Feasibility of Pathways for Transfer of Acyl Groups from Mitochondria to the Cytosol to Form Short Chain Acyl-CoAs in the Pancreatic Beta Cell*†‡

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The mitochondria of pancreatic beta cells are believed to convert insulin secretagogues into products that are translocated to the cytosol where they participate in insulin secretion. We studied the hypothesis that short chain acyl-CoA (SC-CoAs) might be some of these products by discerning the pathways of SC-CoA formation in beta cells. Insulin secretagogues acutely stimulated 1.5–5-fold increases in acetoacetyl-CoA, succinyl-CoA, malonyl-CoA, hydroxymethylglutaryl-CoA (HMG-CoA), and acetyl-CoA in INS-1 832/13 cells as judged from liquid chromatography-tandem mass spectrometry measurements. Studies of 12 relevant enzymes in rat and human pancreatic islets and INS-1 832/13 cells showed the feasibility of at least two redundant pathways, one involving acetoacetate and the other citrate, for the synthesis SC-CoAs from secretagogue carbon in mitochondria and the transfer of their acyl groups to the cytosol where the acyl groups are converted to SC-CoAs. Knockdown of two key cytosolic enzymes in INS-1 832/13 cells with short hairpin RNA supported the proposed scheme. Lowering ATP citrate lyase 88% did not inhibit glucose-induced insulin release indicating citrate is not the only carrier of acyl groups to the cytosol. However, lowering acetoacetyl-CoA synthetase 80% partially inhibited glucose-induced insulin release indicating formation of SC-CoAs from acetoacetate in the cytosol is important for insulin secretion. The results indicate beta cells possess enzyme pathways that can incorporate carbon from glucose into acetyl-CoA, acetoacetyl-CoA, and succinyl-CoA and carbon from leucine into these three SC-CoAs plus HMG-CoA in their mitochondria and enzymes that can form acetyl-CoA, acetoacetyl-CoA, malonyl-CoA, and HMG-CoA in their cytosol.

Mitochondria play two important roles in insulin secretion. One role is ATP production, which in addition to powering numerous cellular processes triggers insulin exocytosis via its acting on the plasmalemmal ATP-sensitive potassium channel. In addition, it is well established that beta cell mitochondria use carbon from glucose-derived pyruvate to synthesize various metabolic intermediates (anaplerosis) (1, 2). About one-half of the pyruvate derived from glucose, the most potent insulin secretagogue, is carboxylated by pyruvate carboxylase to oxaloacetate (2–7). Although most of this oxaloacetate is used for anaplerosis and many studies have suggested that anaplerosis is important for insulin secretion (8–12), the purpose of anaplerosis in the beta cell is unclear. It seems clear, however, that if the carboxylation of pyruvate increases the levels of citric acid cycle intermediates inside the mitochondria, this would alter mitochondrial function because many citric acid cycle intermediates inhibit or activate citric acid cycle enzymes (as described in biochemistry texts and briefly reviewed in Refs. 2 and 13). Therefore, this indicates that these metabolites must be transferred to the extramitochondrial space where they might have signaling or supporting roles in insulin secretion.

To explain part of the role of anaplerosis in insulin secretion, we recently proposed the “succinate mechanism” (14). This hypothesis was based on our own data and data from the literature and speculated that succinate metabolism supplies both NADPH and short chain acyl-CoA precursors of mevalonic acid that have signaling or supporting roles in insulin secretion (2, 14). Although succinate metabolism was instrumental in suggesting the original hypothesis, succinate has become less central to the hypothesis as it has evolved, in part, into a scheme involving the mitochondrial synthesis of multiple short chain acyl-CoAs from the carbon derived from all metabolized insulin secretagogues. Another hypothesis of insulin secretion that has been discussed for 2 decades involves the mitochondrial export of citrate as a carrier of acetyl groups to the cytosol where they are converted to malonyl-CoA, which has a special role in insulin secretion via its inhibition of mitochondrial fatty acid oxidation. This is believed to increase cytosolic long chain acyl-CoA molecules, which might perform signaling roles in insulin secretion (for a review see Ref. 15).

