Exendin-4 inhibits glucolipotoxic ER stress in pancreatic β cells via regulation of SREBP1c and C/EBP β transcription factors

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Abstract

Prolonged exposure to high glucose (HG) and palmitate (PA) results in increased ER stress and subsequently induces β -cell apoptosis. Exendin-4, a glucagon-like peptide-1 agonist, is known to protect β cells from toxicity induced by cytokines, HG, or fatty acids by reducing ER stress. However, the detailed molecular mechanisms for this protective effect are still not known. In this study, we investigated the role of exendin-4 in the inhibition of glucolipotoxicity-induced ER stress and β -cell apoptosis. Exendin-4 treatment protected INS-1 β cells from apoptosis in response to HG/PA (25 mM glucose + 400 μ M PA). HG/PA treatment increased cleaved caspase-3 and induced ER stress marker proteins such as PERK (EIF2AK3), ATF6, and phosphorylated forms of PERK, eIF2 α , IRE1 α (ERN1), and JNK (MAPK8), and these increases were significantly inhibited by exendin-4 treatment. HG/PA treatment of INS-1 cells increased SREBP1 (SREBF1) protein and induced its nuclear translocation and subsequently increased C/EBP β (CEBPB) protein and its nuclear translocation. Exendin-4 treatment attenuated this increase. Knockdown of *SREBP1c* reduced the activation of *C/EBP β* and also blocked the expression of ER stress markers induced by HG/PA treatment. Our results indicate that exendin-4 inhibits the activation of SREBP1c and C/EBP β , which, in turn, may reduce glucolipotoxicity-induced ER stress and β -cell apoptosis.

Key Words

- ▶ Exendin-4
- ▶ ER stress
- ▶ SREBP1c
- ▶ C/EBP β

Journal of Endocrinology
(2013) 216, 343–352

Introduction

β -Cell mass regulation is a critical issue for understanding diabetes, a disease characterized by absolute (type 1) or relative deficiency (type 2) in the number of pancreatic β cells. The loss of β -cell mass can be affected by

environmental factors such as lipotoxicity and glucotoxicity (Poitout & Robertson 2008). The chronic hyperglycemia and hyperlipidemia cause combined detrimental effects on β -cell function and survival,

which is defined as glucolipotoxicity (Robertson *et al.* 2004). Several potential mechanisms underlying β -cell dysfunction caused by glucolipotoxicity have been suggested, including reactive oxygen species-mediated oxidative stress, inflammatory signals, lipid intermediates, and ER stress (Donath *et al.* 2005).

Attention has recently been directed to ER stress because the ER is highly developed in pancreatic β cells due to their insulin secretory activity (Araki *et al.* 2003, Donath *et al.* 2005). Moreover, recent observations suggested that the ER may be responsible for the molecular mechanisms of glucolipotoxicity that contribute to β -cell dysfunction in type 2 diabetes (Weber *et al.* 2004).

To restore normal function after disturbed ER homeostasis, cells activate a protective mechanism known as the unfolded protein response, which involves double-stranded RNA-activated protein kinase RNA-like kinase (PERK), activating transcription factor 6 (ATF6), and inositol requiring enzyme 1 (IRE1) pathways (Wu & Kaufman 2006, Lai *et al.* 2007), which may contribute to β -cell apoptosis.

Transcription factors such as sterol regulatory element binding proteins (SREBPs) and peroxisome proliferator-activated receptor are known to be involved in ER stress-activated cell death (Weber *et al.* 2004, Wang *et al.* 2005). Chronic treatment with high glucose (HG), as well as ER stress inducers, is known to cause SREBP1c processing and activation (Foretz *et al.* 1999, Ye *et al.* 2000), and an increase in SREBP1c is correlated with β -cell glucolipotoxicity (Wang *et al.* 2005). As well, CCAAT-enhancer binding protein (C/EBP), one of the transcription factors regulating lipogenic gene expression, is induced upon chronic exposure to HG concentration (Plaisance *et al.* 2009).

