Decreased Insulin Secretion and Accumulation of Triglyceride in β Cells Overexpressing a Dominant-negative Form of AMP-activated Protein Kinase

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Abstract. Adenosine 5′-monophosphate-activated protein kinase (AMPK) has been implicated in the regulation of energy metabolism, although its role in the pancreatic β cells remains unclear. In the present study, we have overexpressed a dominant negative form of AMPKα1 subunit (Asp57Ala) tagged with c-myc epitope (AMPKα1-DN) in INS-1D cells with an adenoviral vector. After 48 h of adenoviral infection, overexpression of AMPKα1-DN in INS-1D cells was confirmed by Western blot analysis with anti-c-myc antibody. Phosphorylation of the Thr172 in AMPKα1/α2 subunit was progressively decreased in parallel with increasing number of adenoviral titers. Glucose-stimulated insulin secretion in response to 30 mmol/L glucose was decreased in INS-1D cells overexpressing AMPKα1-DN as compared to control cells infected with adeno-LacZ vector. Neither cellular insulin content nor insulin mRNA level was changed between the two groups. Phosphorylation of acetyl-CoA carboxylase (ACC), a downstream substrate of AMPK, was decreased, indicating that ACC activity was increased, due to the decreased AMPK activity. In fact, intracellular triglyceride content was increased as compared to control cells. The β-oxidation of palmitate was decreased at 30 mmol/L glucose. Insulin secretion in response to potassium chloride or glibenclamide was also decreased as compared to control cells. In conclusion, suppression of AMPK activity in β-cells inhibited insulin secretion in response to glucose, potassium chloride or glibenclamide without altering insulin content. Accumulation of triglyceride subsequent to the activation of ACC by suppression of AMPK activity, was suggested to be, at least in part, responsible for the impaired insulin secretion through so called lipotoxicity mechanism.

Key words: AMPK, Insulin secretion, β-oxidation, Triglyceride

Type 2 diabetes is explosively increasing worldwide due to the recent dramatic changes in the lifestyle, such as sedentary behavior and excessive fat intake, since these changes have increased the rate of obesity [1, 2], which often develops both peripheral insulin resistance and pancreatic β cell dysfunction [3, 4]. Multiple mechanisms are proposed to be involved in the development of these disorders [5, 6].

Of these, adenosine 5′-monophosphate-activated protein kinase (AMPK) currently comes into a spotlight as an important regulator for glucose and lipid metabolism in hepatocytes and skeletal muscle [7-10], by modulating expression and activity of key enzymes in the regulation of gluconeogenesis, lipid oxidation and lipid synthesis. AMPK is a heterotrimeric serine/threonine kinase composed of α, β and γ subunits [11-13]. The catalytic activity resides in the α subunit [14-17], which occurs as two isoforms, α1 and α2. When the AMP/ATP ratio is increased by various stimuli including starvation, heat shock, exercise and some of the adipokines such as adiponectin and leptin, AMP binds to the γ subunit of AMPK and the binding causes a conformational change [18-20]. This allows the α subunit to be phosphorylated at Thr172 by the...
upstream kinases, such as LKB1 and CaM kinase kinase, leading to full activation of AMPK [21-23]. By contrast, AMPK activity is suppressed in hyperglycemia and obesity [18, 20, 24]. Thus, AMPK functions as an energy sensor [18, 20]. In liver and skeletal muscle, activated AMPK phosphorylates acetyl-CoA carboxylase (ACC), and the phosphorylation suppresses its activity and increases fatty acid β oxidation [9, 25-29]. This results in inhibition of intracellular triglyceride (TG) accumulation, leading to improvement of insulin sensitivity.

