Glucose-induced repression of PPARα gene expression in pancreatic β-cells involves PP2A activation and AMPK inactivation

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Abstract

Tight regulation of fatty acid metabolism in pancreatic β-cells is important for β-cell viability and function. Chronic exposure to elevated concentrations of fatty acid is associated with β-cell lipotoxicity. Glucose is known to repress fatty acid oxidation and hence to augment the toxicity of fatty acids. The peroxisome proliferator activated receptor α (PPARα) is a key activator of genes involved in β-cell fatty acid oxidation, and transcription of the PPARα gene has been shown to be repressed by increasing concentrations of glucose in β-cells. However, the mechanism underlying this transcriptional repression by glucose remains unclear. Here we report that glucose-induced repression of PPARα gene expression in INS-1E cells is independent of β-cell excitation and insulin secretion but requires activation of protein phosphatase 2A in a process involving inactivation of the AMP-activated protein kinase (AMPK).

Pharmacological activation of AMPK at high glucose concentrations interferes with glucose repression of PPARα and PPARα target genes in INS-1E cells as well as in rat islets. Specific knock-down of the catalytic AMPK-subunit AMPKα2 but not AMPKα1 using RNAi suppressed PPARα expression, thereby mimicking the effect of glucose. These results indicate that activation of protein phosphatase 2A and subsequent inactivation of AMPK is necessary for glucose repression of PPARα expression in pancreatic β-cells.

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Introduction

Tight regulation of β-cell fatty acid metabolism is important for β-cell function. Fatty acids acutely potentiate glucose stimulated insulin secretion (Stein et al. 1996, Itoh et al. 2003, Roduit et al. 2004), whereas chronic exposure of β-cells to elevated concentrations of fatty acids causes β-cell dysfunction known as lipotoxicity (Shimabukuro et al. 1998b, Maedler et al. 2001). The nuclear receptor peroxisome proliferator activated receptor α (PPARα) has previously been shown to be expressed and to activate fatty acid oxidation in pancreatic β-cells (Zhou et al. 1998). This would bring PPARα in a position to antagonize lipotoxicity; however, reports on the role of PPARα in β-cell function have been conflicting (Guerre-Millo et al. 2001, Tordjman et al. 2002, Gremlich et al. 2004). Recent data from our laboratory showed that acute activation of PPARα, but not PPARγ, has the potential to stimulate mitochondrial β-oxidation and potentiate glucose-stimulated insulin secretion (GSIS) in both INS-1E insulinoma cells and rat islets (Ravnskjaer et al. 2005). Thus, regulation of PPARα expression and activity appears essential for adjusting β-cells to metabolic challenges and for maintenance of β-cell function.

The lipotoxicity in β-cells is augmented by hyperglycemia in the pathology of glucolipotoxicity (El Assaad et al. 2003). Glucose interferes with lipid partitioning and redirects the flow from lipid oxidation into lipid accumulation. Instantly, this is accomplished through post-translational modification of numerous metabolic enzymes including acetyl-CoA carboxylase (ACC) (Zhang & Kim 1995), whereas chronic effects of glucose on lipid metabolism are exerted at the transcriptional level (Roche et al. 1998). Both acute and chronic effects of glucose are antagonized by the AMP-activated protein kinase (AMPK). AMPK is considered a central cellular energy gauge activated by energy depletion, cellular stress and adipokines and is known to promote lipid oxidation (Kahn et al. 2005). Similarly, AMPK shuts down energy-consuming processes such as lipogenesis by suppressing expression and activities of lipogenic enzymes like ACC (Zhang & Kim 1995) and key lipogenic transcription factors such as sterol regulatory element binding protein 1 (Zhou et al. 2001) and carbohydrate response element binding protein (ChREBP) (Kawaguchi et al. 2002). Inactivation of AMPK by glucose has previously been described in β-cells and could be a pivotal regulatory event in glucose-induced lipogenesis (Salt et al. 1998b, da Silva et al. 2001).
et al. 2000). In addition to ATP-generation from glucose oxidation, activation of protein phosphatase 2A (or PP2A-like phosphatases) could mediate glucose action through disruption of AMPK subunit interaction (Gimeno-Alcaniz & Sanz 2003, Samari et al. 2005).

