

Critical Role for Cataplerosis via Citrate in Glucose-Regulated Insulin Release

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The molecular mechanisms mediating acute regulation of insulin release by glucose are partially known. The process involves at least two pathways that can be discriminated on basis of their (in)dependence of closure of ATP-sensitive potassium (K^+_{ATP}) channels. The mechanism of the K^+_{ATP} channel-independent pathway was proposed to involve cataplerosis, the export of mitochondrial intermediates into the cytosol and in the induction of fatty acid-derived signaling molecules. In the present article, we have explored in fluorescence-activated cell sorter (FACS)-purified rat β -cells the molecular steps involved in chronic glucose regulation of the insulin secretory response. When compared with culture in 10 mmol/l glucose, 24 h culture in 3 mmol/l glucose shifts the phenotype of the cells into a state with low further secretory responsiveness to glucose, lower rates of glucose oxidation, and lower rates of cataplerosis. Microarray mRNA analysis indicates that this shift can be attributed to differences in expression of genes involved in the K^+_{ATP} channel-dependent pathway, in cataplerosis and in fatty acid/cholesterol biosynthesis. This response was paralleled by glucose upregulation of the transcription factor sterol regulatory element binding protein 1c (SREBP1c) (ADD1) and downregulation of peroxisome proliferator-activated receptor (PPAR)- α and PPAR- β (PPAR δ). The functional importance of cataplerosis via citrate for glucose-induced insulin release was further supported by the observation that two ATP-citrate lyase inhibitors, radicicol and (-)-hydroxycitrate, block part of glucose-stimulated release in β -cells. In conclusion, chronic glucose regulation of the glucose-responsive secretory phenotype is associated with coordinated changes in gene expression involved in the K^+_{ATP} channel-dependent pathway, in cataplerosis via citrate and in acyl CoA/cholesterol biosynthesis. *Diabetes* 51:2018–2024, 2002

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ACL, ATP-citrate lyase; FAS, fatty acid synthase; GLP-1, glucagon-like peptide-1 (7–37)amide; G6PDH, glucose-6-phosphate dehydrogenase; IPPP, isopentenyl pyrophosphate; K^+_{ATP} channel, ATP-sensitive potassium channel; PPAR, peroxisome proliferator-activated receptor; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element binding protein.

Insulin release from pancreatic β -cells is tightly regulated according to metabolic needs (1,2). A key regulator of β -cell function is plasma glucose. Since the process of glucose-regulated insulin release is disturbed in many diabetic patients, it is of major medical interest to know the underlying molecular mechanisms. At least two glucose signaling pathways are known to be present (1,2). A well-defined pathway proceeds via glucose uptake and phosphorylation, mitochondrial pyruvate oxidation, increased [ATP]/[ADP] ratio, closure of ATP-sensitive potassium channels (K^+_{ATP} channels), membrane depolarization, opening of voltage-dependent calcium channels, rise in cytosolic calcium, and activation of calcium-calmodulin-dependent effector proteins. A second, though still poorly defined, pathway occurs independently of K^+_{ATP} channels (1). Although the messengers and targets for regulation are still unclear, this pathway has been proposed to require cataplerosis (3) and the acylation of proteins (4). It was shown before that culturing rat β -cells for 9 days in low glucose causes their desensitization for further glucose activation, decreasing the insulin secretory capacity of the cells (5). The molecular basis for this phenotypic shift is presently unknown. Large-scale analysis of gene expression using oligonucleotide microarrays is a promising new technology to search for genes that are relevant for the β -cell phenotype (6,7). To our best knowledge, glucose effects on the mRNA profile of insulin-secreting cells have thus far only been measured in a β tumor cell (7), the phenotype of which is expected to differ from primary cultures of nontumoral β -cells. In the present article, we have explored the phenotypic changes at the mRNA, metabolic, and secretory levels imposed by comparing two glucose concentrations during 24-h tissue culture: standard (10 mmol/l; G10 cells) and low (3 mmol/l; G3 cells). We observe that a large shift in the insulin secretory phenotype (75% loss in G3 cells) is accompanied by coordinated changes in glucose oxidation and in citrate-derived lipid metabolism, which in turn can be explained by an orchestrated change in gene transcription. The current analysis of the glucose-responsive phenotype of rat β -cells strongly indicates that cataplerosis via citrate and ATP-citrate lyase (ACL) is a major metabolic pathway of β -cell activation, both acutely and as chronic adaptation to changes in extracellular glucose.

RESEARCH DESIGN AND METHODS

Islet cell isolation and culture. Purified rat β -cells were obtained from male Wistar rat collagenase-isolated pancreatic islets using flow cytometry and were cultured in serum-free medium, as previously described (5). β -cells were precultured overnight in Ham's F10 medium (Gibco-BRL, Grand Island, NY), supplemented with 2 mmol/l glutamine, 10 mmol/l glucose (Merck, Darmstadt, Germany), 1% charcoal treated type V BSA (Boehringer Mannheim, Germany), 0.075 g/l penicillin (Sigma, St. Louis, MO), and 0.1 g/l streptomycin (Sigma). After this period, the cells were cultured for 24 h in the same medium containing either 3 or 10 mmol/l glucose. Viability of the cells, assessed by neutral red uptake, was the same for the two cell cultures ($87 \pm 1\%$ G3 cells vs. $87 \pm 1\%$ G10 cells).