Glucagon-like peptide-1 (GLP1), a potent glucorecretin hormone, has an antidiabetic function. It enhances glucose-dependent insulin release, insulin biosynthesis, and β -cell proliferation and suppresses β -cell apoptosis (Doyle & Egan 2007). Exendin-4, a GLP1 receptor agonist, has been reported to enhance β -cell survival when exposed to streptozotocin, cytokine, or fatty acid (Tourrel *et al.* 2001, Ferdaoussi *et al.* 2008), and to have a protective effect on β -cell death through the activation of protein kinase B/AKT via the CREB pathway (Jhala *et al.* 2003).

In this study, we investigated the protective effect of exendin-4 on glucolipotoxic ER stress on INS-1 β cells, particularly on lipogenic transcription factor SREBP1c and C/EBP β (CEBPB). We found that exendin-4 inhibits SREBP1c and subsequent C/EBP β activation, contributing to attenuation of ER stress and β -cell apoptosis.

Materials and methods

Materials

The sources of various reagents and materials were as follows: exendin-4, D-glucose, palmitate (PA), fatty acid-free BSA were from Sigma-Aldrich; anti-C/EBP β and anti-lamin B antibodies were from Santa Cruz Biotechnology, Inc.; polyclonal antibody against SREBP1 (SREBF1) was from Abcam (Cambridge, MA, USA); MAB against ATF6 was from IMGEX (San Diego, CA, USA); and anti-C/EBP homologous protein (CHOP), anti-caspase-3, anti-poly(ADP-ribose) polymerase (PARP), anti-PERK, anti-phospho-PERK, anti-phospho-IRE1 α , anti-phospho-c-Jun N-terminal kinase (JNK), and anti-phospho-eukaryotic initiation factor 2 α (eIF2 α) antibodies were from Cell Signaling Technology (Beverly, MA, USA). All other biochemical reagents were from Sigma or Invitrogen.

Cell culture

INS-1 pancreatic β cells were maintained in RPMI 1640 (11 mM glucose, 1 mM sodium pyruvate, and 10 mM HEPES) containing 10% fetal bovine serum (Gibco), 100 U/ml penicillin, and 100 μ g/ml streptomycin. To examine the effect of exendin-4, cells were plated on six-well plates at 1.2×10^6 cells/well in media containing 25 mM glucose and 400 μ M PA with or without 5 nM exendin-4. The cells treated with vehicle as a control.

PA preparation

PA/BSA conjugates were prepared as described previously (Listenberger *et al.* 2001). Briefly, 20 mM PA in 0.01 M NaOH was incubated at 70 °C for 30 min, and the PA was then mixed with 5% BSA in PBS at a 3:1 molar ratio of BSA to PA.

Cell viability assay

Briefly, the cells were treated with RPMI media containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; 0.5 mg/ml) for 1 h at 37 °C. Supernatants were discarded and isopropanol was then added. After incubating for 30 min at room temperature, cell viability was measured at 570 nm using a microplate reader (Molecular Devices Corp., Menlo Park, CA, USA).

Quantitative real-time PCR

Total RNA was prepared with RNAiso Plus (TaKaRa, Shiga, Japan), and cDNA was synthesized from 5 μ g RNA with

TAKARA PrimeScript 1st strand cDNA synthesis kit. Quantitative real-time PCR (qRT-PCR) analysis was performed using SYBR master mix (Applied Biosystems) using the ABI 7900 Real-time PCR system according to the protocols provided by the manufacturer (Applied Biosystems). The sequences of the primer pairs are as follows: CHOP, (forward) 5'-GGAAGTGCATCTTCATACACCA-CC-3' and (reverse) 5'-TGAAGTGAATCTGGAGAGC-GAGGGC-3'; binding immunoglobulin protein (Bip), (forward) 5'-ACCAATTATCAGCAAACCTCTATGGAA-3' and (reverse) 5'-CATCTTTTCTGCTGTATCCTCTTCA-3'; SREBP1c, (forward) 5'-GGAGCCATGGATTGCACATT-3' and (reverse) 5'-AGGCCAGGGAAGTCACTGTCT-3'; C/EBP β , (forward) 5'-AAGATGCGCAACCTGGAGAC-3' and (reverse) 5'-CCTTCTTCTGCAGCCGCTC-3'; spliced XBP1, (forward) 5'-CTGAGTCCGAATCAGGTGCAG-3' and (reverse) 5'-ATCCATGGGAAGATGTTCTGG-3'; SREBP1a, (forward) 5'-ACACAGCGTTTTGAACGACA-3' and (reverse) 5'-GCATCAATAGGCCAGGGAA-3'; Bax, (forward) 5'-AGACACCTGAGCTGACCTTGGA-3' and (reverse) 5'-CGGAGACACTCGCTCAGCTT-3'; and cyclophilin, (forward) 5'-GGTCTTTGGGAAGGTGAAAGAA-3' and (reverse) 5'-GGTCTTTGGGAAGGTGAAAGAA-3'. The relative mRNA transcript levels were calculated according to the $2^{-\Delta CT}$ method, in which ΔCT represents the difference in threshold cycle values between the target mRNA and the cyclophilin internal control.