Previous reports have described the possible involvement of NADPH in insulin secretion (6, 8, 10, 16, 17). In this study we examined the possibility that multiple short chain acyl-CoAs, not just mevalonic acid or malonyl-CoA, might be some of the many products of anaplerosis in the beta cell. We first showed that acetyl-CoA, acetoacetyl-CoA, HMG-CoA, succinyl-CoA, and malonyl-CoA are increased to various extents in INS-1 cells

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‡The abbreviations used are: HMG-CoA, hydroxymethylglutaryl-CoA; KIC, α-ketoisocaproate; shRNA, short hairpin RNA; RT, reverse transcription.

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stimulated by many different insulin secretagogues as judged from liquid chromatography-tandem mass spectrometry measurements. We then considered the pathways by which multiple short chain acyl-CoAs might be synthesized from insulin secretagogues in mitochondria and their acyl groups transferred to the cytosol where they could be re-activated with coenzyme A and have roles in insulin secretion. Acyl-CoA molecules themselves cannot be transported across the inner mitochondrial membrane and, therefore, after they are synthesized in mitochondria, the coenzyme A moiety is removed, and their acyl groups are transported as carboxylate ions out of the mitochondria to the extramitochondrial space. Outside the mitochondria they are reconverted to their CoA derivatives. The pathways of the original “succinate mechanism” scheme (14) were proposed on the assumption of pathways known to be present in non-beta cells without verification of the presence of the enzymes in the beta cell that might synthesize and metabolize short chain acyl-CoAs or, if present, their intracellular location (mitochondrial versus extramitochondrial). Thus, the scheme did not fully address the pathways by which short chain acyl-CoAs could be synthesized in mitochondria and re-formed in the cytosol. The malonyl-CoA hypothesis seemed to consider citrate as the only carrier of short chain acyl groups from mitochondria to the cytosol (15).

To discern the feasibility of the various possible relevant pathways, we determined the presence or absence, as well as the intracellular locations, of short chain acyl-CoA-synthesizing enzymes in rat and human pancreatic islets and in INS-1 832/13 cells with measurements of their enzyme activities, immunoblot analysis of their protein levels, and/or RT-PCR analysis of their cognate mRNAs. The results showed that beta cells possess enzymes that can form all of the short chain acyl-CoAs studied except malonyl-CoA in their mitochondria and all of the CoAs except succinyl-CoA in their extramitochondrial space. Proof that citrate is not the only carrier of acyl groups from mitochondria to the cytosol was obtained by lowering ATP citrate lyase activity with shRNA and showing that this did not inhibit glucose-induced insulin release. Evidence that acetocetate is a carrier of acyl groups from mitochondria to the cytosol in the beta cell was obtained by lowering acetocetate-CoA synthetase activity with shRNA and observing that this partially inhibited insulin release. Thus, we believe the data suggest that multiple short chain acyl-CoAs formed by anaplerosis could play roles in signaling or supporting insulin secretion and that there are at least two pathways, one involving acetocetate and the other citrate, for translocation of acyl groups from mitochondria to the cytosol in beta cells.

**EXPERIMENTAL PROCEDURES**

**Materials—**[3-14C]Hydroxybutyrate was from American Radiolabeled Chemicals, Inc. Succinic acid monomethyl ester and all other chemicals were from Sigma. Auxillary enzymes used in analysis of enzyme activity were from Roche Diagnostics. An antibody against rat acetocetate-CoA synthetase was a generous gift of Dr. Tetsuya Fukui (18). Rabbit antibodies against the β subunits of rat succinyl-CoA synthetase were from David O. Lambeth (19). A rabbit polyclonal antiserum against the rat peroxisomal acetyl-CoA acetyltransferase (Acaal1a)3 was from Paul Van Veldhoven (20). An antibody to human HMG-CoA lyase was from Grant Mitchell (21). A rabbit polyclonal antibody to human HMG-CoA reductase was from Upstate Cell Signaling Solutions (Lake Placid, NY). A rabbit polyclonal antibody to human cytosolic acetyl-CoA acetyltransferase (ACAT2) was from Abcam. Human pancreatic islets were from the Islet Isolation Core at Washington University School of Medicine, St. Louis. INS-1 832/13 cells were from Chris Newgard (22).