In addition to the activation of lipogenic genes, increasing levels of glucose leads to repression of genes involved in fatty acid oxidation. Pancreatic islets chronically exposed to hyperglycemic conditions show a significant reduction in PPARα expression level (Zhou et al. 1998, Laybutt et al. 2002), and in vitro studies of insulinoma cells and rat islets confirm this repression by glucose as a direct effect on PPARα gene transcription (Roduit et al. 2000). However, the molecular mechanism underlying this repression by glucose remains unknown.

Here we report that glucose repression of PPARα gene expression in pancreatic β-cells is independent of β-cell excitation and insulin secretion but involves activation of PP2A (or PP2A-like phosphatase) and acute inactivation of AMPK. Activation of AMPK using the biguanide metformin or the thiazolidinedione (TZD) compound troglitazone completely reverses the glucose-repression of AMPK. Activation of PP2A (or PP2A-like phosphatase) could mediate glucose action through disruption of AMPK subunit interaction (Gimeno-Alcaniz & Sanz 2003, Samari et al. 2005).

Importantly, AMPK activation also abolished glucose repression of the PPARα gene in isolated rat islets. Similarly, selective inhibition of PP2A activity interferes with the effect of glucose. Specific knock-down of AMPKα2 by shRNA mimics the effect of glucose on the PPARα expression and supports a model where AMPK activity is necessary to maintain β-cell PPARα expression and where AMPK inactivation is instrumental for glucose-induced repression of PPARα gene expression.

Materials and methods

Cell culture and glucose stimulation

The cell line INS-1E was cultured as previously described (Merglen et al. 2004). The cells in use were all at passage numbers between 50 and 70 and used for experiments at 70–80% confluence. Medium and supplements were from Invitrogen-GIBCO and serum from HyClone. In all glucose experiments, cells were pre-incubated 24 h in 5 mM glucose media before addition of glucose and compounds. Rat islets were isolated from adult male Wistar rats by collagenase perfusion and pre-cultured for 24 h in 3 mM glucose medium before addition of glucose and metformin and further incubation for 24 h. Actinomycin D, verapamil and metformin were purchased from Sigma-Aldrich, okadaic acid from Biomol (Plymouth Meeting, PA, USA), and troglitazone was kindly provided by Novo Nordisk ( Bagsvaerd, Denmark).

Adenovirus generation and transduction

pSuper-AMPKα1 and α2 were constructed with specific oligos directed against sequences AGCCCTAGGT A GTGGTAGTA and CGCTCAGTTGATAAGTTCTG, respectively, as described (Brummelkamp et al. 2002). The H1-promoter and oligos were then excised using Smal and HindIII and ligated into pShuttle (EcoRV and klenow-filled Sall). Recombinant adenoviruses containing shRNA against AMPKα1 and α2 were generated using the AdEasy cloning system from Stratagene. The linearized plasmids were transfected into 293-HEK cells and the viruses were amplified and purified using CsCl gradients. Viruses were initially titrated and titers estimated by a plaque assay-based approach. Subsequently, relative titers of functional viruses were equalized based on quantification of the adenoviral transcript AdE4 by real time PCR. Adenoviral vectors expressing simian virus 40 (SV40) small-t antigen and mutated SV40 small-t antigen (C103S) (Porras et al. 1996) were kindly provided by Professor Kathleen Rundell (Feinberg School of Medicine, Chicago). Twenty-four hours after transduction with AdshAMPKα1 or α2, medium was changed to new 5 mM glucose medium and cells were incubated 48 h before harvest. SV40 Small-t antigen transduction was performed in 5 mM glucose medium and glucose added 24 h later. All experiments were made at least in duplicate.

Protein analysis by western blotting and ECL detection

For total protein extraction INS-1E cells were harvested in hypotonic lysis buffer containing SDS. Nuclear extracts were prepared essentially as described in (Roduit et al. 2000). Protein extracts were separated by SDS–PAGE and proteins blotted onto PVDF membranes (Millipore, Billerica, MA, USA) and probed with specific antibodies. Primary antibodies anti-PPARγ (SC-7273) and anti-TFIIIB (SC-225) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and anti-AMPKα (2532) and anti-phosphoAMPKα (Thr172) (#2531) from Cell Signaling (Danvers, MA, USA). Secondary horseradish peroxidase-coupled anti-Fcγ antibodies, anti-Mouse (P0447) and Anti-Rabbit (P0399), were obtained from DAKO Cytomation (Carpinteria, CA, USA). Densitometric quantification was performed using ImageQuant 5.0 software from GE Healthcare (Chalfont St. Giles, UK).