Microarray and RT-PCR analysis of mRNA expression in β -cells. Total RNA (10 μ g) was isolated using the Trizol RNA isolation method (Gibco-BRL) and used as starting material for the cDNA preparation. The first- and second-strand cDNA synthesis was performed using the SuperScript Choice System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, except we used an oligo-dT primer containing a T7 RNA polymerase promoter site. Labeled cRNA was prepared using the MEGAscript in vitro transcription kit (Ambion, Austin, TX). Biotin-labeled CTP and UTP (Enzo, Farmingdale, NY) were used in the reaction together with unlabeled NTPs. After the in vitro transcription reaction, the unincorporated nucleotides were removed using RNeasy columns (Qiagen, Hilden, Germany). Array hybridization and scanning was performed as described in Cordozo et al. (6). cRNA was fragmented at 94°C for 35 min in a fragmentation buffer containing 40 mmol/l Tris-acetate, pH 8.1, 100 mmol/l KOAc, and 30 mmol/l MgOAc. Before hybridization, the fragmented cRNA in a 6 \times SSPE-T hybridization buffer (1 mol/l NaCl, 10 mmol/l Tris, pH 7.6, and 0.005% Triton) was heated to 95°C for 5 min and subsequently to 40°C for 5 min before loading onto the Affymetrix probe array cartridge (Affymetrix, Santa Clara, CA). The probe array was then incubated for 16 h at 40°C at constant rotation (60 rpm). The washing and staining procedure was performed in the Affymetrix Fluidics Station. The probe array was exposed to 10 washes in 6 \times SSPE-T (at 25°C) and 4 washes in 0.5 \times SSPE-T (at 50°C). The biotinylated cRNA was stained with a streptavidin-phycoerythrin conjugate (10 mg/ml, Molecular Probes, Eugene, OR) in 6 \times SSPE-T for 30 min (at 25°C). The labeled RNA was exposed to 10 washes in 6 \times SSPE-T (at 25°C). The probe arrays were scanned at 560 nm using a confocal laser-scanning microscope with an argon ion laser as excitation source (Hewlett Packard GeneArray Scanner G2500A). The readings from the quantitative scanning were analyzed by the Affymetrix Gene Expression Analysis Software.

To assess which genes are responsible for the glucose-responsive phenotype of rat β -cells, mRNA profiles in G3 and G10 cells were compared via Affymetrix rat Genome U34A oligonucleotide arrays, as described previously (6). Genes were considered to be glucose responsive if they fulfilled the following criteria: 1) average difference (AvDiff as defined by Affymetrix software on basis of difference between perfect match/mismatch probes) is ≥ 150 units in the condition of increased gene expression; 2) mean fold change in expression level (three replicate experiments) of ≥ 2 ; and 3) ≥ 1.5 -fold change in expression level between G3 and G10 cells in each of the three replicate experiments. Genes were assigned to functional clusters based on searches on PubMed and the Kyoto Encyclopedia of Genes and Genomes (www.KEGG.com). Expressed sequence tag sequences were further identified by BLAST searches onto databases.

For some selected transcripts, microarray data were confirmed or extended by semiquantitative PCR analysis. This was performed for GLUT2 (forward primer GGTGTGATCAATGCACCTC, reverse primer GTATCTGGGGCTTTCTGGAC, 25 cycles), ACL (forward primer CCATGTCAGCCAAGGCAAT, reverse primer CAGTTCGTTGAGACCTCAA, 30 cycles), SREBP1c (forward primer GGAGCCATGGATTGCACATT, reverse primer AGGAAGGCTTCCAGAGAGGA, 25 cycles), PDX1 (forward primer CTCGCTGGGAACGCTGGAACA, reverse primer CTTTGGTGGATTTCATCCACGG, 25 cycles), PPAR- α (forward primer: CCCTCTCCAGCTTCCAGCCC, reverse primer CCACAAGCGTCTTCTCAGCCATG, 25 cycles), and PPAR- β (forward primer TGACGGCCAGCGAGGGATG, reverse primer GAGGCTGCTGAAGTTGGGATG, 25 cycles). Polymerization reactions were performed in a Perkin-Elmer 9600 thermocycler in a 50- μ l reaction volume containing 2 μ l cDNA, 50 ng RNA equivalent, 20 pmol of appropriate oligonucleotide primers, GeneAmp PCR buffer, and 5 units of AmpliTaq Gold polymerase (Perkin Elmer). The thermal cycle profile used was as follows: a 10-min denaturing step at 94°C, a number of cycles indicated between brackets (cycling temperature at 58°C), and a final extension step (at 72°C). To correct for experimental variations between samples, the gene of interest was amplified with α -tubulin (forward primer CTCGCATCCACTTCCCTC, reverse primer ATGCCCTCACCCAGTAC, 20 cycles) in each sample. Samples were loaded onto 1.5% agarose gels and photographed with the Kodak 120 System. Bands were analyzed with the 1D

Image Analysis Software program (Eastman Kodak). The amount of each specific product was then expressed relative to the internal control, giving a ratio specific product/control gene for each sample. A negative control (RT reaction performed in absence of RT enzyme) was performed to exclude genomic DNA contamination of cDNA.