Western blot analysis

INS-1 cells were solubilized in lysis buffer (50 mM Tris-HCl, pH 7.4, 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 1 mM NaF, 1 mM Na₃VO₄, 1 mM phenylmethylsulphonyl fluoride, and 1 mM protease inhibitor cocktail). Protein (30 μ g) was subjected to SDS-PAGE electrophoresis and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% nonfat milk and incubated with specific antibodies. The blots were then incubated with HRP-conjugated anti-rabbit or anti-mouse antibodies. Signals were detected using the ECL detection system (Pierce, Rockford, IL, USA).

Whole cell and nuclear protein extract preparation

To prepare the whole cell extracts, cells were lysed in buffer containing 1% Triton X-100, 50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, and protease inhibitors. Cell debris was removed after centrifugation (10 000 g) for 10 min at 4 °C. Nuclear fractions were prepared from cells using a modification of the method of Sheng *et al.* (1995).

The cells were homogenized in buffer containing 10 mM HEPES-KOH, pH 7.9, 10 mM KCl, 1.5 mM MgCl₂, 1 mM dithiothreitol, 0.1% NP40, and protease inhibitors. Nuclei were separated by centrifugation (7200 g) for 10 min at 4 °C and resuspended in nuclei resuspension buffer (10 mM HEPES-KOH, pH 7.9, 400 mM KCl, 0.1 mM EDTA, 25% glycerol, and protease). The mixture was stirred gently for 10 min at 4 °C and centrifuged at 1500 g . The nuclear protein extract was recovered in supernatant. Protein concentration was determined using the BCA protein assay kit (Pierce).

Transfection with siRNA

SREBP1 siRNAs were purchased from Dharmacon Research (Lafayette, CO, USA) as ON-target plus SMART pool. They targeted four regions of *SREBP1* mRNA for interference. The target sequences were as follows: #1 GCCCAGC-CUUUGAGGAUAA, #2 GGUCUAGCCUGCAGUUCUA, #3 GGAUAUCUACAGUUGCUGAA, and #4 GAGGCAAAG-CUGAAUAAAU. A synthetic cyclophilin B control pool siRNA (scramble siRNA) was used as a negative control. Cells were plated on six-well plates and transfected with 100 nM siRNA and oligofectamine reagent (Invitrogen), according to the manufacturer's instructions. After 36 h of incubation, cells were treated with 25 mM glucose and 400 μ M PA for 12 h with or without 5 nM exendin-4.

Statistical analysis

Results are expressed as mean $\pm$ S.E.M. of three separate experiments. ANOVA followed by Scheffe's multiple comparison test was used to determine the significance of any differences among more than two groups. $P < 0.05$ was considered significant.