On the other hand, physiological and pathophysiological implications of alterations in AMPK activity in insulin secretion are poorly understood. In agreement with the findings in muscle and liver, up-regulation of AMPK activity has been shown to increase lipid oxidation and reduce TG accumulation in β cells [30], which may interfere with insulin secretion, recognized as lipotoxicity [3, 4, 31, 32]. Indeed, we and others have recently demonstrated that accumulation of TG in the cultured β cells and mouse islets by overexpressing sterol regulatory element binding protein-1c (SREBP-1c), a key transcription factor for lipid synthesis, impairs insulin secretion [33, 34], suggesting that lipotoxicity in β cells indeed induces β cell dysfunction. Interestingly, however, expression of a constitutively active form of AMPK (CA-AMPK) has been reported to rather inhibit glucose-stimulated insulin secretion by unexplored mechanism [35, 36]. We have also shown that expression of CA-AMPK or treatment with AICAR (5-amino-1-beta-D-ribofuranosyl-imidazole-4-carboxamide) in INS-1D cells significantly suppresses insulin secretion associated with a reduction of intracellular insulin content [33, 37]. However, it is obviously important to clarify the role of AMPK in the regulation of insulin secretion under the more physiologically relevant condition, such as obesity and type 2 diabetes in which AMPK activity might be down-regulated by overnutrition.

To address this question, in the present study we overexpressed a dominant-negative form of the AMPKα1 subunit in an INS-1D β cell line, which replaces the endogenous α1 and α2 subunits, resulting in the specific suppression of the AMPK activity [38]. We found that inhibition of AMPK-mediated signaling impaired insulin secretion in response to glucose, potassium chloride and glibenclamide with decreased glucose oxidation and increased intracellular TG accumulation, suggesting that an appropriate amount of AMPK activity is required for the normal insulin secretion.

Materials and Methods

Materials

An antibody against phosphorylated AMPK was purchased from Cell Signaling Technology. An antibody against AMPKα1, an antibody against phosphorylated acetyl-CoA carboxylase, and an antibody against vesicle-associated membrane protein (VAMP) were purchased from Upstate Biotechnology. An antibody against c-myc, and an antibody against syntaxin-1 were purchased from Santa Cruz Biotechnology. The insulin radioimmunoassay kit, ECL Western Blotting Detection Reagents and Hybond-N+ were purchased from Amersham. Express Hyb Hybridization Solution was purchased from CLONTECH, and N,N,N′,N′-Tetramethyl-ethylenediamine, L-type Wako TG·H kit, and an antibody against SNAP-25 were purchased from Wako Chemical. TRIZOL Reagent was purchased from Invitrogen. RPMI-1640, bovine albumin, fetal bovine serum, the ATP assay kit and phosphonopyruvate were purchased from Sigma. Northern Max 10x Denaturing Gel Buffer and Northern Max 10x MOPS Gel Running Buffer were purchased from Ambion. [14C(U)]palmitate, [14C(U)]glucose, and [α-32P]dCTP were purchased from NEN Life Science Products Company. The BCA protein assay kit was purchased from Pierce. Carbosorbe E and Fura 2-AM was purchased from Dojin Chemical. Trypan blue was purchased from Flow Laboratories. The INS-1D cells, derived from insulinoma β-cells, were generously provided by Dr. C. B. Wollheim [39].

Preparation of recombinant adenovirus

The recombinant adenovirus (adeno-AMPKα1-DN), which contains a c-myc epitope tag at the N terminus, was a gift from Drs. Fabienne Foufelle, Pascal Ferre (U465 INSERM, Centre Biomolecular des Cordeliers, F-75270 Paris Cedex 06, France), and David Carling (The Cellular Stress Group, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, London W12 0NN, UK). The adenoviral vector contains a cDNA encoding nucleotides 1-312 of AMPKα1 with a mutation substituted from Asp157 to Ala, which has been reported to exert a dominant negative effect [17].
**Role of AMP Kinase in Insulin Secretion**

INS-1D cells were cultured in RPMI 1640 supplemented with 10% inactivated fetal bovine serum, 100 IU/mL penicillin, 100 IU/mL streptomycin, 10 mmol/L HEPES, 1 mmol/L MEM sodium pyruvate solution, and 50 mmol/L 2-mercaptoethanol at 37°C in a humidified atmosphere (5% CO₂, 95% air). The cells were cultured for 24 hours, and the adenoviruses were added to the culture. After 24 hours of infection, the medium was renewed, and the cells were cultured for another 24 hours. The cells were stained with trypan blue to ensure that all the cells were alive.