Western blotting. To examine stearoyl CoA desaturase gene expression at the protein level, extracts corresponding to 1.4×10^5 pancreatic β -cells were loaded. For immunoblots we used a rabbit polyclonal anti-SCD1 antibody (1:2,000 dilution; gift from Dr. J. Ozols, Farmington, CT; this antiserum also recognizes SCD2 and SCDX) and HRP-labeled donkey anti-rabbit Ig (1:2,000 dilution; Amersham, Buckinghamshire, England) as secondary antibody. As positive controls, rat liver (50 μ g) or SCD1-induced rat liver microsomes (kindly given by Dr. J. Ozols) were loaded in parallel. Protein loading was checked by immunoblotting stripped blots with anti- α -tubulin (1:2,000; Sigma). Protein abundance was visualized using the ECL kit (Amersham) and Kodak Xomat films; intensities of SCD1 bands were quantified using Kodak software (1D Image Analysis Software) and normalized for α -tubulin.

Insulin release. Insulin release from rat β -cells was measured during either 2-h static incubations (5) or dynamic perfusions (8). Samples were assayed for immunoreactive insulin with guinea pig anti-insulin serum. Static incubations were performed with 3×10^4 cells per tube in 0.5-ml volumes of Earle's Hepes medium (pH 7.35). The conditions were indicated below the figure [different glucose concentrations, 10 mmol/l glucose + 10 mmol/l GLP-1, presence or absence of the ACL-inhibitors radicicol, also known as monorden, a C14-macrolide derivative originally isolated from *Monosporium bonorden* (9; kind gift from Dr. Yoshida, Tokyo, Japan) or (-)-hydroxycitrate (Hoffman La Roche, Nutley, NJ; 10)]. The perfusion experiments were carried out in a multiple microchamber module (Endotronics, Coon Rapids, MN). Approximately 2.5×10^5 β -cells were loaded per column and preperfused for 20 min in Ham's F10 medium, 3 mmol/l glucose, supplemented with 0.5% BSA, 2 mmol/l glutamine, and 2 mmol/l CaCl₂ and were equilibrated with 95%O₂/5% CO₂. Perfusion was done by stimulation with 3 mmol/l glucose for 20 min and pulses for 10 min with either 10 mmol/l glucose or 10 mmol/l glucose with 10 nmol/l GLP-1. Flow rate was 0.5 ml/min. The dead space was taken into account when expressing the results as a function of time. Insulin content of the perfused β -cells was measured by sonificating the Biogel P2 containing the cells in 2 mmol/l acetic acid/0.25%BSA.

Metabolic fluxes. Glucose oxidation was measured as conversion of [U-¹⁴C]-labeled glucose into ¹⁴CO₂ (11). The escape of glucose carbon from mitochondrial oxidation (cataplerosis) was measured radiometrically (11). After 2-h labeling in 100 μ l Earle's Hepes medium containing [U-¹⁴C]-labeled glucose (2.5 μ Ci/tube), the cells were washed five times in cold buffer and sonicated in 1 mol/l perchloric acid. Cataplerosis was measured, using a β -scintillation counter, as moles of ¹⁴C-labeled carbon-atoms that were precipitated by perchloric acid and that were solubilized by adding water (0.2 mol/l NaCl), chloroform and methanol (volume ratios 1:2:1) (12) to the precipitate. Whereas the water/methanol phase contains peptides synthesized from glucose-derived amino acids (11), the bottom organic phase contains acyl CoAs and lipids that are synthesized from glucose carbon (12).

RESULTS AND DISCUSSION

To explore the significance and regulation of cataplerosis in insulin release, we have performed a molecular and physiological study of this pathway in FACS-purified β -cells that were cultured for 24 h at 3 mmol/l glucose (G3 cells) and 10 mmol/l glucose (G10 cells). As can be seen in Fig. 1A, the secretory response to a subsequent acute rise in extracellular glucose was severely suppressed in G3 cells ($\sim 75\%$) as compared with G10 cells. In perfusion experiments, the secretory defect was detected during both the first and early second phase of glucose-stimulated insulin release and it could not be abolished by adding the gluco-incretin hormone glucagon-like peptide-1 (7-37)amide (GLP-1; Fig. 1B). The difference in insulin secretory responsiveness from G3 and G10 cells was not due to changes in cellular viability and insulin content (23 ± 2 vs. 20 ± 3 pg/cell, G3 vs. G10 cells). Quantitative differences between G3 and G10 cells were detected at the level of metabolic flux, measured by incubating the cells for 2 h with [U-¹⁴C]-glucose. These differences were present at both the level of ¹⁴CO₂ pro-