RNA isolation and cDNA synthesis

INS-1E cells were harvested in guadinium thiocyanate, and RNA was isolated according to a modified Chomczynski–Sacchi protocol (Chomczynski & Sacchi 1987). cDNA was prepared after DNase treatment (Invitrogen DNaseI) by reverse transcription (Invitrogen...
first-strand-kit) of the isolated RNA primed by four hexamers (dNTP)6.

Real time PCR

Quantitative 3-step real time PCR was performed on the ABI-7700 Prism real time PCR instrument using SyBRgreen master mix (Sigma) and Sigma passive reference according to instructions from the manufacturer. PCR reactions were made in duplicates. Primers for real time PCR were designed using Primer Express 2.0 (Applied Biosystems, Foster City, CA, USA) and specificity and efficacy validated before use. All quantifications were performed with TFIIB as internal standard and presented as fold over control. Primer sequences (forward and reverse, respectively): PPARα gataacactatggaactcagca, gcgaaagaacctgtgc; TFIIB gtt ctgtcctcactggcct, tgttgaagatgtctgccatcctgct; L-PK tggacac atctttgcctct, ctgtaaacaagtcctgtgc; c-fos ccttteccagcat ggcct, gacttgccgacagttccttg acyl-CoA oxidase (ACO) cagataattggccatcgcg; carnitine palmitoyl transferase 1 (CPT-1), ctggaggtgcaacatagc; aggtgataattcttcccaccagc; AdE4 cttcggaaacccacacag aa, gcagacagtggagaaaaattgg; SV40 Small-t-Ag tgcagctaatggaccttctaggt, gaatattcccccaggcactc; AMPKα1 aagccaaatcagggactgctac, agtgctgatggatcccgat; AMPKα2 gcgaaagtgaagattggagaaca, aactgccactttatggcctgtc.

Statistical analysis

Statistical evaluation of the data was performed using the two-tailed Student's t-test on paired data or one-way ANOVA (Fig. 5B). Data (relative to control (5 mM or C)) are presented as means ± S.D. (n≥3) or means ± range (n=2).

Results

Glucose represses transcription of the PPARα gene independently of insulin secretion

Glucose has previously been reported to repress the expression of PPARα mRNA in pancreatic β-cells, and to do so solely at the level of transcription (Roduit et al. 2000). We confirmed this observation in the INS-1E β-cell line and showed that glucose repression of PPARα mRNA level is observed within 30 min (Fig. 1A). In keeping with the notion that this repression occurs at the transcriptional level, inhibition of RNA synthesis with actinomycin D at 25 mM glucose, did not lead to further reduction in the PPARα mRNA level (Fig. 1A).

Glucose is known to induce β-cell excitation, Ca++ influx and insulin secretion. We therefore wanted to examine if glucose-stimulated Ca++ influx or GSIS is required for glucose repression of the PPARα gene in INS-1E cells. INS-1E cells were pre-incubated with verapamil, a selective L-type Ca++ channel blocker, prior to 12-h incubation with 25 mM glucose or with the non-nutrient insulin secretagog KCl (40 mM). Importantly, neither verapamil nor KCl had any effect on the PPARα mRNA level (Fig. 1B). This shows that Ca++ influx and insulin secretion are not implicated in the glucose repression of PPARα expression. Similarly, neither the specific insulin receptor inhibitor HNMPA-AM3 nor wortmannin affected glucose repression of PPARα (data not shown). As a control for the experiment, the expression levels of two glucose-induced genes, liver pyruvate kinase (L-PK) and the immediate early gene c-fos, were quantified. In pancreatic β-cells, L-PK is induced by glucose through activation of the ChREBP independently of β-cell excitation (Wang & Wollheim 2002), whereas c-fos induction by glucose is dependent on Ca++ influx (Susini et al. 1998). As expected, L-PK was induced only by glucose even in the presence of verapamil, while c-fos was induced by both glucose and KCl in a verapamil sensitive manner (Fig. 1B). Thus, these results show that glucose-induced repression of PPARα gene expression in INS-1E cells is independent of β-cell excitation and insulin secretion.