Results

Exendin-4 protects INS-1 cells from HG/PA-induced toxicity

Previously, it was reported that HG and PA synergistically induced glucolipotoxicity in β cells and caused β -cell dysfunction (Zhou & Grill 1994, El-Assaad *et al.* 2003, Donath & Halban 2004, Tanabe *et al.* 2011). Consistent with previous reports, we found that glucose alone enhanced cell viability in a dose-dependent manner, whereas HG induced cell toxicity in the presence of PA (50% cell viability in 25 mM glucose/400 μ M PA; Fig. 1A). Exendin-4 treatment significantly inhibited PA-induced

Figure 1

Exendin-4 treatment protects against HG/PA-induced glucolipotoxicity. (A) INS-1 cells were treated with various concentrations of glucose for 30 min before adding 300 or 400 μ M PA. After 24 h, cell viability was determined by MTT assay. (B) INS-1 cells were treated with 25 mM glucose (HG) and/or 400 μ M PA in the presence of 5 or 10 nM exendin-4 (Ex) or vehicle (CON). After 24 h, cell viability was determined by MTT assay. (C) INS-1 cells were treated as described in (B); after 24 h, cells were harvested and activation of cleaved caspase-3 and PARP was checked by immunoblotting with specific antibodies. Bezafibrate (BEZ) was used as positive control for anti-apoptotic effect. * $P < 0.05$.

or HG/PA-induced cell death on INS-1 cells (Fig. 1B). Immunoblotting analysis showed that exendin-4 treatment inhibited the expression of cleaved forms of caspase-3 and PARP, which were induced by HG/PA treatment

(Fig. 1C). Treatment with bezafibrate, as a positive control for the anti-apoptotic effect, similarly inhibited the expression of cleaved caspase-3 and PARP. These results suggest that exendin-4 has protective effects against HG/PA-induced INS-1 cell apoptosis.

Exendin-4 inhibits ER stress signaling induced by HG/PA treatment

To examine whether the protective effect of exendin-4 against HG/PA-induced apoptosis is related to the inhibition of ER stress, INS-1 cells were treated with HG/PA in

Figure 2

Exendin-4 inhibits HG/PA-induced expression of ER stress markers. (A) INS-1 cells were treated with vehicle (CON) or HG/PA in the absence or presence of 5 nM exendin-4 (Ex) for 6 h. Total RNA was isolated and mRNA expression of *sXBP1*, *CHOP*, and *Bip* were determined by qRT-PCR. Cyclophilin was used as control gene. (B) INS-1 cells were treated as described earlier for 12 or 18 h and total cell extracts were analyzed for PERK, p-PERK, p-eIF2 α , p-IRE1 α , p-JNK, ATF6 (p50), and actin by immunoblotting using specific antibodies. (C) Quantitative analysis of western blots. Relative abundance of each band was estimated by densitometric analysis. Each bar represents the mean $\pm$ S.E.M. from three independent experiments. * $P < 0.05$.

the absence or presence of 5 nM exendin-4 and the levels of ER stress-related markers were analyzed. As the PERK, IRE, and ATF6 pathways are the major signaling pathways of ER stress, we examined the expression level of specific makers of each pathway. HG/PA treatment induced the expression of *sXBP1* and *CHOP* mRNAs, and the expression of these mRNAs was significantly reduced by exendin-4 treatment (Fig. 2A). In addition, PERK, p-PERK, p-eIF2 α , p-IRE1 α , p-JNK, and cleaved ATF6 (p50) protein expression was increased by HG/PA treatment and the expression of these proteins was significantly reduced by exendin-4 treatment (Fig. 2B and C). mRNA expression of *Bip* was not changed by HG/PA or exendin-4 treatment (Fig. 2A and B). These results suggest that exendin-4 inhibits HG/PA-induced ER stress signaling, contributing to protection from β -cell apoptosis.

Exendin-4 inhibits HG/PA-induced activation of SREBP1 and C/EBP β

As it was reported that SREBP1 and C/EBP β are involved in ER stress-induced cell death (Wang *et al.* 2005, Meir *et al.* 2010), we checked whether HG/PA treatment induced activation of these transcription factors. INS-1 cells were incubated with HG/PA for 2, 4, 6, or 12 h and the mRNA expression of *SREBP1c* and *C/EBP β* was examined by qRT-PCR. We found that both genes were increased by HG/PA treatment over time. Induction of *SREBP1c* was higher than that of *C/EBP β* , and induction time was as early as 2 h after treatment (Fig. 3A). Protein expression level was also increased by HG/PA treatment similar to that of mRNA (Fig. 3B). Finally, we observed an increase in the nuclear form of SREBP1 as early as after 2 h of HG/PA treatment, and the levels were sustained up to 12 h of treatment. By contrast, the expression of nuclear C/EBP β did not substantially increase until after 6 h of treatment (Fig. 3C and D). These results indicate that SREBP1 activation precedes activation of C/EBP β in INS-1 β cells in response to HG/PA treatment.