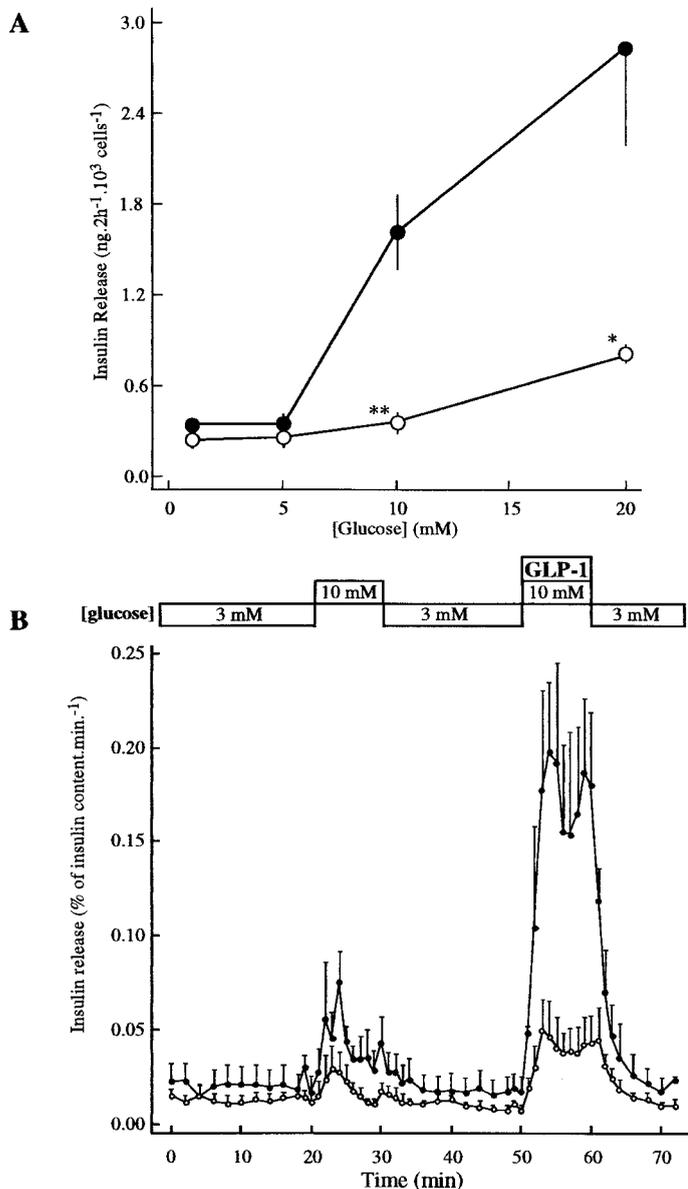


FIG. 1. Effect of a 24-h culture in low (3 mmol/l) versus high (10 mmol/l) glucose on the secretory capacity of purified pancreatic β -cells. **A:** β -cells were cultured for 24 h in 3 mmol/l glucose (○; G3 cells) or 10 mmol/l glucose G10 cells (●; G10 cells), followed by 2-h static incubations at the indicated glucose concentrations to measure insulin release. **B:** Insulin secretory response during a dynamic perfusion experiment in which G3 cells (○) and G10 cells (●) were stimulated for 10 min with either 10 mmol/l glucose alone or 10 mmol/l glucose plus 10 nmol/l GLP-1. Values are means \pm SE of five experiments.

duction (glucose oxidation; Fig. 2A) and cataplerosis, which was detected as accumulation of ¹⁴C-labeled intermediates in the cells (Fig. 2B). These metabolic fluxes are expected to be relevant for, respectively, the K_{ATP} -dependent pathway and cataplerosis (1–4,11,12). Messenger RNA profiling of these cells using Affymetrix GeneChips led to the observations that 76 mRNAs were upregulated in high glucose and that 103 mRNAs were more abundant in cells cultured at low glucose. A molecular correlation to the metabolic changes in Fig. 2 is shown in Fig. 3A, which summarizes the regulated transcripts that are involved in metabolic signaling pathways. Upregulation of mRNA en-

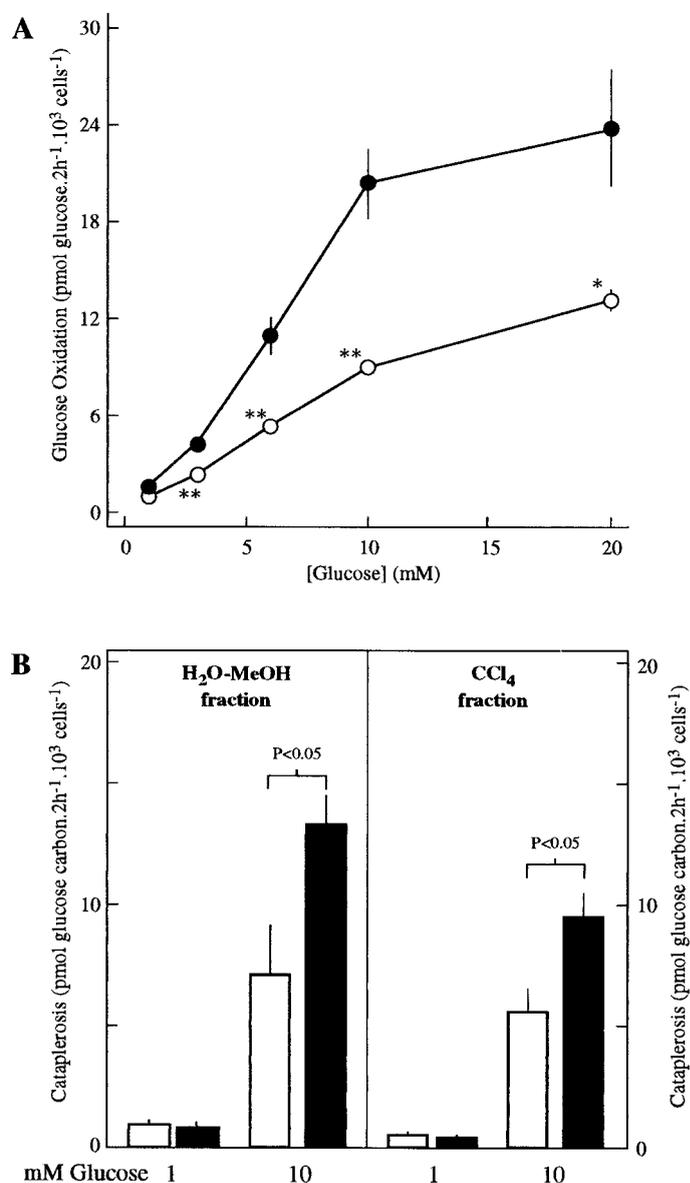


FIG. 2. Metabolic fate of glucose carbon in G3 cells and G10 cells. After a 24-h culture in either 3 mmol/l (○) or 10 mmol/l (●) glucose, β -cells were incubated with [¹⁴C]glucose at the indicated concentrations to follow the metabolic fate of the sugar (11). **A:** Rate of ¹⁴CO₂-production in G3 cells (□) and G-10 cells (■) reflecting mitochondrial glucose oxidation. **B:** Cataplerosis, measured as mol/l of ¹⁴C-labeled carbonatoms precipitated by perchloric acid and solubilized in either water (0.2 mol/l NaCl)/methanol or chloroform (12). □, G3 cells; ■, G10 cells. Data represent the means \pm SE of five experiments.