**Short Chain Acyl-CoAs—**INS-1 832/13 cells were maintained as monolayers on 150-mm diameter plates in INS-1 medium (RPMI 1640 tissue culture medium (the glucose concentration in this medium is 11.1 mM) supplemented with 10% fetal bovine serum, 1 mM pyruvate, 50 μM β-mercaptoethanol, and 10 mM Hepes buffer (23)) and penicillin (100 units/ml) and streptomycin (100 μg/ml). Twenty four hours before an experiment was to be performed, this medium was replaced with fresh medium modified to contain 5 mM glucose. On the day of the experiment plates were washed twice with 10 ml of phosphate-buffered saline and once with 10 ml of Krebs-Ringer bicarbonate solution modified to contain 15 mM sodium bicarbonate and 15 mM sodium Hepes buffer, pH 7.3. Secretagogues were added to the plates in 10 ml of the modified Krebs-Ringer solution, and after 30 min at 37 °C, this solution was quickly withdrawn and replaced with 2 ml of 1% trifluoroacetic acid in 50% methanol. The extracts were then prepared for analysis and analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry exactly as described previously (24).

**Subcellular Fractionation—**Subcellular fractionation of pancreatic islets was as described previously (6). Briefly, islets were homogenized in 220 mM mannitol, 70 mM sucrose, 5 mM potassium Hepes buffer, pH 7.3 (MSH). The homogenate was centrifuged at 600 × g for 10 min to precipitate the nuclei and cell debris fraction, and the resulting supernatant fraction was centrifuged at 5,500 × g for 20 min to obtain the mitochondrial fraction. The resulting supernatant fraction or a 20,000 × g for 20 min supernatant fraction was the cytosol. In some instances, the 5,500 × g supernatant fractions were centrifuged at 20,000 or 50,000 × g for 20 min to obtain a pellet enriched in microsomes and peroxisomes. Liver, kidney, and heart subcellular fractions were prepared as described previously (25). INS-1 cells were fractionated with reagents from the mitochondrial isolation kit (catalog number 8974, Pierce). About 100 μL of loosely packed INS-1 832/13 cells were homogenized in 800 μL of mitochondria isolation reagent A containing a protease inhibitor EDTA-free mixture (Pierce). After vortexing and a 2-min incubation on ice, 10 μL of reagent B was added. The mixture sat on ice for 5 min and was then homogenized with 30 strokes up and down in a Potter-Elvehjem homogenizer. Reagent C (800 μL) containing Halt protease inhibitor was added, and the mixture was centrifuged at 700 × g for 10 min. The resulting supernatant fraction was centrifuged at 12,000 × g for

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3 When the same isoforms of both a human and a rat enzyme are discussed together, the abbreviation for the human enzyme (upper case) is used. When either human or rat enzymes are individually mentioned, the convention of using the upper case for the human enzyme and the lower case for rat enzyme is used.
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10 min to give the mitochondrial pellet that was resuspended in MSH solution and freeze-thawed three times before use. The resulting supernatant fraction (cytosol) and mitochondrial fraction were used for enzyme assays and immunoblot analysis. In some instances, the resulting supernatant fraction was centrifuged at higher speeds to obtain a pellet enriched in microsomes and peroxisomes as described for islets.