Next, to check whether exendin-4 regulates SREBP1c activation, cells were incubated with HG/PA in the absence or presence of exendin-4 for 12 h and mRNA, protein, and nuclear translocated protein levels were examined. We found that exendin-4 significantly reduced the mRNA level (Fig. 4A) and both whole cell (Fig. 4B) and nuclear translocated protein levels (Fig. 4C and D) of SREBP1c induced by HG/PA treatment. Nuclear translocation of C/EBP β was also induced by HG/PA treatment and it was reduced by exendin-4 treatment (Fig. 4C and D).

Figure 3

HG/PA treatment increases expression levels of SREBP1 and C/EBP β in INS-1 cells. INS-1 cells were treated with 25 mM glucose and 400 μ M palmitate (HG/PA) for the indicated times. (A) Total RNA was isolated and mRNA expressions of *SREBP1c* and *C/EBP β* were examined by qRT-PCR. (B) Total cell lysate and (C) nuclear protein extracts were analyzed by immunoblotting for SREBP1 and C/EBP β using specific antibodies. (D) Quantitative analysis of western blots of nuclear protein extracts. The graph represents the fold change in protein levels compared with the control band (lamin B) on western blots that were quantitated by densitometric analysis.

Figure 4

Exendin-4 reduces the expression of SREBP1 and C/EBP β in HG/PA-treated INS-1 cells. (A) INS-1 cells were treated with vehicle (CON) or 25 mM glucose and 400 μ M palmitate (HG/PA) in the absence or presence of 5 nM exendin-4 (Ex) for 12 h. Total mRNA was isolated and mRNA expression of *SREBP1c* was examined by qRT-PCR. (B) INS-1 cells were treated as described in (A) and total cell extract was analyzed by immunoblotting for SREBP1 and β -actin using specific antibodies. (C) Nuclear proteins were harvested and analyzed by immunoblotting for SREBP1, C/EBP β , and lamin B using specific antibodies. (D) Quantitative analysis of western blots of nuclear proteins. The relative fold change in C/EBP β and SREBP1 to its control band (lamin B) was quantitated by densitometric analysis. * $P < 0.05$.

Exendin-4 inhibits HG/PA-induced ER stress by downregulation of SREBP1 activation

To examine whether SREBP1c regulates CEBP β expression, HG/PA-induced ER stress, and subsequently β -cell apoptosis, we inhibited *SREBP1c* by SREBP1c siRNA in INS-1 cells and then treated them with HG/PA. mRNA level of *SREBP1c* in siRNA-transfected INS-1 cells was about 48% of INS-1 cells transfected with scramble siRNA under HG/PA conditions. We also confirmed that *SREBP1a*, another splicing form of SREBP1 (Shimomura *et al.* 1997), was not affected by HG/PA and SREBP1c siRNA treatment (Fig. 5A). We found that inhibition of SREBP1c significantly reduced HG/PA-induced *Bax* mRNA (Fig. 5B) and also reduced cleaved PARP (Fig. 5C), and the effect was

similar to exendin-4 treatment. As shown in Fig. 5D, SREBP1c downregulation recovered cell viability reduced by HG/PA treatment.

When we examined the expression of C/EBP β and related ER proteins, we found that inhibition of SREBP1c significantly inhibited HG/PA-induced nuclear translocation of C/EBP β and expression of PERK, p-PERK, and p-eIF2 α (Fig. 6A and B). In addition, induction of *sXBP* mRNA expression by HG/PA was significantly reduced in *SREBP1c* siRNA-transfected cells similar to exendin-4 treatment of HG/PA-treated cell (Fig. 6C).