coding GLUT2 and mitochondrially encoded subunits of the respiratory chain was observed in G10 cells, while on the contrary, mRNA corresponding to the inactivating kinase of the pyruvate dehydrogenase complex was upregulated fourfold in G3 cells. Amplification of signaling caused by enhanced glucose oxidation in G10 cells may be expected from upregulated mRNA encoding both subunits of the K_{ATP} channels and the calcium sensor protein calmodulin (Fig. 3A). A molecular correlation to the measured cataplerotic flux (Fig. 2B) was also present at the level of microarray analysis (Fig. 3A), because ACL, a cataplerotic enzyme, was upregulated between three- and fourfold at the mRNA level. The resulting citrate efflux

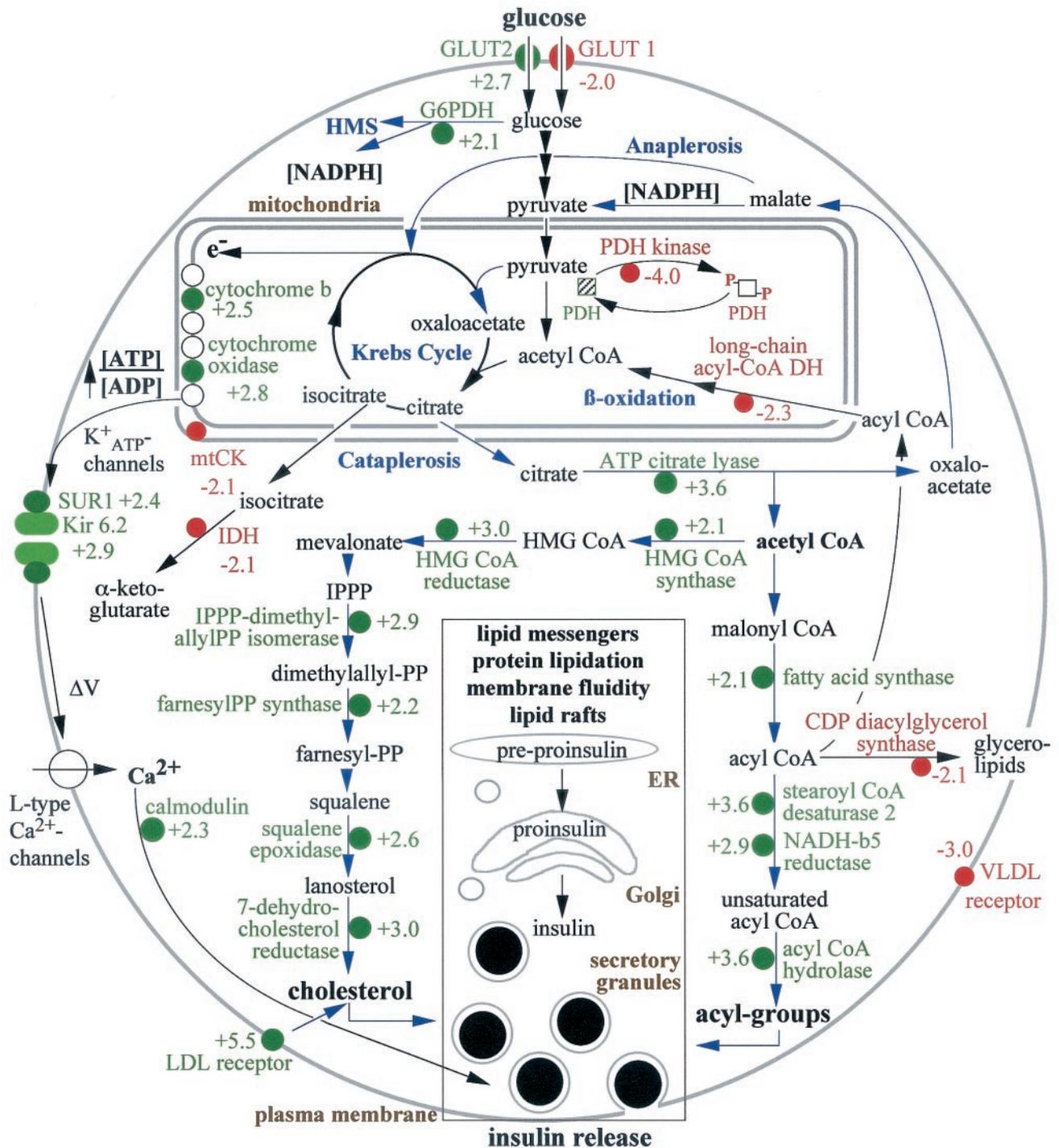


FIG. 3. Glucose regulation of genes involved in metabolic signaling in rat β -cells. **A:** GeneChip microarray data of glucose-regulated mRNAs encoding proteins involved in metabolic signaling pathway involving K_{ATP} channels, cataplerosis as well as lipid metabolism. Data are mean fold-change ($n = 3$) of mRNA abundance of G10 versus G3 cells (G10/G3 ≥ 2 [green; positive numbers] or G3/G10 ≥ 2 [red; negative numbers]). **B:** Western blot analysis of SCD expression in β -cells. Relative abundance of SCD protein was higher in G10 cells (■) than in G3 cells (□). Values are the