Stimulation of Acetyl-CoA Carboxylase Gene Expression by Glucose Requires Insulin Release and Sterol Regulatory Element Binding Protein 1c in Pancreatic MIN6 β-Cells

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Acetyl-CoA carboxylase I (ACCI) is a key lipogenic enzyme whose induction in islet β-cells may contribute to glucolipotoxicity. Here, we provide evidence that enhanced insulin release plays an important role in the activation of this gene by glucose. Glucose (30 vs. 3 mmol/l) increased ACCI mRNA levels ~4-fold and stimulated ACCI (pII) promoter activity ~30-fold in MIN6 cells. The latter effect was completely suppressed by blockade of insulin release or of insulin receptor signaling. However, added insulin substantially, but not completely, mimicked the effects of glucose, suggesting that intracellular metabolites of glucose may also contribute to transcriptional stimulation. Mutational analysis of the ACCI promoter, and antibody microinjection, revealed that the effect of glucose required sterol response element binding protein (SREBP)-1c. Moreover, adenoviral transduction with dominant-negative–acting SREBP1c blocked ACCI gene induction, whereas constitutively active SREBP1c increased ACCI mRNA levels. Finally, glucose also stimulated SREBP1c transcription, although this effect was independent of insulin release. These data suggest that glucose regulates ACCI gene expression in the β-cell by complex mechanisms that may involve the covalent modification of SREBP1c. However, overexpression of SREBP1c also decreased glucose-stimulated insulin release, implicating SREBP1c induction in β-cell lipotoxicity in some forms of type 2 diabetes. Diabetes 51:2536–2545, 2002

Elevated glucose concentrations stimulate the transcription of several genes involved in glucose metabolism in islet β-cells (1–6). These include the liver isoform of pyruvate kinase (l-PK) (1,7) and acetyl-CoA carboxylase I (ACCI) (3). ACCI is transcribed from two promoters (PI and PII) (8), both of which are subject to control by nutrients (9). Although ACCI catalyzes an essential step in fatty acid synthesis in lipogenic tissues, this is usually a minor pathway of glucose usage in β-cells (3). However, malonyl-CoA, the immediate product of ACCI, may play a signaling role in glucose-stimulated insulin secretion (10,11).

We have recently reported that proinsulin and l-PK gene expression is regulated by glucose in MIN6 β-cells largely through the activated release of endogenous insulin (12), consistent with findings in primary β-cells (13). Moreover, homozygous inactivation of the insulin receptor specifically in islet β-cells leads to diabetes in mice (14), emphasizing the probable importance of insulin for the regulation of genes in the β-cell (15). However, the molecular mechanisms through which gene expression is regulated by insulin in this cell type are poorly understood (5). In particular, the identity of the transcription factors involved remains a matter of controversy (2,9).

Sterol regulatory element binding proteins (SREBPs) (1) are members of the basic/helix-loop-helix/leucine zipper family of transcription factors and play a central role in the regulation of lipid synthesis in other mammalian tissues (16,17). Alternative transcriptional start sites and splicing give rise to two isoforms of SREBP1: SREBP1a and SREBP1c (16). SREBP1c, which is preferentially expressed in liver and adipose tissue, has recently been implicated in the regulation by insulin of lipogenic genes in these tissues (18,19). Thus, in isolated hepatocytes, insulin increases the level of SREBP1c mRNA (18), the endoplasmic-reticulum–bound precursor (M, ~125), and the NH2-terminal fragment of SREBP1 (M, ~50) present in the nucleus. Moreover, SREBP1c is indispensable for the induction by insulin of the fatty acid synthase and l-PK and ACCI genes in the liver (18). Finally, expression of the constitutively active NH2-terminal fragment of SREBP1c (corresponding to amino acids 1–403) was sufficient to activate glucokinase gene expression in the absence of
and, at low glucose concentrations, in liver cells (19).

Recent studies have also shown that SREBP1c mRNA is present at low levels in islets from normal rats and at elevated levels in diabetic fa/fa rats (20). Islets from these animals also show enhanced deposition of triglyceride (21). However, up to now, neither the presence of SREBP1 protein nor its regulation or functional importance has been explored in pancreatic β-cells.

