report a time-dependent phosphorylation of p47phox, a cytosolic subunit of Nox2, by cytomix (IL-1 β +TNF α +IFN γ) in insulin-secreting INS-1 832/13 cells. Furthermore, cytomix induced the expression of gp91phox, a membrane component of Nox2. 2-Bromopalmitate (2-BP), a known inhibitor of protein palmitoylation, markedly attenuated cytokine-induced, Nox2-mediated reactive oxygen species (ROS) generation and inducible nitric oxide synthase-mediated nitric oxide (NO) generation. However, 2-BP failed to exert any significant effects on cytomix-induced CHOP expression, a marker for endoplasmic reticulum stress. Together, our findings identify palmitoyltransferase as a target for inhibition of cytomix-induced oxidative (ROS generation) and nitrosative (NO generation) stress in the pancreatic β -cell.

Keywords

Pancreatic β -cell; NADPH-oxidase; 2-Bromopalmitate; Cytokines; Oxidative stress; Nitrosative stress

1. Introduction

Type 1 diabetes is characterized by an absolute insulin deficiency arising from progressive autoimmune destruction of insulin-secreting pancreatic β -cells [1–3]. During the progression of this disease, several proinflammatory cytokines, including IL-1 β , TNF α and IFN γ are secreted into the islets by infiltrated, and activated T cells and macrophages, which, in turn, elicit damaging effects on pancreatic β -cells. However, putative mechanisms and the underlying signaling mechanisms involved in cytokine-induced loss of β -cells remain only partially understood [4, 5]. Among numerous mechanisms proposed, IL-1 β has been demonstrated to mediate its effects via the induction of inducible nitric oxide synthase

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(iNOS), which, in turn, promotes cytotoxic NO release culminating in β -cell demise. In addition, TNF α and IFN γ elicit regulatory effects on β -cell function via regulation of individual metabolic signaling steps leading to cell death [6–8]. Along these lines, previous studies have suggested that IFN γ sensitizes human islets to the effects of IL-1 β [9].

In addition to NO, several recent studies including our own have suggested that phagocyte-like NADPH oxidase (Nox2) contributes to loss of β -cell function, metabolic dysregulation and the loss of β -cell mass under the duress of pathological conditions such as glucolipotoxicity and exposure to cytokines. Specifically, we have demonstrated that chronic exposure of isolated β -cells to cytomix leads to Nox2 holoenzyme activation, ROS generation, mitochondrial dysfunction and cell death [10–14]. Moreover, using selective inhibitors of Rac1, a small G-protein, which is a member of Nox2 holoenzyme, we have been able to demonstrate that inhibition of Rac1 activation leads to prevention of ROS generation and mitochondrial dysregulation in isolated β -cells [14–16]. Interestingly however, we failed to see any clear effects of Rac1 inhibitors on cytomix-induced NO generation suggesting involvement of a Rac1-independent mechanism for cytomix-induced NO release in these cells.

Jastroch recently pointed out a need for the development of pharmacological probes/reagents that can block both ROS and NO generation in pancreatic β -cells exposed to cytomix as simultaneous release of both ROS and NO could lead to the formation of peroxynitrite, which may be more damaging to the β -cell compared to ROS or NO alone [17]. Therefore, as a logical extension to our recently published evidence [14], we undertook an investigation to assess the roles of protein palmitoylation in the cascade of events leading to cytomix-induced ROS and NO generation in INS-1 832/13 cells. We describe evidence to suggest that 2-bromopalmitate, a selective inhibitor of protein palmitoylation, markedly attenuates cytomix-mediated ROS and NO generation in these cells. Our findings identify palmitoyltransferase as a target for inhibition of cytomix-induced oxidative (ROS generation) and nitrosative (NO generation) stress in the pancreatic β -cell.

2. Materials and methods

2.1. Materials

IL-1 β , TNF α and IFN γ were purchased from R & D Systems (Minneapolis, MN). Antisera directed against p-p47phox (*NCF1*) and gp91phox were from Abcam (Abcam Inc, Cambridge MA). Anti-p47phox was from Santa Cruz Biotechnology (Santa Cruz, CA). 2', 7-dichlorofluorescein diacetate (DCHF-DA), 2-bromopalmitate, Griess reagent were obtained from Sigma-Aldrich (St. Louis, MO). Anti-rabbit IgG-horseradish peroxidase conjugate and enhanced chemiluminescence (ECL) kits were from Amersham Biosciences (Piscataway, NJ).