means \pm SE of three experiments; $P < 0.05$. **C:** Semiquantitative RT-PCR analysis of mRNA isolated from G3 cells (□) or G10 cells (■). Relative intensity of ethidiumbromide-stained electrophoretically separated amplified cDNA fragments was normalized for α -tubulin cDNA abundance, amplified from the same samples. Values are the means \pm SE of n independent RNA extracts. Significance of differences between fragment abundance in G3 and G10 cells was assessed via paired Student's t tests. Relative (normalized) cDNA fragment abundance significantly higher in G10 cells: * $P < 0.01$; abundance significantly higher in G3 cells: \$ $P < 0.05$ and \$\$ $P < 0.001$. HMS, hexose monophosphate shunt; IDH, NADP⁺-dependent isocitrate dehydrogenase; mtCK, ubiquitous mitochondrial creatine kinase; PDH, pyruvate dehydrogenase; PP, pyrophosphate.

may be used in the cells for several purposes. First, the produced acetyl CoA produced by ACL could serve as precursor for lipid biosynthesis. In agreement with this idea, we identified 12 lipogenic transcripts that were

upregulated in G10 cells encoding enzymes for de novo synthesis of acyl CoAs and metabolites on the mevalonate pathway. The specific upregulated mRNAs in acyl-group synthesis encode fatty acid synthase (FAS), stearoyl CoA

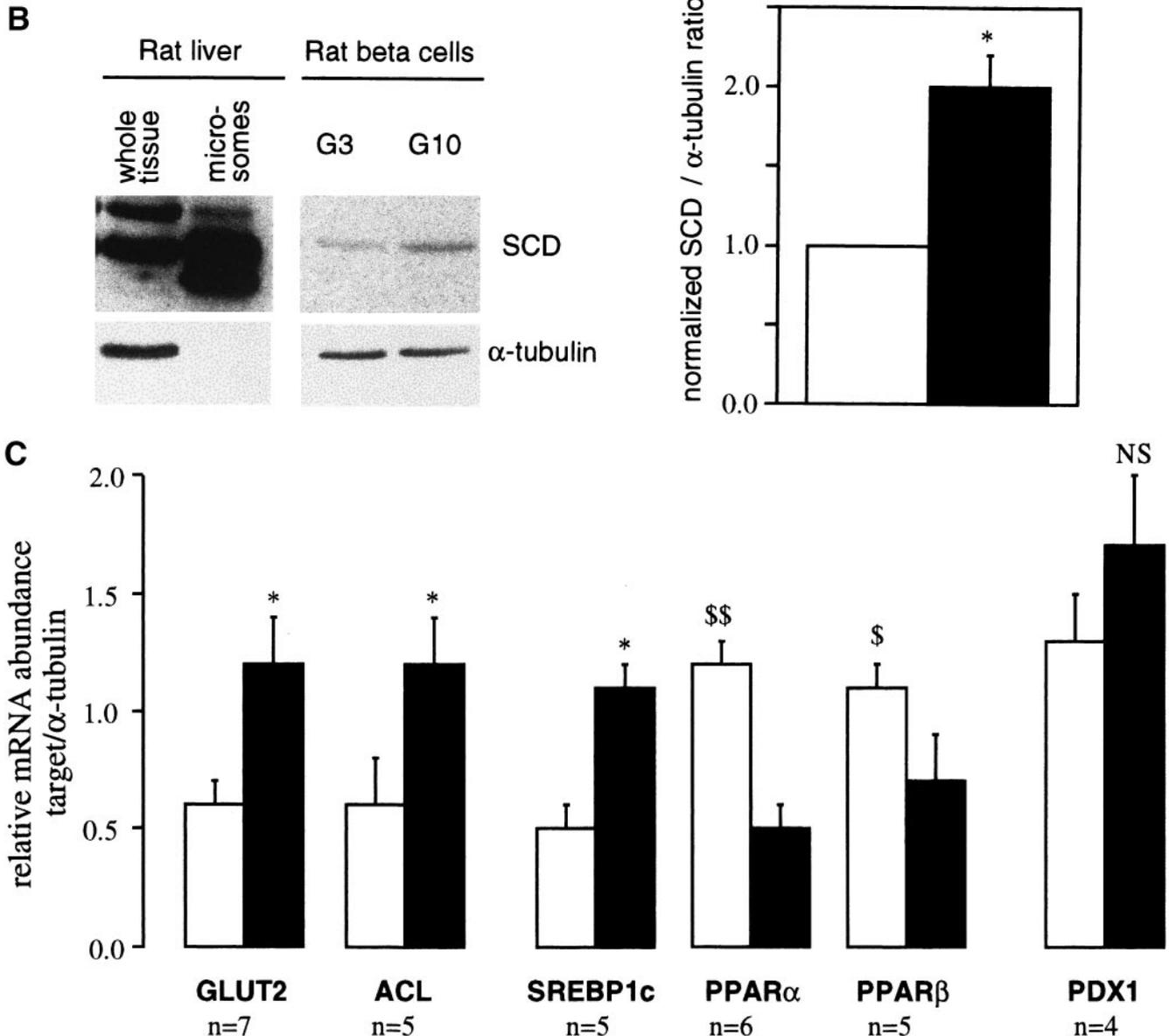


FIG. 3—Continued.