**Western Blotting**

INS-1D cells were lysed in a lysis buffer (25 mmol/L Tris-HCl, 2 mmol/L sodium orthovanadate, 10 mmol/L pyrophosphate, 10 mmol/L sodium fluoride, 1 mmol/L EDTA, 1 mmol/L EGTA, 0.1 mmol/L PMSF, 1 µg/mL aprotinin, 1 µg/mL leupeptin, and 1% Triton x100), and the protein contents were measured using a BCA protein assay kit. For the Western blot analysis, the samples were boiled in Laemmli’s sample buffer for 10 minutes, and separated on SDS polyacrylamide gels, transferred to polyvinylidene difluoride membrane, and blotted with the indicated antibodies. Signal detection was performed using an ECL kit.

**Measurement of insulin secretion and content**

After adenoviral infection, INS-1D cells were washed with Krebs-Ringer bicarbonate (KRb) buffer composed of 129 mmol/L NaCl, 4.8 mmol/L KCl, 1.2 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 2.5 mmol/L CaCl₂, 5 mmol/L NaHCO₃, 10 mmol/L HEPES, and 0.2% BSA (pH 7.4), preincubated at 37°C for 30 minutes in KRb buffer containing 0.1 mmol/L glucose, and incubated at 37°C for 60 minutes in KRb buffer containing 30 mmol/L glucose. The insulin secreted into the KRb buffer was measured using a radioimmunoassay with rat insulin as a standard. The cellular insulin content was extracted with acid ethanol (0.18 mmol/L HCl in 95% ethanol) overnight at 4°C, and its concentration was determined by radioimmunoassay after appropriate dilutions.

**Measurement of glucose oxidation**

Glucose oxidation by INS-1D cells was determined by measuring the generation of [¹⁴C]CO₂ from [U-¹⁴C]glucose [41]. INS-1D cells were preincubated at 37°C for 30 minutes in KRb buffer containing 0.1 mmol/L glucose, then incubated at 37°C for 60 minutes in KRb buffer containing 0.1 mmol/L or 30 mmol/L glucose in the presence of 1.2 µCi of [U-¹⁴C]glucose. The generated [¹⁴C]CO₂ was made volatile by adding HCl, captured by Carbosorb E, and measured with liquid scintillation counting.

**Measurement of cytosolic [Ca²⁺]**

Cytosolic [Ca²⁺] was measured using a previously described method [40]. INS-1D cells were loaded with 10 µmol/L fura-2/acetoxymethylester at 37°C for 30 minutes in KRb buffer containing 0.1 mmol/L glucose. INS-1D cells on a glass coverslip were placed on the stage of an inverted fluorescence microscope equipped with a charged-coupled device camera. INS-1D cells were then viewed under a water-immersion objective lens with alternate excitation wavelengths of 340 nm and 380 nm, and the ratio (F340 nm/380 nm) was calculated.

**RNA preparation and Northern blot analysis**

Total RNA was extracted from INS-1D cells with Trizol, separated in a denaturing formaldehyde agarose gel (1%), and transferred to Hybond N⁺ nylon membranes. Insulin cDNA probe was labeled by a random priming method using the Megaprime DNA labeling system and [α-³²P]dCTP. After overnight hybridization, the membrane was stringently washed and the
signals were detected and quantified with BAS 2000.

**Measurement of palmitate oxidation**

Palmitate oxidation in INS-1D cells was determined by measuring the generation of $^{14}$CO$_2$ from [U-1$^4$C]palmitate [44]. INS-1D cells were preincubated at 37°C for 30 minutes in KRB buffer containing 0.1 mmol/L glucose. The cells were then incubated at 37°C for 60 minutes in KRB buffer containing 0.1 mmol/L or 30 mmol/L of glucose in the presence of 1.2 µCi of [U-1$^4$C]palmitate. The generated $^{14}$CO$_2$ was made volatile by adding HCl, captured by Carbosorb E, and measured using liquid scintillation counting.