We show here that, in clonal MIN6 β-cells, the effects of glucose on the ACCI promoter are likely to be mediated by the release of insulin. Moreover, suppression of SREBP1c function with a dominant-negative—acting form of the protein completely inhibits glucose-induced transcription of the ACCI gene, whereas overexpression of the nuclear fragment of SREBP1c to high levels activated ACCI gene expression and promoter activity. These results suggest that insulin-stimulated changes in the activity of nuclear SREBP1c are important for the regulation of the ACCI gene by glucose in islets, but leave open the possibility of involvement from other factors.

RESEARCH DESIGN AND METHODS

Materials. Culture media and antibiotics were obtained from Life Technologies (Paisley, U.K.). Other reagents were from Sigma (Poole, Dorset, U.K.) or BDH (Poole).

Cell culture. MIN6 cells (22) were used between passages 20 and 30 and grown in Dulbecco’s modified Eagle’s medium containing 15% (vol/vol) heat-inactivated FCS, 25 mmol/l glucose, 2 mmol/l glutamine, 50 μmol/l β-mercaptoethanol, 100 μg/ml penicillin, and 100 mg/ml streptomycin in a humidified atmosphere at 37°C with 5% CO₂, unless specified otherwise. MIN6 cells were maintained and infected with viruses at 25 mmol/l glucose. Cells were transferred to medium containing 3 mmol/l glucose 16 h before exploring the subsequent effects of glucose.

Amplification of recombinant adenosviruses and cell infection. Adenoviruses expressing constitutively active SREBP1c (amino acids 1–403, wild-type sequence) or dominant-negative (1–403, Y320A) SREBP1c were constructed and amplified as described (18,23). Virus particles were purified on CsCl gradients before infection (24) at a multiplicity of infection of 30 viral particles per cell. Infection efficiency was judged using enhanced green fluorescent protein, also present in the adenoviral genome; >95% of individual MIN6 cells were infected after 24–48 h. In all experiments, adenovirus expressing enhanced green fluorescent protein cDNA only (null) was used as control.

Assay of insulin secretion. MIN6 cells (1.5 x 10⁵) were perfused (0.5 ml/min) at 37°C in Krebs-Ringer bicarbonate HEPES buffer (1 ml) comprising 125 mmol/l NaCl, 3.5 mmol/l KCl, 1.5 mmol/l CaCl₂, 0.5 mmol/l MgSO₄, 0.5 mmol/l KH₂PO₄, 2.5 mmol/l NaHCO₃, and 10 mmol/l HEPES-Na⁺, pH 7.4, and containing initially 3 mmol/l glucose and equilibrated with 95:5 O₂/CO₂. Fractions (0.5 ml) were collected and stored at −20°C. Total cellular insulin was extracted in acidified ethanol (25). Radioimmunoassay was performed by competition with [125I]-labeled rat insulin (Linco Research, St. Charles, MO) (24).

Assay of lipid deposition with oil red O. Staining of neutral lipid in MIN6 cells was performed as described (26). Lipid-specific staining was visualized with a Leica TCS NT confocal microscope (excitation at 564 nm).

Semiquantitative RT-PCR. Total RNA was isolated by cell lysis in TRI Reagent (Sigma). First-strand cDNA synthesis was performed as described (27). RT reactions (1 h) were performed at 42°C using 3.5 μg RNA (determined by absorbance at 260 nm) and 50 μl RT mix (1.25 μg/ml, 0.2 mmol/l MgCl₂, and 0.1% (wt/vol) sodium deoxycholate, and 0.1% (vol/vol) proteinase K), also present in the adenoviral genome; >95% of individual MIN6 cells were infected after 24–48 h. In all experiments, adenovirus expressing enhanced green fluorescent protein cDNA only (null) was used as control.

Electrophoretic mobility shift assay. Nuclear extracts were prepared from MIN6 cells treated with 10 mmol/l HEPES, 0.5 mol/l NaCl, 0.1 mmol/l EDTA, pH 7.4, and transferred to immobilized membranes using a discontinuous buffer system as described (22). Blots were probed with monoclonal antibody to SREBP1 (1:1,000 dilution) and revealed with horseradish peroxidase–conjugated anti-mouse IgG (1:10,000) using an enhanced chemiluminescence detection system (Roche Diagnostics).