desaturase-2 (as well as a homologous protein), NADH-cytochrome b5-reductase, and acyl CoA hydrolase. In the mevalonate pathway, higher abundance in G10 cells was detected of mRNA encoding HMG-CoA synthase, HMG-CoA reductase, isopentenyl pyrophosphate (IPPP)/dimethylallylpyrophosphate isomerase, farnesylpyrophosphate synthase, squalene epoxidase, as well as 7-dehydrocholesterol reductase. Second, the enhanced citrate efflux could serve to accelerate the citrate pyruvate cycle (3), which reconverts the produced oxaloacetate into malate and pyruvate that re-enters the mitochondria (anaplerosis). The metabolic flux through this cycle was recently observed to correlate well to glucose-regulated insulin release (13). Both malic enzyme and pyruvate carboxylase are highly expressed in β -cells (11,14). Together with glucose-6-phosphate dehydrogenase, malic enzyme produces cytoplasmic NADPH that is required for reductive biosynthesis. Whereas transcript encoding malic enzyme was not upregulated in G10 cells, the glucose-6-phosphate

dehydrogenase mRNA was elevated consistently (2.1-fold higher than in G3 cells).

Glucose stimulation of stearoyl CoA desaturase-gene expression was also noted at the protein level (Fig. 3B). Because, on the one hand, the antiserum does not discriminate between the different stearoyl CoA desaturase isoforms and, on the other hand, SCD1 probes on the rat U34A chip scored below the detection threshold in G3 and G10 cells), we interpret the protein changes in Fig. 3B to reflect changes in SCD2 and/or SCD2-related mRNA. Higher abundance in G3 cells was observed for transcripts encoding mitochondrial long-chain acyl CoA dehydrogenase and CDP-diacylglycerol synthase, two enzymes that are expected to use substrate from the pool of available acyl CoAs and mitochondrial creatinine kinase, which may buffer cellular ATP. Incubation in 3 mmol/l glucose increased the abundance of mRNA encoding NADP⁺-dependent isocitrate dehydrogenase (2.1 ± 0.1 -fold increase), a change that could divert mitochondrial citrate from

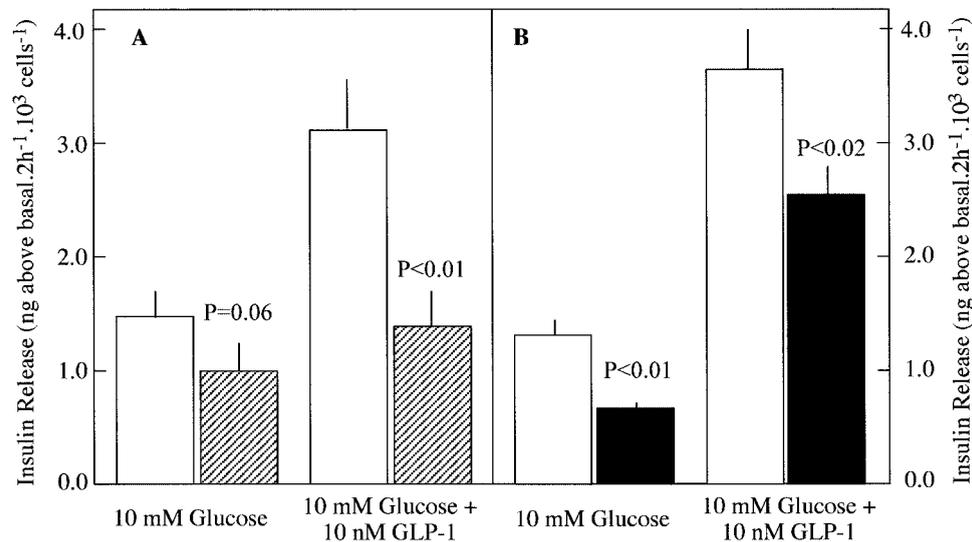


FIG. 4. The ACL inhibitors radicicol and (-)-hydroxycitrate suppress regulated insulin release. After a 16-h preculture in HAMF10 medium containing 10 mmol/l glucose, β -cells were stimulated to release insulin for 2 h in the presence of either 10 mmol/l glucose or 10 mmol/l glucose plus 10 nmol/l GLP-1. Basal release was measured at 1 mmol/l glucose. Release from control cells (\square) is compared with that of the inhibitors radicicol (25 μ mol/l; hatched bars in A) or (-)-hydroxycitrate (2 mmol/l; black bars in B), which were present during the 2-h incubation. Data are means \pm SE from four (A) or six (B) independent experiments. Significance of differences between release from control and experimental conditions was assessed via paired Student's *t* tests. NS, not significant.

cataplerosis and citrate/pyruvate cycling in G3 cells. Finally, 24-h culture in 10 mmol/l glucose switched lipoprotein receptor gene expression from VLDL receptors (3.0 ± 1.1 -fold decrease) to LDL receptors (5.5 ± 1.7 -fold increase), contributing to the cellular possibilities to enlarge the pool of cholesterol in G10 cells.