**Determination of triglyceride content**

TG content was extracted three times with chloroform and methanol. The extraction solvent was evaporated, and the TG was resuspended in isopropanol and measured using an L-type TG kit with triolein as a standard [45].

**Statistical analysis**

The statistical analysis was performed using a Student t-test for unpaired comparisons. Values are presented as the mean ± SE.

**Results**

**Overexpression of AMPKα1-DN in INS-1D cells infected with adenoviral vector**

The expression of AMPKα1-DN in INS-1D cells was examined using Western blot analysis with anti-AMPKα1 antibody, anti-c-myc antibody or anti-phospho AMPK antibody. The expression of the AMPKα1 signal was similar at all multiplicity of infection (MOI) levels (Fig. 1A), because the forced overexpression of AMPKα1-DN leads to the displacement of the endogenous α1 and α2 subunits from the heterotrimer and the degradation of the free α subunit [25]. The c-myc epitope was detected only in the protein from adenovirus-vector overexpressed cells (Fig. 1B), and the amount of phosphorylated AMPKα protein was reduced at MOI 10 (Fig. 1C), indicating that the activity of AMPK was decreased in INS-1D cells overexpressing AMPKα1-DN. To assess whether overexpression of AMPKα1-DN indeed exerted a dominant negative effect in our system, we examined phosphorylation of Acetyl-CoA carboxylase (ACC), since AMPK is known to directly phosphorylate and deactivate ACC.

As shown in Fig. 1D, overexpression of AMPKα1-DN at MOI 30 significantly attenuated phosphorylation of ACC, indicating that AMPKα1-DN had a dominant negative effect at this level in our system. Therefore, we used the adenoviruses at MOI 30 in all experiments hereafter.

**Impact of AMPKα1-DN overexpression on glucose-induced insulin secretion in INS-1D cells**

Insulin secretion from INS-1D cells overexpressing AMPKα1-DN in response to 30 mmol/L glucose was 22% lower than that in the control at MOI 30, while overexpression of AMPKα1-DN did not affect insulin secretion under the low glucose condition (Fig. 2A). The insulin content and the level of insulin mRNA were not reduced (Fig. 2B and C). In INS-1D cells overexpressing AMPKα1-DN, however, glucose oxidation was 27% lower than that in the control in the presence of 0.1 mmol/L of glucose, and 21% lower than that in the control in the presence of 30 mmol/L of glucose (Fig. 2D). The ATP/ADP ratio was also 22% lower than that in control cells overexpressing LacZ in the presence of 30 mmol/L glucose, although the difference did not reach statistical significance (Fig. 2E). Cytosolic [Ca$^{2+}$] was monitored using the fura-2 fluorescence ratio at 340/380 nm. Stimulation with 30 mmol/L of glucose triggered a sharp cytosolic [Ca$^{2+}$] rise in both groups, but the peak was markedly suppressed in INS-1D cells overexpressing AMPKα1-DN (Fig. 2F). These data suggest that glucose-stimulated insulin secretion is impaired at the level of glucose metabolism by inhibition of AMPK activity.

**Impact of AMPKα1-DN overexpression on lipid metabolism in INS-1D cells**

As noted above, AMPK regulates lipid oxidation through inhibition of ACC. Thus, overexpression of AMPKα1-DN is likely to affect lipid oxidation in INS-1D cells. To assess this possibility, we investigated the palmitate oxidation in INS-1D cells overexpressing AMPKα1-DN. In INS-1D cells overexpressing AMPKα1-DN, however, palmitate oxidation was 19% lower than that in the control in the presence of 0.1 mmol/L of glucose, and 24% lower than that in the control in the presence of 30 mmol/L of glucose (Fig. 3A). Interestingly, the TG content was 46% higher than that in the control cells (Fig. 3B). These data suggest that inhibition of AMPK results in intracellular lipid accumulation, a state of lipotoxicity, at least in
ROLE OF AMP KINASE IN INSULIN SECRETION