Plasmids. To generate the SREBP1c promoter: luciferase vector (pSREBP.Luc crud), genomic DNA was isolated from MIN6 cells by lysis in TRI reagent. The independent 5′ flanking region of the SREBP1c gene, lying between exons 1 and 1–2 (23), was amplified by PCR using the primer pair: forward, 5′-TTTTAGATATCATCCTGGATCCATTAG; reverse, 5′-TCTATCACGATATCATTGAGC, corresponding to nucleotides 4021–4040 and 4481–4500, respectively, of human ACCI cDNA (accession no. U19822) (30). PCR products were separated on 2% agarose gels containing ethidium bromide (0.5 μg/ml) and quantitated by digital imaging (ImageQuant; Molecular Dynamics).

Immunoblot (Western) analysis. MIN6 cells (1.2 x 10⁶) were extracted into radio-immunoprecipitation assay buffer comprising PBS supplemented with 1.0% (vol/vol) Nonidet P40, 0.5% Na⁺ deoxycholate, and 0.1% (wt/vol) sodium dodecylsulfate. Protein concentration was determined by a commercially available kit using BSA as standard (BCA, catalog no. 23225; Pierce, Rockford, IL). To obtain nuclear extracts, cells were lysed by swelling in hypotonic buffer (10 mmol/l HEPES, pH 7.9, 10 mmol/l KCl, and 0.1 mmol/l Na⁺ EDTA) as described (31). Proteins were separated by SDS-PAGE (7.5% wt/vol) and transferred to immobilized membranes using a discontinuous buffer system as described (32). Blots were probed with monoclonal antibody to SREBP1 (1:1,000 dilution) and revealed with horseradish peroxidase–conjugated anti-mouse IgG (1:10,000) using an enhanced chemiluminescence detection system (Roche Diagnostics).

Micro-injection and single-cell imaging of gene expression. Intraocular micro-injection of plasmids was performed using an Eppendorf 512/524 microinjection system (27) at plasmid concentrations of 0.1 mg/ml (Luc crud–based vectors) or 0.05 mg/ml (pCMV.RL, pcDNA3.SREBP1c [1–403]) in injection buffer (2 mmol/l Tris-HCl, 0.2 mmol/l Na⁺ EDTA, pH 8.0) (38). SREBP1 antibody was dialyzed into injection buffer and co-injected into the nuclear compartment at 0.1 mg/ml as described (39). Individual experiments involved the injection of 100–200 separate cells per condition, with an efficiency of 5–20% productive injection. Cells were imaged 6 h after micro-injection and cultured under the conditions given in the figure legends.

Electrophoretic mobility shift assay. Assays were performed on nuclear extracts (see above) according to the study by Daniel and Kim (35).

Statistics. Data are expressed as means ± SE. Comparison of groups was performed with a one-tailed Student’s t test.
FIG. 1. Regulation of ACCI gene expression by insulin and glucose. A: INS cells were incubated for 24 h at 3 mmol/l glucose and subsequently for 6 h at 3 or 30 mmol/l glucose, plus the indicated concentrations of insulin. Extraction of total RNA and semiquantitative PCR was performed as given in RESEARCH DESIGN AND METHODS. The results of a typical experiment (upper panel) and combined data from three separate experiments (lower panel) are shown.

B: MIN6 cells were co-microinjected with plasmids ACCIP-II-1082.LucFP and pCMV.RL (see RESEARCH DESIGN AND METHODS) before culture for 6 h at either 3 or 30 mmol/l glucose. Where indicated, insulin was added to 20 nmol/l, and KCl was added to 13.0 mmol/l (vs. 5.5 mmol/l), 100 μmol/l verapamil (verap), and 200 μmol/l diazoxide (diazo). ACCI (pII) promoter activity, measured as the ratio of firefly:Renilla reniformis luciferase activity, was then determined by single-cell imaging (images; scale bar = 50 μm) and is presented as the means ± SE for 20–60 productively injected cells in each case (three to five separate experiments). Neither a control promoter (cytomegalovirus) nor empty promoter vector (pGL3, Promega) was affected by [glucose] in this assay (not shown).

C: Cells were microinjected with plasmids and incubated as in B, with the added presence of clonidine (5 μmol/l) or insulin (20 nmol/l) as indicated.