2.2. Insulin-secreting INS-1 832/13 cells and culture conditions

INS-1 832/13 cells were cultured in RPMI-1640 medium containing 10% heat inactivated FBS supplemented with 100 IU/ml penicillin and 100 IU/ml streptomycin, 1mM sodium pyruvate, 50 μ M 2-mercaptoethanol and 10mM HEPES (pH 7.4). The cultured cells were subcloned twice weekly following trypsinization and passages 53–61 were used for the study. INS-1 832/13 cells were incubated with cytomix (IL-1 β , TNF α and IFN γ ; 10 ng/ml each) or individual cytokines (25ng/ml) for 0–24 hrs as indicated in the text. In select studies, INS-1 832/13 cells were incubated with cytomix (IL-1 β , TNF α and IFN γ ; 10 ng/ml each) in the presence and absence of 2-bromopalmitate (100 μ M) for 24hrs. At the end of the incubation period the cells were harvested and lysed in RIPA buffer containing 1 μ g/ml protease inhibitor cocktail, 1mM NaF, 1mM PMSF, and 1mM Na₃VO₄.

2.3. Isolation of membrane fraction

INS-1 832/13 cells were treated with cytomix for the designated time points as indicated in the text. At the end of the incubation period, cells were washed with ice-cold PBS, harvested by scraping and pelleted by centrifugation at 2,000 rpm for 5 min at 4°C. After re-suspending in the homogenizing buffer (50mM Tris-HCl, pH 7.4, 250mM sucrose, 1mM EDTA, 1mM DTT, 1mM PIC, 1mM PMSF and 1mM Na₃VO₄) and sonication, unbroken cells and nuclei were separated by centrifugation at 1300 rpm for 10min. The cleared supernatant was further centrifuged at 40,000 rpm for 30 min (Optima™ MAX Ultracentrifuge). The cytosol fraction was separated and the membrane fraction was dissolved in 2% CHAPS.

2.4. ROS generation assay

INS-1 832/13 cells were plated in a 6 well plate and treated with cytomix (IL-1 β , TNF α and INF γ ; 10 ng/ml each) or individual cytokines (25ng/ml) for 24 hrs as indicated in the text. Subsequently, the media was removed and the cells were incubated further with 2'-dichlorofluorescein diacetate (DCHF-DA) at 37°C for 30 min. The cells were washed twice with ice-cold PBS and harvested, followed by loading equal amount of protein (50 μ g) and reading the fluorescence using luminescence spectrophotometer (Ex:485nm and Em:535nm) (PerkinElmer, Waltham, MA).

2.5. NO release assay

INS-1 832/13 cells were plated in a 6 well plate and treated with cytomix (IL-1 β , TNF α and INF γ ; 10 ng/ml each) for 24 hrs as indicated in the text. At the end of the incubation period, the media was collected and centrifuged at 1000 g for 5 min. Equal amount of media and Griess reagent were mixed and the absorbance (540nm) was measured using a microplate reader (Molecular Devices, Sunnyvale, CA).

2.6. Western blotting

Proteins (~ 30–40 μ g/lane) were separated by SDS-PAGE on 10% (w/v) polyacrylamide mini gels and electro transferred to nitrocellulose membrane. The membranes were blocked with 5% BSA in 10mM Tris-HCl, pH 7.6, 1.5M NaCl and 0.1% Tween 20 followed by incubation with corresponding primary antibody and secondary polyclonal rabbit antibody conjugated to horseradish peroxidase. The protein signal was enhanced by chemiluminescence system and developed using Kodak Pro Image 400 R (New Haven, CT) and Carestream Molecular Imaging Software was used to measure the band density. The same blots were used to probe for β -actin and total p47phox to ensure equal loading and transfer of the proteins.