Pharmacological activation of AMPK relieves glucose repression of the PPARα gene in INS-1E cells and isolated rat islets

As AMPK activation is known to interfere with glucose induction of gene expression, we wanted to investigate whether activation of AMPK would also counteract the repressive effect of glucose on β-cell PPARα expression. INS-1E cells were cultured for 24 h in medium containing 25 mM glucose in the presence or absence of troglitazone (5–20 µM) or metformin (0.25–1 mM), both of which have been demonstrated to activate the AMPK in muscle and β-cells (Fryer et al. 2002, Leclerc et al. 2004). Total RNA was extracted and real time PCR was used to quantify the PPARα mRNA level relative to that of TFIIB. High concentrations of glucose (25 mM) repressed PPARα expression to approximately 25% of the expression at 5 mM glucose (P<0.01), whereas increasing concentrations of troglitazone (Fig. 2A) or metformin (Fig. 2B) dose-dependently restored PPARα expression. We next wanted to see if AMPK activation could also attenuate glucose repression of the PPARα gene in primary β-cells. To do so, freshly isolated rat islets were pre-incubated for 24 h at 3 mM glucose and further incubated for 24 h at 3 mM or 20 mM glucose, respectively, in the presence or absence of metformin. RNA was extracted and PPARα levels were quantified by real time PCR and presented relative to TFIIB expression. In line with the effects of AMPK activation in INS-1E cells, metformin completely abolished glucose...
repression of PPARα but had no effect at 3 mM glucose (Fig. 2C). These results indicate that inactivation of AMPK activity is necessary for glucose-induced repression of PPARα gene expression in pancreatic β-cells.

AMPK activation by metformin restores PPARα protein level and expression of PPARα target genes at high glucose

To verify that the effects of AMPK activation on PPARα mRNA level were translated into an increase in PPARα protein level, nuclear extracts were prepared from INS-1E cells cultured for 24 h in medium containing 25 mM glucose in the absence or presence of 0.5 mM metformin, respectively. The nuclear extracts were subjected to SDS–PAGE analysis and western blotting. Total cell extracts from cells ectopically expressing mouse PPARα was used as a positive control. PPARα protein levels reflected the changes in mRNA level in that 25 mM glucose markedly reduced PPARα protein, whereas metformin activation of AMPK counteracted glucose repression of PPARα protein (Fig. 3A). To confirm that the glucose regulation of PPARα protein is functionally significant, we investigated expression levels of the known PPARα target genes ACO (Tugwood et al. 1992) and liver CPT-1 (Napal et al. 2005). Notably, expression of both target genes is reduced by glucose (P<0.05) and dose dependently restored by AMPK activation with metformin (0.25–1 mM) (Fig. 3B). Thus, the changes in PPARα mRNA expression in response to glucose and AMPK activation are reflected by parallel changes in PPARα protein level and expression of PPARα target genes.
Pharmacological activation of AMPK and inhibition of PP2A blocks glucose inhibition of β-cell AMPK activity

The finding that glucose represses PPARα expression in a manner counteracted by pharmacological activation of AMPK, points to a central role for AMPK in this response. To address this, AMPK activity was evaluated in INS-1E cells incubated for 0.5–24 h at 5 and 25 mM glucose alone or in the presence of troglitazone (5, 10 and 20 μM) (A) or metformin (0.25, 0.5 and 1 mM) (B). Cells were subsequently cultured for 24 h before total RNA was extracted. Freshly isolated rat islets were pre-cultured for 24 h in 3 mM glucose medium before 24-h incubation at 3 or 20 mM glucose in the presence or absence of metformin (1 mM) (C). Expression of PPARα was quantified using real time PCR, normalized to TFIIB expression and presented relative to expression at 5 mM glucose. RNA was harvested in duplicates, and range is indicated (*, significantly different from 5 mM glucose P<0.05). The presented results are representative of at least 3 independent experiments.

AMPK activity (Fig. 4B), as did metformin (Fig. 4C), without affecting the total protein level of AMPKα subunit. Glucose is known to activate PP2A. Interestingly, inhibition of PP2A by okadaic acid totally blocked the negative effect of glucose on AMPK activity (Fig. 4C). These results confirm that AMPK activity is acutely repressed by glucose in the β-cell line INS-1E and show that this repression is counteracted by metformin and troglitazone as well as by inhibition of PP2A.