D: Cells were microinjected with luciferase reporter plasmids plus either empty pcDNA3 or phosphatase and tensin homologue (PTEN)-expressing vector, as shown, before incubation and luciferase assays. *P < 0.05 and ***P < 0.001 for the effect of the indicated stimulus vs. 3 mmol/l glucose; ##P < 0.01 and ###P < 0.001 for the effect of the indicated agents vs. control.
RESULTS

Regulation of ACCI gene expression by glucose and insulin. ACCI mRNA was strongly (approximately fourfold) induced by incubation of MIN6 cells for 6 h at 30 vs. 3 mmol/l glucose (Fig. 1A) or at 3 mmol/l glucose in the presence of insulin (>1 mmol/l). Glucose- and insulin-induced changes in ACCI mRNA level largely reflected a stimulation of mRNA synthesis, and 30 mmol/l glucose caused a >30-fold activation of an ~1-kb fragment of the ACCI (pII) promoter assayed in single cells after microinjection and imaging of a firefly luciferase reporter construct (plasmid ACCI pII-1082.Luc FF; Fig. 1B).

We previously reported that incubation of MIN6 cells under the conditions used here increases insulin levels, measured in the medium at the end of the 6-h incubation, from ~7 to 14 mmol/l (12). To determine whether the effects of high [glucose] on ACCI mRNA levels and promoter activity could largely be explained by activated insulin release, we suppressed the latter with the L-type Ca\(^{2+}\) channel blocker, verapamil (41), or with an opener of these agents, diazoxide. Either agent completely inhibited ACCI (pII) promoter activation by 30 mmol/l glucose (Fig. 1B). To exclude the possibility that these drugs acted via a decrease in intracellular free [Ca\(^{2+}\)] rather than a blockade of insulin release per se, we determined the effect of the \(\alpha\)2-adrenoreceptor agonist, clonidine, which inhibits glucose-induced insulin secretion but not increases in [Ca\(^{2+}\)] (12,42). Clonidine completely reversed the stimulation of ACCI promoter activity by 30 mmol/l glucose, and this effect was rescued by 20 mmol/l insulin (Fig. 1C).

We next explored the possibility that a factor other than insulin, present within secretory vesicles and cosecreted with the hormone (e.g., ATP), may be responsible for triggering ACCI promoter activation. Arguing against this possibility, the effects of 30 mmol/l glucose were completely suppressed by blockade of signaling by insulin, but not ATP receptors, with the phosphatidylinositol 3’ kinase inhibitor, LY294002 (not shown), or through coexpression of the phosphoinositol 3’ phosphatase phosphatase and tensin homologue (43) (Fig. 1D).

Role of SREBP1c in the activation of the ACCI promoter by glucose. To determine which cis-acting element(s) was involved in its regulation, we next performed 5’ deletion analysis of the ACCI (pII) promoter. Truncation of the promoter as far as nucleotide ~285 and consequent removal of proximal SREs (an E-box and an Sp1 binding site) had no effect on ACCI promoter activity at 30 mmol/l glucose but led to a small increase in basal promoter activity (Fig. 2). By contrast, further removal of nucleotides ~285 to ~217 and thus two SRE half-sites (44) led to a marked (>85%) reduction in glucose-stimulated promoter activity (Fig. 2).

To explore the potential role of SREBP1, acting at the SRE half-sites, we next co-microinjected a monoclonal antibody specific for SREBP1 (Fig. 2, right panel). This led to a complete inhibition of the stimulation by 30 mmol/l glucose of the full-length (~1,082 kb) and ~285-truncated ACCI promoters (Fig. 2, top). By contrast, anti-SREBP1 antibodies had no further inhibitory effect on the construct in which the two SRE half-sites had been removed (truncation at ~217 bp; Fig. 2).

To further dissect the role of the two SRE half-sites, we mutated each of these individually (44). Mutation of either site led to a strong glucose-independent induction of promoter activity (Fig. 2). Suggesting that SREBP1c may bind to either half-site as a monomer (44,45), anti-SREBP1 antibodies caused substantial inhibition of the promoter mutated at either SRE half-site (Fig. 2).