Enzyme Assays—Succinyl-CoA:3-ketoacid CoA transferase was measured in the presence of 50 mM sodium acetate, 0.2 mM succinyl-CoA, 5 mM MgCl₂, 5 mM iodoacetamide in 50 mM Tris chloride buffer, pH 8.0 (26), at 30 °C. The rate of acetoacetyl-CoA formation was followed by measuring the increase in absorbance at 310 nm. Acetyl-CoA acetyltransferase was measured in the presence of 25 μM acetyl-CoA, 60 μM coenzyme A, 5 mM MgCl₂ in 50 mM Tris chloride buffer, pH 7.6, at 30 °C. The disappearance of acetoacetyl-CoA was followed spectrophotometrically by monitoring the decrease in absorbance at 303 nm (27). Acetoacetyl-CoA synthetase was measured with slight modifications of a radiochemical assay (28). The complete assay mixture contained final concentrations of 0.5 mM β-[3-¹⁴C]hydroxybutyrate (specific radioactivity, 3 mCi/mmol), 1 mM coenzyme A, 1.5 mM ATP, 3 mM NAD, 10 mM MgCl₂, 100 mM KCl, and 0.05 units/ml of β-hydroxybutyrate dehydrogenase from Rhodobacter sphaeroides in Tris chloride buffer, pH 7.5. The mixture was maintained at 37 °C for 10 min to generate [3-¹⁴C]acetocetate before pancreatic islet cytosol or INS-1 cytosol was added to bring the final volume to 50 μl. After 20 more min the enzyme reaction was stopped by the addition of 12 μl of acetic acid to the reaction mixture, and part of or the entire volume was spotted on Whatman SG81 chromatography paper. Chromatography was run in a mixture of ethyl ether/formic acid (7:1) for 60—90 min. Acetoacetyl-CoA remains at the origin and acetocetate and 3-hydroxybutyrate migrate with the solvent front. Liquid scintillation spectrometry was used to measure radioactivity in squares of paper cut from the origin to estimate synthetase activity. Radioactivity in blanks containing cytosol and reaction mixtures minus ATP was subtracted from the radioactivity in samples containing the complete reaction mixture plus cytosol to calculate activity attributable to the synthetase. The activity with ATP present in the enzyme assay mixture was 17—26-fold higher than with no ATP present or when a blank with no cellular extract was present in the reaction mixture when INS-1 cytosol was used and 5-fold higher when rat islet cytosol was used. ATP citrate lyase activity was measured in a reaction mixture containing 5 mM citrate, 0.3 mM coenzyme A, 3 mM ATP, 0.15 mM NADH, 10 mM MgCl₂, 10 mM dithiothreitol, and 6 units/ml of malate dehydrogenase from pig heart mitochondria in Tris chloride buffer, pH 8.5, at 37 °C (29). The disappearance of NADH, which corresponded to oxaloacetate formation, was monitored spectrophotometrically at 340 nm. Glutamate dehydrogenase activity was measured in the presence of 10 mM α-ketoglutarate, 50 mM NH₄Cl, 100 μM NADH, 2 mM ADP, 100 μM EDTA, 1 mM KCN, 0.1% Triton X-100, and 50 mM imidazole buffer, pH 7.0 at 30 °C (30). NADH disappearance was monitored spectrophotometrically at 340 nm. In each spectrophotometric assay, the background rate measured in the absence of one substrate was subtracted from the rate in the presence of the complete reaction mixture to give the rate attributable to the enzyme. Usually for positive controls, enzyme activities were measured in non-islet tissues known to contain the enzyme of interest (data not shown).

RT-PCR—Total RNA was isolated from cultured INS-1 (832/13) cells and from freshly isolated rat islets and liver using TRIzol reagent (Invitrogen) and further purified on an RNeasy Mini column (Qiagen). cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Invitrogen) and oligo(dT) primers (Promega, Madison, WI). PCR was performed with the primers shown in supplemental Table 1. cDNAs from tissues known to contain specific transcripts were run as positive controls (data not shown).