Inhibition of PP2A blocks glucose repression of PPARα expression

To further investigate the role of PP2A in glucose action on β-cell PPARα expression, PP2A activity was chemically inhibited in INS-1E cells stimulated with 25 mM glucose and PPARα mRNA levels were analyzed by real time PCR. Resembling the stimulating effect on AMPK-activity, chemical inhibition of PP2A using the specific inhibitor okadaic acid (12.5 or 25 nM)
dose-dependently restored PPARα levels (Fig. 5A, \( P < 0.05 \)). This was confirmed by adenoviral transduction (titer 40 pfu/cell) of INS-1E cells with SV40 small-t antigen—a protein known to form a stable complex with PP2A and inhibit its catalytic activity (Yang et al. 1991). Similarly to okadaic acid, SV40 small-t antigen blunted the glucose effect on PPARα expression (Fig. 5B, \( P < 0.05 \)). A mutated form of SV40 small-t antigen (C103S) expressed to the same level but unable to interact with PP2A (Porras et al. 1996) did not affect PPARα expression nor did Ad-CMV (C). These results indicate that glucose activation of PP2A or PP2A-like kinases is necessary for glucose-induced repression of PPARα gene expression.

### Specific knock-down of the catalytic AMPK α2-subunit mimics glucose-induced PPARα repression

To confirm the role of AMPK activity in maintenance of PPARα gene expression under low glucose conditions, we generated adenoviral vectors expressing shRNA for specific knock-down of the catalytic subunits α1 and α2. INS-1E cells were transduced with these adenoviral shRNA constructs targeting AMPKα1, α2, or a combination of both, respectively, for 72 h in 5 mM glucose medium. Control cells were transduced with an empty adenoviral shRNA-vector (C). Total RNA and protein was extracted and AMPKα mRNA (Fig. 6A) and protein levels (Fig. 6B) were investigated. Specific knock-down of the AMPKα1 and α2 genes was confirmed by quantitative real time PCR (50% and 60% mRNA reduction respectively, \( P < 0.05 \)). Also AMPKα protein level was reduced by knock-down as assessed by western blotting and ECL-detection of total level of AMPKα subunit. Whereas knock-down of AMPKα1 did not affect PPARα expression level, AMPKα2 knock-down reduced the PPARα mRNA level by \(~50\%\) (Fig. 6C, \( P < 0.05 \)). These results show that the activity of AMPKα2, but not that of AMPKα1, is important for maintenance of PPARα gene expression.

### Discussion

During the last years, our knowledge about the family of AMPKs has expanded dramatically. Once a glucose sensor in budding yeast (Celenza & Carlson 1986) the AMPK is now considered a central fuel gauge upholding mammalian energy homeostasis and controlling fuel selection in various metabolically important tissues (reviewed in Kahn et al. 2005). Metabolic signals such as...
adipokines (Minokoshi et al. 2002, Yamauchi et al. 2002, Banerjee et al. 2004), hormones (Minokoshi et al. 2004) and nutrients (da Silva et al. 2000, Leclerc & Rutter 2004, Koo et al. 2005) are integrated by the AMPK centrally and peripherally resulting in increased food intake, reduced hepatic glucose output and a general shift

Figure 4 AMPK activity is repressed by glucose but restored by troglitazone, metformin and PP2A inhibition. (A) and (B) INS-1E cells were pre-cultured for 24 h in 5 mM glucose medium before addition of glucose to 25 mM with or without with troglitazone (5, 10, 20 μM). (C) INS-1E cells pre-cultured at 5 mM glucose were treated with metformin (0.5 mM) or okadaic acid (25 nM) at 5 mM or 25 mM glucose, respectively. Cells were harvested and whole cell extracts subsequently prepared at the indicated time points A or after 24 h incubation B and C. Proteins were separated by SDS-PAGE and levels of phosphoAMPKα (Thr172) and AMPKα visualized by immunoblotting and densitometric quantification. The presented results are representative of at least 3 independent experiments.