Transcriptional regulation of genes involved in lipid and glucose metabolism is coordinated by members of the SREBP and PPAR families. Consistent with this knowledge, we observed upregulation of SREBP1c mRNA in G10 cells, using both Affymetrix U34A microarrays (2.7 ± 0.6 -fold difference with G3 cells) and RT-PCR analysis (Fig. 3C). SREBP2 mRNA (probe not on U34A array) was very low in RT-PCR analysis (data not shown). In liver, transcription of the SREBP1c gene depends on a permissive action of insulin (15); furthermore, in primary cultures of rat hepatocytes, SREBP1c-gene transcription is directly enhanced by glucose (16) and dominant-negative SREBP1c suppresses glucose stimulation of lipogenic gene expression in liver (16). Because the insulin levels that had accumulated at the end of the 24-h tissue culture were 5- to 10-fold higher in the G10 culture than in the G3 culture (data not shown), it is conceivable that the observed stimulation is the combination of direct glucose signaling (14) and autocrine insulin signaling, as was reported to exist in vitro (17). It is known that PPAR- α and PPAR- β are expressed in β -cells (18) and that glucose represses PPAR- α gene transcription (19). Because PPAR- β and PPAR- γ probes were below the detection limit, and because PPAR- α probes were not on the U34A microarray, we assessed PPAR-mRNA abundance via RT-PCR analysis. Consistent upregulation of PPAR- α and PPAR- β mRNA was observed in G3 cells (Fig. 3C) while PPAR- γ mRNA expression level remained below detection limits after 35 PCR cycles (data not shown). The effect of low glucose on PPAR- α expression (2.5-fold higher level in G3 cells than in G10 cells; $P < 0.001$) may well be responsible for the observed upregulation of mRNA en-

coding both PDH kinase and mitochondrial long-chain acyl CoA dehydrogenase (Fig. 3A). The observed PPAR- α -dependent metabolic adaptation may explain (at least in part) the switch from glucose oxidation to fatty acid oxidation that is known to occur when cells are chronically exposed to low glucose (19).

The coordinated glucose-regulated change in cataplerosis of citrate, induction of the glucose 6-phosphate dehydrogenase gene, and lipogenic gene transcription may be an accidental correlate to the changes in the secretory phenotype. To test the idea that continuous cataplerotic flux is necessary for normal stimulation of the insulin secretory response, we measured the acute regulation of insulin release from rat β -cells with or without pharmacological inhibition of ACL. We tested this site of intervention, since ACL represents the cataplerotic enzyme that is common for both citrate/pyruvate cycling and acyl CoA/cholesterol production (Fig. 3A). For this purpose, we used two different, chemically unrelated, ACL inhibitors: radicicol, a macrolide antibiotic (9) and (-)-hydroxycitrate, a citrate analog (10); both compounds effectively blocked enzymatic activity in extracts from purified rat β -cells (data not shown). As illustrated in Fig. 4, radicicol lowered insulin release stimulated by 10 mmol/l glucose and GLP-1 by $44 \pm 13\%$ ($P < 0.01$). Moreover, (-)-hydroxycitrate (2 mmol/l) reduced the amount insulin release stimulated by 10 mmol/l glucose alone ($48 \pm 6\%$ inhibition; $P = 0.002$) or 10 mmol/l glucose plus GLP-1 ($28 \pm 6\%$ inhibition; $P < 0.02$). Therefore, in agreement with earlier observations in the intact perfused pancreas (10), it can be concluded from our data that the acute metabolic flux through the cataplerotic ACL reaction is critical for normal stimulation of insulin release by glucose and GLP-1. Orchestrated upregulation of genes involved in acyl CoA and cholesterol synthesis and the effects of ACL inhibition on insulin release both suggest that a rapid flux through ACL is essential for insulin release. ACL both delivers acetyl CoA for lipid biosynthesis (e.g., chole-

terol, terpenes, or acyl groups) and brings oxaloacetate into the citrate/pyruvate cycle, which generates NADPH in concert with NADPH produced by flux through the glucose 6-phosphate dehydrogenase step. Direct measurement of pyruvate cycling in insulin-producing cells via nuclear magnetic resonance (NMR) spectroscopy (13) indicates that the metabolic flux through the citrate/pyruvate cycle is closely linked to insulin release. Pancreatic islet secretory granules have been observed to contain numerous cholesterol-rich complexes (20). The participation of cholesterol in the process of granule fusion was observed in neuroendocrine PC-12 cells, where cholesterol was observed to be responsible for the concentration of the SNARE protein SNAP-25 (21). The glucose-induced upregulation of genes involved in acyl CoA synthesis and fatty acid desaturation may also be linked to granule biogenesis and/or exocytosis. Acyl CoAs have been proposed to be directly involved in the regulation of exocytosis (4,22), possibly because they act as substrates for protein lipidation. Another possibility is that glucose regulation of cholesterol, phospholipid, and sphingolipid synthesis alters the composition of membrane domains that are critical for exocytosis, for instance by influencing membrane fluidity or lipid raft density. Regulation of membrane fluidity via SREBP and/or SCD gene transcription has been proposed to be important in other eukaryotic systems (23). In this context, it is interesting to mention that $\Delta 9$ -stearoyl desaturase exists in liver as a short-lived cellular protein whose abundance is sensitive to nutritional signals (24).

In conclusion, our present analysis of part of the glucose-induced transcriptome in FACS-purified rat β -cells identifies a coordinated glucose-regulated gene cluster that may be involved in citrate/pyruvate cycling, NADPH formation, lipid signaling, granule biogenesis, and/or maintenance of lipid membrane domains into an exocytosis-competent state. It should be further investigated if these results can be extrapolated to regulatory mechanisms that operate in human β -cells. If so, they can aid in the identification of β -cell abnormalities in diabetic patients and in the identification of new targets for oral antidiabetic drugs.

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