Immunoblotting—For immunoblotting of HMG-CoA synthase, rat islets and INS-1 cells, or human islets, were washed twice in phosphate-buffered saline and lysed with M-PER mammalian protein extraction reagent (Pierce) containing protease inhibitors. Rat liver and heart tissue were homogenized, and protein was extracted using T-PER tissue protein extraction reagent (Pierce) containing protease inhibitors. Whole rat islets were either homogenized in MSH and boiled in sample buffer or boiled directly in sample buffer (1% SDS, 5% glycerol, 65 mM Tris chloride, pH 6.8, 0.01% bromphenol blue, and either 100 mM dithiothreitol or 2.5% β-mercaptoethanol). Proteins were separated by electrophoresis on 7.5% SDS-polyacrylamide gels and electrotransferred to nitrocellulose membranes. The membranes were blocked with a mixture of 5% nonfat milk and 1% bovine serum albumin and 0.05% Tween 20 in Tris-buffered saline (TBST) and incubated overnight at 4 °C with primary antiserum diluted 1:500 to 1:2000 in blocking buffer. Primary antisera were raised in rabbits immunized with internal peptides of the rat cytosolic HMG-CoA synthase (HIPSPA-KKVPRLPAT) and the rat mitochondrial HMG-CoA synthase (CSTIPPAPLAKTDT) conjugated to keyhole limpet hemocyanin (the amino-terminal cysteine was added to the native sequence to facilitate conjugation). The membranes were washed and treated with the secondary antibody labeled with goat anti-rabbit horseradish peroxidase diluted 1:15,000 in blocking buffer, and proteins were visualized with the Supersignal West Pico chemiluminescent substrate kit (Pierce). Luminescence was captured on a Chemi Doc XRS Gel documentation system (Bio-Rad) or on KSR-B Luc Ultra Autorad x-ray film (ISC Bioexpress). To demonstrate equal loading of proteins per lane, the membranes were then washed in TBST and incubated in Restore Western blot stripping buffer (Pierce) for 30 min at 42 °C and re-probed with anti-β-actin antibody (Sigma). Immunoblotting to detect other proteins was done similarly using dilutions of antibody recommended by the supplier. For each enzyme as appropriate, proteins were also measured in subcellular fractions from kidney, liver, or heart as positive or negative controls (data not always shown).

Silencing of ATP Citrate Lyase and Acetoacetyl-CoA Synthetase Genes—Five different 65-bp inserts containing 19-bp sequences targeted against the ATP citrate lyase gene were cloned into the BamHI and HindIII sites of the pRNA-U6.1/Hygro plasmid downstream of the U6 promoter (Genescript) and transfected into INS-1 832/13 cells with Lipofectamine 2000 reagent (Invitrogen). Hygromycin-resistant cells were
Selected and maintained in INS-1 tissue culture medium containing 150 μg/ml of hygromycin. The target sequence that provided the largest decrease in enzyme activity and enzyme protein was CAACAGACCTATGACTACG (nucleotides 1012–1030 of GenBank™ accession number NM_016987). Three 64–69-bp inserts containing 19–22 bp sequences targeted against the acetoacetyl synthetase gene were cloned into the BamHI and HindIII sites of plasmid pSilencer 2.1-U6 Hygro (Ambion) downstream of the U6 promoter and transfected into the INS-1 cells and selected with hygromycin. The target sequence that provided the largest decrease in enzyme activity and insulin release was ACAGCATGTTCCTGGATGA (nucleotides 872–891 of GenBank™ accession number NM_023104). Sequences that gave less knockdown of enzyme activity and insulin release were GGAAATCAGTAGACTTATA and GGAAG-GCTTACCTTCCAATA. As an additional control, cells were also transfected with a vector containing a nontargeting shRNA sequence.

Data Analysis—Statistical significance was confirmed with Student’s t test.

RESULTS

Short Chain Acyl-CoA Measurements—The first phase of insulin release normally begins within 3 min of an increase in glucose or other insulin secretagogues that come in contact with the beta cell. The second phase of insulin release, in which the largest amount of insulin is released, is caused by metabolism of insulin secretagogues (31) and lasts for several hours or until the stimulus stops. We stimulated INS-1 832/13 cells with various insulin secretagogues, as well as nonsecretagogues as negative controls, and selected 30 min as a time point to measure short chain acyl-CoAs in beta cells when the cells would likely be showing changes in metabolite levels related to a stimulated state. Relative insulin release caused by the various secretagogues and nonsecretagogue controls is listed in the legend of Fig. 2. In INS-1 832/13 cells used for these studies numerous concomitant measurements of insulin release showed that glucose, pyruvate, and leucine plus glutamine were the most potent stimulants of insulin release from these cells, closely followed by 2 mM α-ketoisocaproate plus 10 mM monomethyl succinate (24). Leucine provides a moderate stimulus of insulin release in these cells. Incubation of INS-1 832/13 cells for 30 min under conditions that stimulate insulin release caused large relative increases in several short chain acyl-CoAs. The unstimulated concentration of acetoacetyl-CoA was the lowest of any of the CoAs measured, and the relative increases in acetoacetyl-CoA were the largest (2–5-fold). Succinyl-CoA, HMG-CoA, and malonyl-CoA were present at intermediate levels. Succinyl-CoA and malonyl-CoA were increased 2–3-fold. HMG-CoA was increased 30–60% and acetyl-CoA, which was present at the highest level of the CoAs studied, was increased 55–80%. Pyruvate, which is as potent an insulin secretagogue as glucose in INS-1 832/13 cells (24, 32, 33), also caused large increases in the acyl-CoA levels (Fig. 2). In fresh rat pancreatic islets α-ketoisocaprate at a 10 mM concentration is as potent an insulin stimulant as glucose, but α-ketoisocaprate alone does not stimulate insulin in INS-1 832/13 cells (24). α-Ketoisocaprate alone does not stimulate insulin release about equal to that produced by glucose (24). Leucine plus glutamine caused large increases in the CoAs. As shown previously (24), a high concentration of glucose caused the levels of various CoA molecules to increase, but somewhat less so than the other conditions (Fig. 2).