2.7. Statistical analysis

Results are expressed as mean $\pm$ SEM. Statistical significant difference between groups was evaluated by ANOVA followed by SNK Post-Hoc test where appropriate. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. IL-1 β , but not TNF α or INF γ promotes ROS generation in insulin-secreting INS-1 832/13 cells

We recently reported a significant increase in Nox2-mediated generation of ROS in INS-1 832/13 cells. In the current set of experiments we examined effects of individual cytokines on ROS generation in order to determine which of the three cytokines exert stimulatory effects on ROS generation in INS 832/13 cells. Data in Figure 1 indicated that of all the

three cytokines tested, namely IL-1 β , TNF α or IFN γ (25 ng/ml each; 24 hr) only IL-1 β significantly augmented (~2.5 fold) ROS generation; these values were comparable to those demonstrated in the presence of all the three cytokines combined (referred to as cytomix throughout). A modest, but insignificant, effect of TNF α (bar 1 vs. 3) or IFN γ (bar 1 vs.4), was demonstrated under these conditions. Therefore, we determined the effects of cytokines on Nox2 activation in all the studies described from here on since the cytomix represents an appropriate model to determine effects of cytokines on islet β -cell dysfunction.

3.2. Cytomix stimulates the phosphorylation of p47phox in a time-dependent manner

Recent studies from our laboratory and others have shown that cytokines induce the expression of p47phox, and siRNA-mediated depletion of endogenous pools of p47phox markedly attenuated cytokine-induced Nox2-mediated ROS generation in insulin-secreting cells [14]. Furthermore, evidence in other cell types indicates that p47phox is phosphorylated subsequent to agonist activation [18, 19] and that the phosphorylation step is necessary for its translocation to the membrane fraction for association with other members of the Nox2 core proteins to complete holoenzyme assembly leading to the activation of Nox2. This has not been examined before in islet β -cells exposed to cytokines. Data in Figure 2 (Panel A) suggested a time-dependent activation of p47phox (0–60 min) following exposure to cytokines. We observed nearly a 2-fold increase in the phosphorylation of p47phox by cytokines within an hour of incubation (Panel B). These data suggest that cytokines induce phosphorylation of p47phox.

3.3. Cytomix stimulates the expression of gp91phox in a time-dependent manner

We next investigated alterations, if any, in the expression of gp91phox in cells following exposure to cytomix. To address this, INS-1 832/13 cells were incubated in the presence of cytomix for different time intervals (0–60 min) as above (Figure 2). Relative abundance of gp91phox was determined in the total membrane fraction isolated by a single-step centrifugation method [see Methods] by Western blotting for gp91phox (Figure 3; Panel A) and densitometry (Figure 3; Panel B). These data indicated a time-dependent increase in the expression of gp91phox in INS-1 832/13 cells following exposure to cytomix. A significant increase in the expression was seen as early as 20 min (~2 fold), which appear to plateau with time. Together, these data are indicative of positive modulatory effects of cytomix on Nox2 in INS-1 832/12 cells. Such effects are comprised of increase in the activation of Rac1 [14, 16], phosphorylation of p47phox and expression of gp91phox (this study); such conditions are essential for activation of Nox2 enzyme.

3.4. A protein palmitoylation step is necessary for cytomix-induced ROS generation in INS-1 832/13 cells

Our recent studies have demonstrated that activation of Rac1 is necessary for cytokine-induced Nox2 activation and ROS generation. In this context we reported a marked increase in Rac1-GTP (active conformation) formation in INS-1 832/13 cells following exposure to cytomix [14]. We also showed that prior incubation of INS-1 832/13 cells with GGTI-2147, an inhibitor of geranylgeranylation of Rac1 or NSC23766, a known inhibitor of Rac1 mediated by Tiam1, a guanine nucleotide exchange factor for Rac1 markedly attenuated cytomix-induced ROS generation [14]. Therefore, we determined the effects of 2-bromopalmitate, a known inhibitor of protein palmitoylation on cytomix-induced ROS generation. These studies are based on the recent reports of palmitoylation of Rac1 at a cysteine residue upstream to the C-terminal cysteine. To address this cytomix-induced ROS generation was quantitated in INS-1 832/13 cells incubated in the presence of diluent alone or 2-BP (100 μ M; 24 hr). Data depicted in Figure 4 indicated no significant effects of 2-BP on basal ROS generation (bar 1 vs. bar 2). However, cytomix-induced ROS generation was

completely abolished by 2-BP (bar 3 vs. bar 4). These findings suggest that a palmitoylation-dependent signaling step is necessary for cytomix-induced ROS generation.