Our next experiments explored the impact of SREBP1 inhibition on the activity of the endogenous ACCI gene. Suggesting that SREBP1c is probably the predominant SREBP1 isoform in MIN6 \(\beta\)-cells, SREBP1c mRNA was clearly quantifiable after 20 PCR cycles, whereas SREBP1a mRNA was undetectable after 20 PCR cycles and only weakly detectable after 25 cycles (data not shown). Correspondingly, infection with an adenovirus expressing a dominant-negative–acting form of SREBP1c (SREBP1c

FIG. 2. Deletion analysis of the ACCI promoter. Single MIN6 cells were microinjected with the indicated promoter constructs plus pCMV.RL plus either IgG or anti-SREBP1 antibody (SREBP1-ab) (1 mg/ml) as shown. Normalized promoter activity was measured after a 6-h culture at the indicated glucose concentrations.
DN) (residues 1–403, Y320A), which is unable to bind DNA (46), completely suppressed the glucose-induced increase in ACCI mRNA (Fig. 3A). This inhibition could be ascribed to the blockade of ACCI transcription because microinjection of a plasmid encoding SREBP1c DN totally abrogated the glucose-induced increase in ACCI (pII) promoter activity (Fig. 3B).

**Regulation of ACCI expression by constitutively active SREBP1c.** To test whether an increase in SREBP1c amount or activity in response to glucose may be responsible for the stimulation of the ACCI gene, MIN6 cells were infected with an adenovirus bearing the constitutively active NH2-terminal fragment of SREBP1c (SREBP1c CA) (SREBP1c 1–403) (19). Infection with this virus caused a marked increase in ACCI mRNA levels in cells incubated for 6 h at either 3 or 30 mmol/l glucose (Fig. 4A). Similarly, microinjection of single cells with a plasmid construct bearing SREBP1c CA caused a marked increase in ACCI (pII) promoter activity (Fig. 4B) at either glucose concentration.

We next determined whether the effects of SREBP1c CA on ACCI gene expression might be due to enhanced insulin secretion. As evidence against this possibility, SREBP1 CA expression caused a near-complete abolition of glucose-stimulated insulin release (Fig. 4C, right panel) and was associated with a marked accumulation of intracellular lipid (Fig. 4C, left panel).

**Regulation of SREBP1c expression by glucose and insulin.** Elevated glucose concentrations (30 vs. 3 mmol/l) increased the levels of SREBP1c mRNA (Fig. 5A), total and mature SREBP1 protein (Fig. 5B), and SREBP1c transcription (Fig. 5C). However, in contrast to the regulation of the ACCI gene (Fig. 1), exogenously added insulin had no effect on any of the above parameters (Fig. 5). Moreover, neither high [glucose] nor insulin (20 nmol/l) enhanced SREBP1c processing (Fig. 5B). Finally, the effects of

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**FIG. 3. Regulation of ACCI gene expression by SREBP1c DN.** A: MIN6 cells were infected with the indicated adenoviruses before incubation at the given glucose concentrations. ACCI and \( \beta \)-actin mRNA levels were assessed by semiquantitative RT-PCR. Means (±SE) of three separate experiments are shown. B: ACCI (pII) promoter activity was measured in single cells (see Fig. 1) co-injected with either empty pcDNA3 or plasmid pcDNA3.SREBP Y320A (SREBP1c DN) as indicated. Luminescence ratio of typical single cells (left panel) and combined data (means ± SE) from five experiments involving 20–60 productively injected cells is shown (right panel). Scale bar = 30 μm. *P < 0.05 and ***P < 0.001 for the effect of 30 mmol/l glucose; #P < 0.05 and ##P < 0.01 for the effect of SREBP1c DN vs. control plasmid.
glucose on the SREBP1c promoter were unaltered by blockade of insulin secretion with verapamil or diazoxide (Fig. 5C), and neither exogenously added insulin nor the insulin secretagogue KCl affected SREBP1c promoter activity (Fig. 5C).

To further explore the role of the induction of SREBP1c or other transcription factors in the stimulation of the ACCI gene by glucose, we tested the impact of inhibiting protein synthesis globally. Although incubation of cells for 6 h with cycloheximide led to a stimulation of ACCI mRNA levels at 3 mmol/l glucose, a significant further increase in ACCI [mRNA] in response to 30 mmol/l glucose was still observed (ratios of ACCI: β-actin mRNA in the absence of cycloheximide 0.59 ± 0.10 and 0.96 ± 0.14 at 3 and 30 mmol/l glucose, respectively; corresponding values with 50 µg/ml cycloheximide were 1.08 ± 0.02 and 1.68 ± 0.19, n = 3 experiments in each case, P < 0.05 for the effect of 30 mmol/l glucose).