Pathways That Form Acyl-CoAs in Beta Cells—Because all of the known anaplerotic pathways that can form short chain acyl-CoAs or their precursors from glucose or other physiologic insulin secretagogues originate in mitochondria, it was assumed that increases in acyl-CoA levels can only arise from their initial synthesis in mitochondria. The inner mitochondrial membrane is not permeable to acyl-CoAs themselves. Therefore, in order for acyl-CoAs to increase in the cytosol, their acyl groups must be transported as carboxylates from the mitochondria to the cytosol where the coenzyme A is added back to the carboxylates. To discern the pathways by which this might occur, we looked at the intracellular location of relevant enzymes that can interconvert carboxylic acids with acyl-CoA molecules. The general approach was to use measurements of enzyme activity and/or immunoblotting to show the presence of enzymes and RT-PCR to prove the absence or confirm the presence of enzymes.

HMG-CoA Synthase and HMG-CoA Lyase—A mitochondrial and a cytosolic isoform of HMG-CoA synthase are known. Both RT-PCR (Fig. 3A) and immunoblot analysis (Fig. 3B) showed that the cytosolic isoform of the enzyme (Fig. 1, reaction 10) is the only isoform present in rat pancreatic islets and INS-1 832/13 cells. Thus, glucose carbon can be incorporated

4 M. J. MacDonald, unpublished data.

5 M. J. MacDonald, unpublished data.
into HMG-CoA only in the cytosol as shown in Fig. 1. However, mitochondrial HMG-CoA formed from leucine metabolism can be converted to acetyl-CoA and acetoacetate by HMG-CoA lyase (Fig. 1, reaction 4), which was detected in mitochondria of both rat and human pancreatic islets as well as INS-1 cells, as judged from immunoblot analysis (Fig. 4). HMG-CoA lyase has also been reported to be present in peroxisomes (21). Subcellular fractionation of islets and INS-1 832/13 cells showed this enzyme to be present predominantly, if not exclusively, in the mitochondrial fraction (data not shown).