Discussion

ER stress participates in β -cell dysfunction and death during the development of type 2 diabetes (Ozcan *et al.* 2004, Laybutt *et al.* 2007). High levels of glucose and increased lipids (glucolipotoxicity) are known to be a cause of β -cell damage and induce ER stress (El-Asaad *et al.* 2003, Poitout 2008). GLP1 and GLP1 receptor agonists have been reported to inhibit ER stress and prevent β -cell death induced by these toxicities. However, the molecular mechanisms regarding how GLP1 receptor activation reduces ER stress and prevents β -cell apoptosis are not well known. In our study, we found that expression level of markers of the PERK, IRE1, and ATF6 ER stress pathways were elevated in HG/PA-treated cells and reduced by treatment with exendin-4, a GLP1 agonist. These results are consistent with previous studies showing that exendin-4 reduces ER stress and prevents β -cell apoptosis (Tsunekawa *et al.* 2007).

Although three branches of ER stress signaling were involved in the anti-apoptotic effect of exendin-4, we found that *Bip* mRNA expression was not changed in our experimental condition, in which we treated INS-1 cells with HG/PA for 6 h, although mRNA levels of *CHOP*, one of the transcription factors activated by ATF6, was increased by glucolipotoxicity and was reduced in exendin-4-treated cells. Others have shown that different stimulation conditions can have differential effects on the regulation of *Bip* mRNA and protein. For example, 30 mM glucose for 48 h induced *Bip* mRNA levels (Wang *et al.* 2005), but 0.5 mM PA treatment for 14 h did not alter *Bip* mRNA levels (Cunha *et al.* 2009). Therefore, the transcriptional regulation of ER stress signaling might be different depending on the cell types or stimulus condition. Further studies will be required to understand the detailed molecular mechanisms for these differential effects.

Studies on the mechanisms showed that the anti-apoptotic protein, JunB, was involved in the protective

Figure 5

SREBP1c downregulation inhibits HG/PA-induced β -cell apoptosis. (A) INS-1 cells were transiently transfected with cyclophilin B control pool siRNA as a negative control (scramble si) or *SREBP1* siRNA (*SREBP1* si). After 36 h, cells were treated with 25 mM glucose and 400 μ M palmitate (HG/PA) or vehicle (CON). Total RNA was isolated and mRNA expression of *SREBP1c* and *SREBP1a* was determined by qRT-PCR. (B) After transfection with siRNA, INS-1 cells were treated with HG/PA in the absence or presence of

5 nM exendin-4 (Ex). After 6 h, total RNA was isolated and mRNA expression of *Bax* was determined by qRT-PCR. (C) Cells were treated as described earlier and harvested. Total cell lysate was extracted and western blotting was performed to detect cleaved PARP and actin protein. (D) Cells were treated as described earlier, and cell viability was determined by MTT assay. Each bar represents the mean $\pm$ s.e.m. from three independent experiments. * $P < 0.05$.

effect of GLP1 receptor agonists against ER stress (Cunha *et al.* 2009). As well, exendin-4 significantly induced ATF4 expression by ER stress and accelerated recovery from ER stress-mediated translational repression in a protein kinase A-dependent manner (Yusta *et al.* 2006). However, the exact mechanisms for ER stress regulation by GLP1 in glucolipotoxicity are not clear.

Activation of transcription factors that control lipogenic gene expression, particularly SREBP, is implicated in ER stress. Previous studies demonstrated that overexpression of the active nuclear form of SREBP1c in insulin-secreting cells and isolated islets results in β -cell lipotoxicity (Andreolas *et al.* 2002, El Saadawi *et al.* 2010). ER stress inducers enhance SREBP1 binding to the insulin receptor substrate-2 promoter and suppress its expression, suggesting that SREBP1 activation caused by ER stress is involved in β -cell glucolipotoxicity (Wang *et al.* 2005). As well, treatment with some antidiabetic drugs or hormones including metformin, troglitazone, and leptin can prevent diabetes-associated lipotoxicity in diabetic animals through inhibition of SREBP1c activation