Effects of glucose and insulin on SREBP1c binding DNA activity. The above data suggest that glucose acts to stimulate ACCI gene expression through a mechanism that requires preexisting SREBP1c protein but not the activation of SREBP1 gene expression or SREBP1c processing. Therefore, to determine whether glucose or insulin enhances the DNA binding activity of preexisting SREBP1c, we used electrophoretic mobility shift assay. However, binding to an oligonucleotide corresponding to the two SRE half-sites delineated as the likely site of SREBP1c binding by promoter deletion analysis (Fig. 2) was too weak to be detected in extracts from cells incubated at either 3 or 30 mmol/l glucose (not shown).

DISCUSSION

Involvement of insulin release in the regulation of ACCI gene expression by glucose. Similar to proprioninsulin (13,38,47) and I-PK (12) gene expression, we show here that, at relatively early time points (≤6 h), ACCI gene expression in β-cells is probably stimulated by high glucose concentrations through a mechanism principally involving stimulated insulin release (see Fig. 1 and RESULTS). Thus, addition of the hormone was as efficient as high [glucose] in increasing ACCI mRNA levels (Fig. 1A). However, suggesting that intracellular metabolites of glucose such as glucose-6-phosphate (3) may also play some role in transcriptional stimulation, added insulin (20 mmol/l) was less effective than high glucose in stimulating ACCI promoter activity (Fig. 1B). Furthermore, 30 mmol/l glucose still activated the ACCI promoter after expression of active SREBP1c (Fig. 4A and B)—conditions under which glucose-stimulated insulin release was substantially, although not completely, inhibited (Fig. 4C). However, under these conditions, where the ACCI promoter was “super-activated” by active SREBP1c, it is conceivable that even the modest stimulation of insulin release by glucose (Fig. 4C) may contribute to further transcriptional activation of the ACCI gene. Moreover, the failure of elevated glucose concentrations to affect ACCI transcription when insulin release was completely inhibited with verapamil or diazoxide (Fig. 1B) suggests that the impact of intracellular metabolites of glucose may be limited to a potentiation of the effects of released insulin. These findings thus contrast results previously reported in INS1 cells (3), where non-glucose nutrients and other secretagogues (KCl and forskolin) had no apparent effect on ACCI mRNA levels. One possible explanation of this apparent discrepancy is that glucose regulates ACCI gene expression by distinct mechanisms in INS1 and MIN6 cells. However, we have noted that incubation with KCl has deleterious effects on MIN6 cell morphology and does not provoke the accumulation of ACCI mRNA at the 24-h time point used by Brun et al. (3). Moreover, the effects of nutrient secretagogues on the release of insulin were not examined by these authors. Thus, it would seem difficult to exclude a role for secreted insulin in these earlier studies, especially because the effects of blocking insulin release (while preserving glucose metabolism) were not explored.

Role of SREBP1c in the regulation of ACCI gene expression by glucose and insulin. Consistent with a mechanism involving the action of secreted insulin, the effects of glucose on ACCI gene expression reported here required the presence of nuclear SREBP1c (Figs. 2 and 3) (18). However, induction of SREBP1c gene expression by glucose should not be considered responsible for the effects of glucose on the ACCI gene at early time points because ACCI mRNA was still induced by glucose when the synthesis of new proteins was suppressed (see RESULTS). Moreover, high glucose concentrations stimulated SREBP1c promoter activity independently of insulin release (Fig. 5C), whereas the latter apparently activated ACCI transcription (Fig. 1). In this respect, the regulation of the SREBP1c gene in β-cells is different from that in the liver, where insulin alone, but not glucose, induces SREBP1c expression (19).

Our data suggest instead that an insulin-stimulated modification of the NH2-terminal fragment of SREBP1c, and thus the transactivation or DNA binding capacity of this factor, may be responsible for the activation of the ACCI gene in response to high glucose. Although the nature of this modification is unknown, it is of note that SREBP1c can be phosphorylated in vitro by mitogen-activated protein kinases (48,49), a family of enzymes strongly activated by glucose in β-cell lines (50,51). Further studies of the phosphorylation state of SREBP1c in intact cells will be required to resolve these issues. The mechanisms whereby glucose may regulate the ACCI and other genes in the β-cell are summarized in Fig. 6.