Figure 5 Inhibition of PP2A interferes with glucose repression of PPARα gene expression. (A) INS-1E cells were pre-cultured for 24 h at 5 mM glucose. The glucose concentration was increased to 25 mM, DMSO or okadaic acid (12.5 or 25 nM) was added, and cells were cultured for 24 h before harvesting of RNA. (B) Cells were adenovirally transduced with SV40 small-t-antigen wt or mutant (C103S) or Ad-CMV as control (C) at 5 mM glucose. After 24 h, the glucose concentration was increased to 25 mM and cells were subsequently cultured for 24 h before total RNA was extracted. Expression of PPARα was quantified by real time PCR, normalized to TFIIB expression and presented relative to expression at 5 mM glucose. RNA was harvested in duplicates, and range is indicated. Means not sharing same superscript differ significantly (P<0.05). The presented results are representative of at least 3 independent experiments.
from energy expenditure to energy production. Also pharmacologically, the AMPK is of substantial interest. Anti-diabetic compounds such as metformin and rosiglitazone of the biguanide and thiazolidinedione classes, respectively, have been shown to activate AMPK (Zhou et al. 2001, Fryer et al. 2002). Metformin was recently reported to activate hepatic AMPK through activation of the upstream AMPK-kinase LKB1 (Shaw et al. 2005).

Here we describe a novel role for AMPK in the pancreatic β-cell. In keeping with AMPK stimulation of fatty acid oxidation in various tissues, we show that AMPK is important for the maintenance of PPARα gene expression in INS-1E cells and primary β-cells. Furthermore, we provide evidence that AMPK inactivation is a key event in glucose-induced repression of PPARα expression. While acutely decreasing AMPK activity, glucose represses PPARα expression independently of β-cell excitation and insulin secretion in both the INS-1E β-cell line and isolated rat islets. Importantly, repression of PPARα gene expression is reflected at PPARα protein level together with expression levels of the known PPARα target genes ACO and CPT-1 in a manner completely reversed by pharmacological activation of AMPK. Specific knock-down of AMPKα2, but not AMPKα1, reduces PPARα expression even at a low glucose level, suggesting that inactivation of AMPKα2 by glucose is upstream to PPARα gene repression. This is in agreement with our finding that metformin did not have any effect on islet PPARα expression under non-repressive low glucose conditions. To further explore the underlying mechanism, we chemically inhibited phosphatases known to be activated by glucose and found that inhibition of PP2A using okadaic acid attenuated glucose-induced inactivation of AMPK as well as repression of PPARα expression. This was confirmed by specific SV40 small-t antigen mediated inhibition of PP2A activity. SV40 small-t antigen forms a stable complex with the PP2A A-subunit replacing the regulatory B-subunit and inhibiting PP2A activity (Yang et al. 1991). The relative contribution of ATP generation from glucose oxidation to AMPK inactivation was not assessed in our experiments. Notably however, PP2A inhibition with okadaic acid entirely restored AMPKα phosphorylation level at 25 mM glucose (Fig. 4C), suggesting that glucose could suppress AMPKα activity by activating PP2A rather than through an increase in the ATP/AMP ratio. A model where glucose activates PP2A leading to AMPK

Figure 6 Specific knock-down of AMPKα2 represses PPARα expression. INS-1E cells were transduced with a control virus AdshEmpty (C), adenoviral shRNA vectors AdshAMPKα1, AdshAMPKα2 or both and cultured for 72 h at 5 mM glucose. Total mRNA and protein was extracted. (A) Relative knock-down of either AMPKα subunit α1 and α2 was quantified by real time PCR and normalized to TFIIB expression. (B) Knock-down of AMPKα protein was confirmed by western blotting and ECL. (C) PPARα expression was quantified by real time PCR and normalized to TFIIB expression. RNA was harvested in duplicates, and range is indicated (*, significantly different from control P<0.05). The presented results are representative of at least 3 independent experiments.
inactivation and PPARα repression (outlined in Fig. 7) is attractive as homologous pathways controlling energy substrate selection are known from yeast (Jiang & Carlson 1996). Glucose has previously been shown to inactivate AMPK in β-cells (Salt et al. 1998b, da Silva et al. 2000), and recent findings that glucose and PP2A can disrupt mammalian AMPK subunit association (Gimeno-Alcaniz & Sanz 2003) support the depicted model.