(Shimomura *et al.* 1999, Kakuma *et al.* 2000, Lin *et al.* 2000, Xu *et al.* 2004). Therefore, we examined the activation and expression of SREBP1c in HG/PA-treated INS-1 cells and regulation by exendin-4. We found that exendin-4 treatment inhibited the mRNA and protein levels of SREBP1 induced by HG/PA treatment. Consequently, nuclear translocated SREBP1c induced by HG/PA treatment was reduced by exendin-4 treatment. We also found that SREBP1c was involved in the anti-apoptotic effect of exendin-4 on another insulin-secreting cell line, MIN-6, suggesting that exendin-4 generally prevented glucolipotoxicity through suppression of SREBP1c in β cells (data not shown).

C/EBP β is increased in diabetic islets, and accumulation of C/EBP β increases the vulnerability of cells to ER stress (Matsuda *et al.* 2010, Meir *et al.* 2010). It was reported that LIP, a dominant-negative, truncated C/EBP β variant form, augmented thapsigargin- or tunicamycin-induced cell death via forming a LIP/LAP heterodimer and inducing CHOP expression (Meir *et al.* 2010). Moreover, accumulation of C/EBP β reduced glucose-regulated

Figure 6

SREBP1 downregulation inhibits HG/PA-induced expression of C/EBP β and ER stress markers. INS-1 cells were treated as described in Fig. 5C, and cells were harvested. (A) Nuclear protein was extracted and C/EBP β , SREBP1, and lamin B were analyzed by western blotting. Total cell lysate was extracted and western blotting was performed to detect PERK, p-PERK, p-eIF2 α , and actin protein. (B) Quantitative analysis of western blots. Relative abundance of each band was estimated by densitometric analysis. (C) Total RNA was isolated and mRNA expression of *sXBP1* was determined by qRT-PCR. Each bar represents the mean $\pm$ S.E.M. from three independent experiments. * $P < 0.05$.

protein of 78 kDa (GRP78, Bip) due to the suppressed transactivation of ATF6 α , thereby enhancing susceptibility to ER stress (Matsuda *et al.* 2010). In our study, C/EBP β translocation to the nucleus was observed in HG/PA-treated INS-1 cells, and exendin-4 treatment inhibited HG/PA-induced activation of C/EBP β .

We found that the increase in SREBP1c levels in the nucleus was seen before increases in C/EBP β . The expression of *SREBP1c* and C/EBP β is closely correlated in several models of adipogenesis; however, the sequence of activation is still debated (Le Lay *et al.* 2002, Payne *et al.* 2010). Therefore, we investigated whether

downregulation of SREBP1c affects activation of C/EBP β and ER stress-induced β -cell death. We found that downregulation of SREBP1c decreased the activation of C/EBP β and also reduced sXBP, PERK, p-PERK, and p-eIF2 α , resulting in increased cell viability in HG/PA-treated cells. These results suggest that downregulation of SREBP1c by exendin-4 is one mechanism for protection against glucolipotoxic ER stress. As the regulation of SREBP1c by exendin-4 has not been investigated, the detailed mechanisms are not known. However, activation of protein kinase A, one of the major signaling pathways of exendin-4, represses *SREBP1c* expression via phosphorylation of liver X receptor, which causes impaired DNA binding activity by preventing LXR/RXR dimerization (Yamamoto *et al.* 2007). Therefore, protein kinase A-dependent signaling might be involved in the regulation of SREBP1c by exendin-4.

In summary, we have demonstrated that SREBP1c-mediated C/EBP β activation is one of the mechanisms of glucolipotoxic ER stress in INS-1 cells. Moreover, exendin-4 protects β cells against ER stress through downregulation of SREBP1c. The present identification of the apoptotic ER stress pathways and downstream signaling modulated by GLP1 is an important step for the development of targeted approaches to alleviate β -cell ER stress in type 2 diabetes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This study was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (no. 2009-0079342), the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (no. 2010-0009378), and the Innovative Research Institute for Cell Therapy (A062260).

Acknowledgements

The authors thank Dr Ann Kyle for editorial assistance.

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Received in final form 27 November 2012

Accepted 19 December 2012

Accepted Preprint published online 19 December 2012