3.5. A protein palmitoylation step is necessary for cytomix-induced nitric oxide generation in INS-1 832/13 cells

It is widely felt that both oxidative (ROS) and nitrosative (NO) stress could contribute to impairment of the overall health of the islet β -cell [7, 20–23]. Such concerns were raised indeed by investigators in the area warranting additional studies to investigate potential targets which could regulate both ROS and NO generation signaling steps under the duress of cytokines. To this end, we have previously reported that a palmitoylation-mediated signaling step may be necessary for cytokine-induced iNOS gene expression and NO release [24]. Herein, we revisited those earlier studies to demonstrate that inhibition of protein palmitoylation by 2-BP inhibits cytomix-induced NO release in INS-1 832/13 cells. Data in Figure 5 suggested no direct effects of 2-BP on basal NO release (bar 1 vs. bar 2). However, as in the case with ROS generation (Figure 4), 2-BP treatment also attenuated cytomix-induced NO release (bar 3 vs. bar 4). Together, our findings highlight the importance of palmitoylation as a unifying mechanism involved in the induction of Nox2-mediated oxidative stress and iNOS-mediated nitrosative stress in pancreatic β -cells (see Discussion).

3.5. A protein palmitoylation step is not necessary for cytomix-induced CHOP expression, a marker for endoplasmic reticulum stress

It is well established that ER stress underlies cytokine-mediated metabolic dysregulation of the islet β -cell [25–27]. Furthermore, recent studies by Baldwin and associates have suggested a critical requirement for protein palmitoylation in palmitate-induced CHOP expression (a marker for ER stress) in that 2-BP significantly attenuated palmitate-induced CHOP expression in insulin-secreting RINm5F cells [28]. Therefore, we investigated if cytomix-induced CHOP expression in INS-1 832/13 cells is sensitive to 2-BP. To address this, cytomix-induced CHOP expression was measured in cells incubated in the presence of diluent alone or 2-BP. Data depicted in Figure 6 represents a Western blot for the expression of CHOP. It suggested no clear effects of 2-BP on CHOP expression under basal conditions (lane 1 vs. lane 2). Exposure of these cells to cytomix significantly increased CHOP expression (lane 1 vs. lane 3). However, provision of 2-BP to cytomix-treated cells did not exert any significant effects on CHOP expression (Lane 3 vs. lane 4) suggesting that protein palmitoylation is not necessary for cytomix-induced CHOP expression.

4. Discussion

Emerging evidence from multiple laboratories including our own implicates regulatory roles for Nox2 in the metabolic dysfunction under the duress of glucolipotoxicity and cytokines [10, 14–16, 21]. The overall goal of this study was to further understand Nox2 regulation in pancreatic β -cells exposed to cytomix. Our studies yielded some novel clues suggesting that: (i) cytokines induce p47phox phosphorylation and gp91phox expression under acute regulatory conditions; (ii) 2-BP, a classic inhibitor of protein palmitoylation significantly attenuated cytomix-induced, Nox2-mediated ROS generation and iNOS-mediated NO generation. Interestingly, 2-BP failed to affect cytomix-induced ER stress (CHOP expression). We believe that our current data provide clues that protein palmitoylation step may represent a novel therapeutic target for cytokine-induced ROS and NO generation.

Nox2 holoenzyme is comprised of cytosolic and membranous cores. The cytosolic core is comprised of p47phox, p40phox, p67phox and Rac1 whereas the membranous core is comprised of gp91phox/p22phox and Rap1 [12, 13, 21]. It is well established that the cytosolic core translocates to the membrane for association with the membranous core to

complete the holoenzyme assembly and catalytic activation. Several lines of evidence from multiple laboratories including our own implicated Nox2 in dysregulation of the islet β -cell exposed to a wide variety of pathological conditions, including chronic exposure to high glucose, palmitate and proinflammatory cytokines [10, 11, 14, 15]. More recent studies from our laboratory have also suggested hyperactivation of Nox2 in islets derived from type 2 diabetic human donors and animal models such as the Zucker Diabetic Fatty rat [29]. Together, the above studies implicate novel regulatory roles for Nox2 in the pathophysiology of diabetes.

In the context of the currently described studies, we have recently published evidence to suggest that exposure of isolated β -cells to proinflammatory cytokines results in increased expression of p47phox, a member of the cytosolic core of Nox2, within 12–24 hr. We have also shown that Rac1, another cytosolic component of Nox2, gets activated transiently (~15 min) following exposure to cytokines [14]. More importantly, our data also suggested that inhibition of p47phox function via siRNA-p47phox or Rac1 functions using inhibitors of Rac1 prenylation (i.e., GGTI-2147) or its GTP-exchange functions by Tiam1 (e.g., NSC23766) significantly attenuated cytokine-induced ROS generation and loss of mitochondrial membrane potential. Interestingly, neither GGTI-2147 nor NSC23766 prevented cytokine-induced NO generation [14] suggesting that Nox2 activation is not upstream to iNOS induction and NO release and the two signaling steps are regulated by two distinct mechanisms.