AMPK activity is known to counteract glucose-stimulated gene expression through inhibition of ChREBP (Kawaguchi et al. 2002), whereas possible involvement of AMPK in repression of mammalian genes by glucose has not previously been described. However, treatment of islets from hyperglycemic Zucker diabetic fatty (ZDF) rats with leptin resulted in increased fatty acid oxidation, islet delipidation and functional restoration (Zhou et al. 1997, 1998). Strikingly, the lipopenic effect of leptin was associated with an increase in expression of both PPARα and PPARδ target genes. Leptin is known to promote fatty acid oxidation in various tissues but only in skeletal muscle this has been shown to involve AMPK activation (Minokoshi et al. 2002). Also troglitazone, here used to activate AMPK, has a similar potential to counteract glucolipotoxicity in isolated ZDF rat islets through delipidation (Shimabukuro et al. 1998a, Higa et al. 1999). Both observations are in keeping with our data showing that activation of AMPK leads to increased PPARα expression. Of note, PPARγ is not expressed at significant levels in INS-1E cells (data not shown). Therefore it is unlikely that troglitazone, which is also a PPARγ agonist, affects PPARα expression through activation of PPARγ in the present study.

Our finding that specific knock-down of the α2 but not the α1 subunit of AMPK in the β-cell suppresses PPARα expression indicates a functional specialization of the two catalytic isoforms. This was previously addressed by specific α1 and α2 knock-out models (Viollet et al. 2003). Whereas AMPKα1−/− mice did not display any phenotypic abnormalities, AMPKα2−/− mice were highly glucose intolerant with impaired GSIS. The β-cell dysfunction observed in vivo was not found in isolated AMPKα2−/− islets, suggesting that GSIS impairment was either not a primary defect, or that β-cell AMPKα2 depletion is critical only in the in vivo AMPKα2−/− environment with chronically increased levels of fatty acids and catecholamines. Other studies functionally comparing AMPKα1 and α2 revealed that the α1 subunit is cytoplasmatic, whereas AMPKα2 is also found in the nucleus of β-cell lines (Salt et al. 1998a, da Silva et al. 2000). AMPKα1 is hence likely to phosphorylate cytoplasmatic substrates, whereas AMPKα2 may be involved in the conversion of metabolic signals into transcriptional regulation. This model is supported by our data showing that AMPKα2, but not AMPKα1, is involved in the maintenance of PPARα expression. In addition, our RNAi based approach supports previous studies in MIN6 insulinoma cells where inactivation of AMPKα2 by antibody injection at low glucose mimicked glucose induction of the L-PK (da Silva et al. 2000). Of note, a similar approach in primary hepatocytes, where a dominant negative AMPKα subunit was used to inactivate endogenous AMPK at low glucose, showed no effect on L-PK expression (Woods et al. 2000). Whether this discrepancy reveals a general difference between β-cells and hepatocytes or simply reflects different experimental conditions is still to be resolved. In a recent study, ectopic expression of a constitutive active AMPKα subunit in rat islets did not affect PPARα expression (Diraison et al. 2004). However, as the authors note, the experiments were performed at 3 mM glucose, reducing the impact of ectopic AMPKα expression. This would be in agreement with our present data (Fig. 2C). Furthermore, substrate recognition of a deregulated catalytic AMPKα subunit expressed alone could be compromised by the relative lack of regulatory subunits.

In conclusion, we describe here for the first time the involvement of AMPK in repression of gene expression by glucose. We find that repression of PPARα gene expression by glucose in INS-1E insulinoma cells involves activation of PP2A and inactivation of AMPK.
This mechanism is likely to apply also to primary β-cells, since AMPK activation totally abolishes glucose repression of the PPARα gene in isolated rat islets. Our result that AMPKα2 but not AMPKα1 is necessary for maintenance of PPARα gene expression suggest that glucose acts primarily by inactivation of AMPKα2. Hence, acute inactivation of the AMPKα2 appears to be a critical step in both induction and repression of genes by glucose in pancreatic β-cells. PPARα is a known key regulator of fatty acid oxidation, and PPARα expression in the β-cell is important for β-cell function (Zhou et al. 1998, Ravenskaer et al. 2005). Thus, the ability of AMPK to increase PPARα expression under hyperglycemic conditions indicates that pharmacological activators of AMPK may exert direct beneficial effects on β-cell lipid partitioning and contribute to β-cell protection.

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