Impact of AMPK\(\alpha\)-DN overexpression on insulin secretion in response to potassium chloride or glibenclamide

Next, we studied whether the overexpression of AMPK\(\alpha\)-DN affected the response of insulin secretion to potassium chloride or glibenclamide, to assess whether inhibition of AMPK affected the secretory mechanism downstream of K\(^+\)-ATP channel in addition to the defect in glucose metabolism. Insulin secretion in response to 50 mmol/L of KCl with 0.1 mmol/L of glucose was 30% lower in the AMPK\(\alpha\)-DN cells (Fig. 4A). In addition, when stimulated by 5 \(\mu\)mol/L of glibenclamide with 0.1 mmol/L of glucose, insulin secretion decreased by 16% in the AMPK\(\alpha\)-DN cells, compared to that in the control LacZ cells (Fig. 4B). The response of cytosolic \([Ca^{2+}]\) to these secretagogues, however, was similar in the AMPK\(\alpha\)-DN and control cells (Fig. 4C and D). These data suggest the possibility that the secretory machinery downstream of Ca\(^{2+}\) release in response to glucose is also impaired by inhibition of AMPK.

Discussion

AMPK has been shown to play a pivotal role in glucose and lipid metabolism in liver and muscle, sensing energy balance. However, its role in the pancreatic \(\beta\) cells is largely unknown. In the present study, we overexpressed a dominant-negative form of the AMPK\(\alpha\) subunit in the INS-1D \(\beta\) cell line to assess the role of AMPK in \(\beta\) cells. Insulin secretion in response to high glucose was lower in INS-1D cells overexpressing AMPK\(\alpha\)-DN than that in control cells. This reduction in insulin secretion was associated with a reduction in glucose oxidation (Fig. 2D), a reduced ATP/ADP ratio (Fig. 2E) and impairment in the elevation of cytosolic \([Ca^{2+}]\) in response to glucose (Fig. 2F). Thus, the insufficient increase in the ATP/ADP ratio is likely to result in the insufficient elevation of cytosolic \([Ca^{2+}]\), thereby leading to the suppression of glucose-stimulated insulin secretion.

In the current study, glucose oxidation was lower in INS-1D cells overexpressing AMPK\(\alpha\)-DN in the presence of both 0.1 mmol/L of glucose and 30 mmol/L of glucose than that in the control. AMPK has been shown to activate glycolytic pathway by multiple mechanisms, such as phosphorylating 6-phospho-
in the control (Fig. 3B), at least in part, through the decrease in β oxidation and the increase in lipogenesis as a result of the increased ACC activity. In fact, the β oxidation of palmitate was reduced by exposure to both low and high concentration of glucose (Fig. 3a), presumably because of the inhibition of CPT-1 caused by the increase in malonyl-CoA due to the increased ACC activity. Recently, we have shown that AMPK fructo-2-kinase (PFK-2), the enzyme responsible for the synthesis of fructose 2,6-bisphosphate, a key stimulator of glycolysis[46]. Therefore, suppression of AMPK down-regulates the influx of glycolysis, thereby decreasing glucose oxidation and subsequent ATP production.

The intracellular TG content was 46% higher in INS-1D cells overexpressing AMPKα1-DN than that in the control (Fig. 3B).