**Succinyl-CoA Synthetase and Succinyl-CoA:3-Ketoacid CoA Transferase**—There are two isoforms of succinyl-CoA synthetase, which is also called succinate thiokinase, a GTP-specific form and an ATP specific form. Succinyl-CoA synthetases catalyze the reversible interconversion of succinate and succinyl-CoA (Fig. 1, reaction 6). These isoforms possess identical catalytic $\alpha$ subunits, and their regulatory $\beta$ subunits confer the nucleotide specificity. Both isoforms are present in the mitochondria in varying ratios in all body tissues studied (19). With immunoblot analysis with antibodies specific for each $\beta$ subunit, we found that rat pancreatic islets and INS-1 832/13 cells each possess both isoforms of succinyl-CoA synthetase in their mitochondrial fraction. Because the presence of either isoform in the cytosol would indicate that succinate formed in mitochondria and exported to the cytosol could be converted to succinyl-CoA in the cytosol, we attempted to show the presence of either isoform of the enzyme in the extramitochondrial space that can convert succinate into succinyl-CoA. By this approach, we failed to show the presence of either isoform of succinyl-CoA synthetase in the cytosol of INS-1 832/13 cells and rat pancreatic islets. Numerous immunoblot analyses such as the one shown in Fig. 5, as well as measurements of enzyme activity (data not shown), failed to demonstrate the presence of either isoform of the enzyme in the cytosol fraction of INS-1 832/13 cells and rat pancreatic islets. Of the various enzymes in rat pancreatic islets, INS-1 832/13 cells, and when possible human islets. This figure shows pathways by which short chain acyl-CoAs can be synthesized from insulin secretagogue carbon in mitochondria and their acyl groups transported across the inner mitochondrial membrane as either acetoacetate or citrate, which are re-converted to short chain acyl-CoAs in the extramitochondrial space. In the mitochondria, both acetyl-CoA acetyltransferase 1 (ACAT1) (previously called acetoacetyl-CoA thiolase) and acetyl-CoA acetyltransferase 2 (ACAT2) catalyze the same reaction (reaction 2). In the cytosol, acetyl-CoA acetyltransferase 2 (ACAT2) and acetyl-CoA acetyltransferase 2 (ACAT2) catalyze the same reaction (reaction 2).
Acetyl-CoA Acetyltransferases and Acetyl-CoA Acyltransferases—Acetyl-CoA acetyltransferase, previously called acetoacetyl-CoA thiolase, catalyzes the reversible conversion of one molecule of acetoacetyl-CoA to two molecules of acetyl-CoA. An enzyme assay showed that acetyltransferase enzyme activity was present in both mitochondria and cytosol of rat pancreatic islets and INS-1 cells (Table 1). As judged from RT-PCR analyses (Fig. 6) and immunoblotting (Fig. 4, 2nd panel from bottom), cytosolic acetyl-CoA acetyltransferase (ACAT2) (Fig. 1, reaction 9) is present in rat and human pancreatic islets and INS-1 832/13 cells. Although transcripts for the mitochondrial acetyl-CoA acyltransferase (Acaa2) (also called 3-ketoacyl-CoA thiolase) transcripts were present in INS-1 832/13 cells (Fig. 6). This enzyme can catalyze the same reaction as ACAT1 and can explain the presence of acetyl-CoA acetyltransferase activity in INS-1 cell mitochondria. As judged from immunoblot analysis, peroxisomal acetyl-CoA acyltransferase (ACAA1) (Fig. 1, reaction 9) is present in rat and human pancreatic islets and INS-1 832/13 cells. 

Acetoacetyl-CoA Synthetase—Acetoacetyl-CoA synthetase is found exclusively in the cytosol of cells. It catalyzes the unidirectional conversion of acetoacetate and coenzyme A to acetoacetyl-CoA. The breakdown of ATP to AMP plus pyrophosphate drives the reaction (Fig. 1, reaction 8). As judged from

Acetyl-CoA Concentration (pmol acyl-CoA/mg total cell protein)

Acetyl-CoA

Acetoacetyl-CoA

Succinyl-CoA

Hydroxymethylglutarly-CoA

Malonyl-CoA

Incubation Condition

FIGURE 2. Increases in various short chain acyl-CoAs in secretagogue-stimulated INS-1 cells. INS-1 832/13 cells were incubated in the presence of various insulin secretagogues or nonsecretagogues for 30 min, and short chain acyl-CoAs were measured by liquid chromatography-tandem mass spectrometry. Results are the mean ± S.E. with the number of replicate incubations per condition shown in the insets in the bars. Typical insulin release from INS-1 832/13 cells in our hands expressed as fold times the 1.5 mM glucose control is as follows: 16.7 ± 2.6% glucose (12–30), 5.5 ± 2.4% pyruvate (12–30), 2.0 ± 0.5% KIC + 10 mM monomethylsuccinate (MMS) (8–16), 2.0 ± 0.5% KIC (10–20), 10 ± 2.4% KIC (10–30), 10 ± 2.4% glutamine (10–30), p < 0.05; p < 0.01; p < 0.0001 versus 1.5 mM glucose control.