Original studies from our laboratory have suggested that a protein palmitoylation step controls cytokine-induced iNOS gene expression and NO release in insulin-secreting β -cells [8, 24]. Therein, we identified H-Ras, a small G-protein, as one of the palmitoylated proteins, which is involved in cytokine-induced NO release. These conclusions were confirmed via the use of two selective inhibitors of protein palmitoylation, namely cerulenin and 2-BP [24]. Furthermore, we have also been able to demonstrate that selective inhibitors of protein farnesylation (e.g., allylfarnesols, manumycin, damnacanthal, FTI-277) markedly attenuated cytokine-induced NO release thereby suggesting that farnesylation as well as palmitoylation of H-Ras may be necessary for cytokine-mediated effects on NO release [21]. Identity of H-Ras as one of the regulatory proteins involved in cytokine-induced NO release in β -cells was also confirmed through the use of bacterial toxins [8]. Interestingly, our current findings demonstrated that 2-BP also inhibited cytokine-induced Nox2 activation and ROS generation suggesting that protein palmitoylation might be crucial to cytokine-mediated effects. While these data do not directly address the question of potential identity of the protein, it may be Rac1 since recent studies [30] suggest that Rac1 undergoes palmitoylation at cysteine residues upstream to the prenylated cysteine. This remains to be verified in the context of current studies. Along these lines, recent studies from Corbett's laboratory have demonstrated a requirement for a palmitoylation step in palmitate-induced metabolic dysfunction of the islet β -cell [28]. Interestingly, they reported a significant inhibition in palmitate-induced CHOP expression and associated metabolic dysfunction of the islet β -cell in the presence of 2-BP. While our results further validate such a model in the context of cytokines, we failed to observe any significant effects of inhibition of protein palmitoylation with 2-BP on cytokine-induced CHOP expression suggesting distinct mechanisms underlying palmitate and cytokine-induced CHOP expression and ER stress. Additional studies are needed to further understand these signaling steps under these experimental conditions.

Our current findings also implicate alterations in the cytosolic and membranous core proteins under acute conditions in the presence of cytokines. For example, we have demonstrated that exposure of INS-1 832/13 cells to cytomix resulted in a time-dependent phosphorylation of p47phox and expression of gp91phox within 20 minutes of exposure.

Interestingly, we have reported a significant activation of Rac1 by cytokines under short-term exposure conditions [14]. These kinetics are consistent with translocation of cytosolic components to the membrane fraction for holoenzyme assembly. Together, our studies further validate regulation of Nox2 function by cytokines under short- and long-term exposure conditions leading to metabolic dysregulation of the islet β -cell.

Based on the data accrued thus far we propose a working model (Figure 7) for cytokine-induced metabolic dysregulation of the islet β -cell with an emphasis on Nox2 and iNOS signaling pathways. We propose that exposure of isolated β -cells to proinflammatory cytokines leads to generation of oxidative (increase in ROS) and nitrosative (increase in NO) stress mediated by Nox2 and iNOS, respectively. Previously published evidence from our laboratory suggested marked attenuation of cytokine-induced Nox2 activation and ROS generation by siRNA-p47phox, apocynin, NSC23766 and GGTI-2147. We have also demonstrated that the above manipulations prevented mitochondrial dysfunction induced by cytokines [14]. Published evidence from our laboratory also suggested a marked diminution in cytokine-induced iNOS gene expression and NO release by Clostridial toxins [8, 31], farnesyl transferase inhibitors [21], and inhibitors of palmitoylation such as cerulenin and 2-BP [24]. Our current observations further implicate a palmitoylation-dependent signaling step in cytokine-induced Nox2-mediated ROS generation. Together, these data suggest that protein palmitoyltransferase might serve as a novel therapeutic target for the prevention of cytokine-induced oxidative and nitrosative stress leading to mitochondrial dysfunction and accelerated cell death. In addition, our current studies are also suggestive of acute regulation of Nox2 subunits by cytokines, which has not been demonstrated before in the islet β -cell. While our earlier studies identified H-Ras as the palmitoylated protein which regulates NO release induced by cytokines, the identity of the palmitoylated protein that regulates cytokine-induced ROS generation remains to be determined. It would be interesting to see such a protein that regulates Nox2 activation and ROS generation under the duress of various pathological stimuli including high glucose, saturated fatty acids and ceramide. These are being investigated in our laboratory currently.