**Fig. 2.** Effects of overexpression of AMPKα1-DN on glucose metabolism, insulin contents and insulin mRNA level in INS-1D cells. (A) Glucose-stimulated insulin secretion in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. Values are expressed as the means±SE (n=4). *P<0.01. (B) Insulin content in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. Values are expressed as the means±SE (n=3). (C) Insulin mRNA level in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ using Northern blot analysis. (D) Glucose oxidation in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. Values are expressed as the means±SE (n=4). *P<0.01, **P<0.05. (E) ATP/ADP in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. Values are expressed as the means±SE (n=3). P<0.10 (F) Glucose-stimulated cytosolic [Ca²⁺] levels in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. A representative profile from four repeated experiments is shown for each group.
Fig. 3. Effects of AMPKα1-DN overexpression on lipid metabolism in INS-1D cells. (A) Palmitate oxidation in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. Values are expressed as the means±SE (n=4). *P<0.05. (B) Triglyceride content in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. Values are expressed as the means±SE (n=3). *P<0.05.

Fig. 4. Effects of AMPKα1-DN overexpression on insulin secretion stimulated by depolarization or glibenclamide in the presence of 0.1 mmol/L of glucose. (A,B) Insulin secretion stimulated by depolarization (A) or glibenclamide (B) in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. Values are expressed as the means±SE (n=4). *P<0.01. (C,D) Cytosolic [Ca^{2+}] stimulated by depolarization (C) or glibenclamide (D) in INS-1D cells overexpressing adeno-AMPKα1-DN or adeno-LacZ. A representative profile from four repeated experiments is shown for each group.
activity negatively regulates SREBP-1c expression in liver [47]. Thus, suppression of AMPK in β cells may also increase production of fatty acid through this mechanism, contributing to the accumulation of TG.

There are some reports that glucose-stimulated insulin secretion can be impaired by TG accumulation in pancreatic β cells [33, 37, 48-51]. This phenomenon is called lipotoxicity, although its mechanism is unclear. Indeed, we and others previously reported that overexpressing a constitutive active form of sterol regulatory element binding protein (SREBP)-1c, a master transcription factor for lipogenesis in INS-1D cells increased TG content and concomitantly decreased glucose-stimulated insulin secretion [33, 37]. Importantly, when INS-1D cells overexpressing SREBP-1c were treated with AICAR, an activator of AMPK, the TG content was reduced and glucose-stimulated insulin secretion was partially restored. Furthermore, it has recently been shown that cultured rat β cells treated with shRNS for adipose triglyceride lipase (ATGL), whose expression is regulated by AMPK [52], or isolated islets of ATGL deficient mice exhibit impaired glucose-stimulated insulin secretion associated with TG accumulation [53]. These findings provide correlative evidence that increased TG content in INS-1D cells may play a role in impaired insulin secretion by suppression of AMPK activity. On the other hand, Rutter et al. reported that when MIN6 cells were treated with a dominant-negative form of AMPK, insulin secretion was not affected by 30 mmol/L of glucose stimulation compared to the situation in control MIN6 cells [35]. They infected MIN6 cells for 40 hours, while we infected INS-1D cells for 72 hours, but their report did not mention whether they measured the TG content. The difference in insulin secretion may be due to the different experimental conditions in their report and ours. Nevertheless, there are many reports that glucose-stimulated insulin secretion can be impaired by TG accumulation in pancreatic β cells [33, 37, 48-51]. However, there are some reports that TG accumulation in pancreatic β cells does not decrease glucose-stimulated insulin secretion [54, 55]. Thus the causal role of TG accumulation in impairment of glucose-stimulated insulin secretion remains to be further examined.

It is also possible that other pathways than intracellular TG accumulation are involved in impaired insulin secretion, since suppression of AMPK also inhibits glibenclamide stimulated insulin secretion, suggesting the defects in the steps downstream of the K_ATP channel. Indeed, stimulation of AMPK by AICAR in the isolated murine islets has recently been reported to inhibit the K_ATP channel activity thereby increasing insulin secretion [56], and this may also contribute to the impaired insulin secretion by suppression of AMPK. However this mechanism does not appear to account for the fact that suppression of AMPK also inhibits KCl stimulated insulin secretion in the present study. On the other hand, another report has demonstrated that activation of AMPK by glucose deprivation inhibits insulin secretion by increasing the traffic of the K_ATP channel from intracellular pool to cell surface [57]. In the current study, however, intracellular Ca2+ concentration was not affected by expression of DN-AMPK at low glucose concentration, suggesting that the number of K_ATP channel at the cell surface was not significantly affected by suppression of AMPK.