Lopez et al. (52) have previously shown that purified recombinant SREBP1c is capable of binding oligonucleotides corresponding to the two half-site SREs in the region of −285 to −247 with respect to the ACCI transcriptional

FIG. 4. Effect of overexpression of SREBP1c CA on ACCI gene expression, lipogenesis, and insulin secretion. A: ACCI mRNA levels were assessed after infection with the shown adenoviruses. B: ACCI (pII) promoter activity was assessed in cells co-microinjected with plasmids ACCIpII-H11082LucP and pCMV-RL, plus either empty pcDNA3 or pcDNA3.SREBP1c (1–403) (SREBP1c CA) before culture for 6 h at either 3 or 30 mmol/l glucose. Scale bar = 75 µm. Mean data from three separate experiments are given (±SE) in A and B. *P < 0.05, **P < 0.01, or ***P < 0.001 for the effect of 30 vs. 3 mmol/l glucose: #P < 0.05 and ###P < 0.01 for the effect of SREBP1c CA—expressing vs. null adenovirus. C: Adenoviral-mediated expression of SREBP1c (1–403) protein (left, upper panel) and the impact on lipid deposition (left, two lower panels) in cells incubated at 30 mmol/l glucose were assessed as described in RESEARCH DESIGN AND METHODS. Insulin secretion (right panel) was measured during perfusion with Krebs-Ringer bicarbonate medium at the indicated glucose concentrations. *P < 0.01 vs. null virus.

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FIG. 5. Regulation of SREBP1c gene expression by glucose and insulin. A: After incubation of cells at the indicated concentrations of glucose and added insulin, SREBP1c mRNA levels were assessed by semiquantitative RT-PCR, as given in RESEARCH DESIGN AND METHODS. Data (±SE) are given from three separate experiments.

B: SREBP1 protein was quantitated by Western analysis in cells incubated as in A, and the amounts of SREBP1 precursor (125 kDa) and mature SREBP1 (50 kDa) were quantitated by densitometric analysis of the immunoblots obtained in three separate experiments.

C: SREBP1c promoter activity was quantitated by single-cell analysis (see RESEARCH DESIGN AND METHODS) after microinjection and incubation for 6 h at either 3 or 30 mmol/l glucose and in the presence of the indicated additions (see Fig. 1 legend). Means (±SE) of data from 20–60 productively injected cells (three to five experiments) are given in the histogram. *P < 0.05 and ***P < 0.001 for the effects of 30 vs. 3 mmol/l glucose. verap, verapamil; diazo, diazoxide.
ROLE OF SREBP1c IN PANCREATIC ISLET β-CGS

FIG. 6. Scheme. Glucose regulates SREBP1c and ACCI transcription by distinct mechanisms. Elevated glucose concentrations lead to the activation of insulin release and binding to the insulin receptor (IR). Signaling via a phosphatidylinositol 3-kinase (PI 3K)-dependent pathway leads to activation of SREBP1c or another unknown transcription factor, perhaps by phosphorylation. Activation of SREBP1c transcription involves an exclusively intracellular signaling cascade and may potentiate the effects of released insulin on ACCI promoter activity long term (>6 h). G6P, glucose-6-phosphate; IRS, insulin receptor substrate.

start site. We show here that this region is required for the effect of glucose on the ACCI promoter in MIN6 cells (Fig. 3) and that mutation of either half-site leads to an increase in ACCI promoter activity. However, we were unable to detect the presence of any activity capable of binding these oligonucleotides in nuclear extracts from MIN6 cells by electrophoretic mobility shift assay (see RESULTS). This result was unsurprising because DNA binding activity of SREBP1c is difficult to demonstrate, even in nuclear extracts where SREBP1c is abundant, such as liver (F.F., P.F., unpublished observations). Nevertheless, our preliminary data suggest that the binding activity of adenovirally overexpressed SREBP1c to a consensus oligonucleotide derived from the fatty acid synthase (FAS) promoter (53) may be stimulated by elevated glucose concentrations (F.D., G.A.R., unpublished observations; data not shown).

An important finding made during these studies is that overexpression of SREBP1c leads to a dramatic deposition of intracellular lipid and suppression of insulin secretion (Fig. 4C). Thus, by regulating ACC and FAS gene expression, glucose-induced increases in SREBP1c may play an important role in β-cell glucolipotoxicity (54) in some forms of type 2 diabetes.

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REFERENCES