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Abbreviations

2-BP	2-Bromopalmitate
CHAPS	3-[3-cholamidopropyl] dimethylammonio]-1-propanesulfonate
CHOP	C/EBP homologous protein
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
NO	nitric oxide

Nox2	phagocyte-like NADPH oxidase
Na₃VO₄	Sodium orthovanadate
PIC	Protease inhibitor cocktail
PMSF	Phenylmethanesulfonylfluoride
RIPA	Radioimmuno-precipitation assay
ROS	Reactive oxygen species

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Figure 1. IL-1 β , but not TNF α and INF γ , activates ROS generation in INS-1 832/13 cells
INS-1 832/13 cells were treated with individual cytokines (IL1 β , TNF α and INF γ ; 25ng/ml each) for 24 hrs and the amount of ROS generation was measured with 2',7'-dichlorofluorescein diacetate assay. Data represent mean $\pm$ SEM from three independent experiments and expressed as % of control. * $P < 0.05$ versus control.

Figure 2. Cytomix induces time-dependent phosphorylation of p47phox in INS-1 832/13 cells
 INS-1 832/13 cells were treated with cytomix (IL1 β , TNF α , and INF γ , 10ng/ml each) for different time intervals as indicated in the figure. Degree of phosphorylation of p47phox was determined by Western blot (Panel A) followed by densitometry (Panel B). Data are normalized to total p47phox content in corresponding lanes. Data represent mean $\pm$ SEM from three independent experiments and expressed as percent control of the ratios between phosphorylated and total p47phox. * $P < 0.05$ versus control.

Figure 3. Cytomix increases time-dependent expression of gp91phox in INS-1 832/13 cells
 INS-1 832/13 cells were treated with cytomix (IL1 β , TNF α , and INF γ , 10ng/ml each) for different time points as indicated in the figure. Degree of gp91phox expression was measured by Western blot (Panel A) followed by densitometry (Panel B). Data are normalized to β -actin content in individual lanes. Data represent mean $\pm$ SEM from three independent experiments.

* $P < 0.05$ versus control and # $P < 0.05$ versus 20min and 40min.

Figure 4. 2-BP attenuates cytomix-induced ROS generation in INS-1 832/13 cells

INS-1 832/13 cells were treated with cytomix (IL1 β , TNF α and INF γ ; 10ng/ml each) for 24 hrs in the presence and absence of 2-BP (100 μ M) and the amount of ROS generation was measured with 2'7-dichlorofluorescein diacetate assay. Data represent mean $\pm$ SEM from three independent experiments and expressed as % of control. * $P < 0.05$ versus control and # $P < 0.05$ versus cytomix.

Figure 5. Cytomix-induced NO release is inhibited by 2-BP

INS-1 832/13 cells were treated with cytomix (IL1 β , TNF α and INF γ ; 10ng/ml each) for 24 hrs time point in the presence and absence of 2-bromopalmitate (100 μ M) and the amount of nitric oxide release was measured using Griess assay. 2-bromopalmitate significantly decreased cytomix-induced nitric oxide release. Data represent mean $\pm$ SEM from three independent experiments. * $P < 0.05$ versus control and # $P < 0.05$ versus cytomix.

Figure 6. Cytomix-induced CHOP (C/EBP homologous protein) expression is not inhibited by 2-bromopalmitate

INS-1 832/13 cells were treated with cytomix (IL1 β , TNF α and INF γ ; 10ng/ml each) for 24 hrs time point in the presence and absence of 2-bromopalmitate (100 μ M). The level of CHOP expression, a marker for endoplasmic reticulum (ER) stress, was measured by Western blot. Equal protein loading was confirmed by β -actin content in individual lanes. A representative blot from three independent experiments is shown here.

Figure 7. Proposed model to halt cytokine-induced nitrosative and oxidative stress by 2-BP

A model depicting roles of Nox2-mediated ROS generation and iNOS-mediated NO generation in the cascade of events leading to the metabolic dysfunction of the islet β-cell is depicted herein. Data from our current investigation identify protein palmitoyltransferase as a novel therapeutic target for the prevention of cytokine-mediated effects on the islet β-cell (see Discussion for additional details).