Insulin secretion in response to secretagogues other than glucose revealed that insulin secretion in response to KCl and glibenclamide was lower in INS-1D cells overexpressing AMPKα1-DN than that in the control (Fig. 4A and B). Importantly, since the degree of cytosolic [Ca2+] elevation was basically unchanged (Fig. 4C and D), the impairment in insulin secretion stimulated by depolarization or glibenclamide was likely to be due to a dysfunction in the downstream event of the influx of Ca2+. Although the mechanism responsible for the last step of insulin secretion from β cells has not been fully elucidated, SNARE proteins, such as VAMP, syntaxin 1 and SNAP-25, are known to be involved. The expression of SNAP-25 was markedly lower in cells overexpressing AMPKα1-DN than that in control cells (data not shown), although the expression levels of VAMP and syntaxin 1 were not altered between these two groups. Interestingly, the expression levels of syntaxin 1 and SNAP-25 were reduced in diabetic GK rat islets [58, 59], and the expression levels of SNAP-25, syntaxin-1A, VAMP2 were reduced in fa/fa rat islets (a rat model for obesity) [60]. Therefore, the lower levels of SNAP-25 protein may be, at least in part, responsible for the impaired exocytosis from β cells and may play a direct role in Ca2+ evoked insulin secretion. Thus, the depressed exocytotic mechanism might contribute to the impairment in glucose-stimulated insulin secretion, but further study is needed to unravel the role and mechanism of the reduction in SNAP-25 expression in insulin secretion in cells overexpressing AMPKα1-DN. For instance, it remains un-
known whether AMPK directly regulate the expression levels of SNARE proteins or indirectly affect them by modulating cellular metabolites, such as lipid products. Indeed, β cell specific hormone sensitive lipase knock-out mice show decreased insulin secretion due to a defect in exocytosis [61]. Nevertheless, the mechanism regulating exocytosis machinery by AMPK should be further explored in the future.

Lastly, AMPK is also known to sensitize insulin signaling [20], which has been shown to play a pivotal role in the regulation of glucose-stimulated insulin secretion and cell proliferation in β cells [53-55]. Indeed, it has been reported that activation of AMPK in INS-1E cells inhibits high glucose-stimulated apoptosis associated with impaired insulin secretion [62]. Thus, another possible mechanism underlying inhibition of glucose stimulated insulin secretion by AMPKα1-DN might be the down-regulation of insulin action in β cells, although the further studies are needed to assess this possibility.

In summary, the present study has revealed that inhibition of AMPK activity impairs glucose-stimulated insulin secretion partly through suppression of glucose oxidation associated with an increase in intracellular lipid accumulation. Together with the previous findings, this may suggest that AMPK protects β cells from lipotoxicity, an excess of lipid accumulation, thereby maintaining their responsiveness of insulin secretion to glucose. Moreover, our data also suggest that AMPK plays a role to maintain the normal secretory machinery, such as the level of SNAP-25, thereby providing β cells with the maximal ability of insulin secretion. Thus, AMPK appears to regulate insulin secretion in β cells at least by these two mechanisms and an alteration in its activity and/or expression by physiological and pathophysiological responses could affect plasma insulin levels, which must play a key role in the development of diabetes. Considering that AMPK activity is known to increase in the presence of a low glucose level and to decrease in the presence of a high glucose level, β cells chronically exposed to high glucose levels in diabetes or insulin resistant state may lose the full ability of insulin secretion in response to glucose at least in part through down-regulation of AMPK activity, leading to further exacerbation of hyperglycemia and making a vicious cycle. Thus, our data provide the novel therapeutic clue for diabetes that activation of AMPK may be useful not only to improve peripheral insulin sensitivity but also maintain insulin secretion by β cells.

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