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FIGURE 3. HMG-CoA synthase is present in cytosol but not in mitochondria of rat pancreatic islets and INS-1 cells. A depicts an RT-PCR experiment that shows that transcripts from the gene, which encodes the cytosolic isoform of HMG-CoA synthase but not transcripts from the gene which encodes the mitochondrial isoform of the enzyme, are present in islets and INS-1 832/13 cells. Glutamate dehydrogenase gene transcripts (Glutamate DH) were estimated to show uniform loading of cDNA. B depicts immunoblot experiments that show that cytosolic HMG-CoA synthase protein, but no protein of the mitochondrial isoform of the enzyme, is present in rat islets and INS-1 832/13 cells. β-Actin was probed to show uniform loading of protein across lanes. There was 40 µg of protein/lane. In the RT-PCR and immunoblot experiments, liver tissue was used as a positive control.

Liver

Liver, starved

Islet

INS-1

Liver

Liver, starved

Islet

INS-1

β-Actin

Cytosolic HMG-CoA Synthase

Mitochondrial HMG-CoA Synthase

Glutamate DH

reaction 2

reaction 9

reaction 8

reaction 9
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**FIGURE 4.** RT-PCR or immunoblot analysis shows that various enzymes are present in rat and/or human pancreatic islets and INS-1 832/13 cells. The presence of succinyl-CoA:3-ketoacid CoA transferase is shown by RT-PCR analysis in the top panel. The presence of the other enzymes is shown with immunoblot analysis in the other panels. Succinyl-CoA:3-ketoacid CoA transferase gene transcripts: the concentration of glucose in which INS-1 cells were maintained did not influence the amount of PCR product obtained. HMG-CoA lyase: the membrane (20 μg of protein/lane) was probed with anti-human HMG-CoA lyase antibody that reacts well with the protein from the rat. The enzyme is a mitochondrial enzyme, and because a homogenate (Homog) of whole human islets was used, whereas mitochondria (Mito) from other tissues were used, the enzyme was less concentrated in the human islet sample, and the band in this sample appears fainter. Acetoacetyl-CoA synthetase: whole rat islets (15 μg of protein/lane), rat islet cytosol (20 μg of protein/lane), INS-1 832/13 cytosol (25 μg of protein/lane), human islet whole cell homogenate (50 μg of protein/lane), and rat liver cytosol (25 μg of protein/lane) as a control were probed with an antibody to human HMG-CoA reductase that also reacts against the rat protein. As controls, protein from livers of fed rats or rats starved 24 h, as well as from rat kidney cytosol, are also shown.

**FIGURE 5.** Both the GTP and the ATP isoform of succinyl-CoA synthetase are present in mitochondria but not cytosol of rat pancreatic islets and INS-1 cells. The upper panel shows immunoblots in which antibodies against the ATP β subunit (A-β) and GTP β subunit (G-β) of succinyl-CoA synthetase react with the proteins (20 μg protein/lane) in mitochondria (Mito) and whole cell homogenates (Homog) but not cytosol (Cyto) from rat pancreatic islets and INS-1 832/13 cells. Protein from kidney mitochondria, which are known to contain both β isoforms of the protein, was probed as a positive control. The lower panels show RT-PCR analysis in which transcripts for both the isoforms were detected in INS-1 832/13 cells and rat islets as well as rat liver as a positive control.

Measurements of enzyme activity (Table 1), immunoblot (Fig. 4, 3rd panel down), and RT-PCR analyses (data not shown), this enzyme is present in rat and human islets and INS-1 cells, and its level in these cells is as high or higher than in liver.

**ATP Citrate Lyase and Acetoacetyl-CoA Synthetase Knockdown**—ATP citrate lyase enzyme activity was, of course, found in islets and INS-1 cells (Table 1). Another indication that there is more than one pathway for the export of short chain acyl groups from mitochondria to the cytosol and their conversion to short chain acyl-CoAs in the cytosol comes from studies in which the expression of ATP citrate lyase (Fig. 1, reaction 12) was almost eliminated with shRNA in INS-1 832/13 cells without inhibiting insulin release. ATP citrate lyase mRNA was decreased 87% versus the parent INS-1 832/13 cells and 80% versus INS-1 832/13 cells transfected with a vector containing no target sequence, as judged by quantitative PCR. Enzyme activity was knocked down 88 ± 1% (mean ± S.E., n = 7) in our stable cell line ACL 940-12 (passages 12–28) versus the control cells transfected with either no vector or a vector containing a nontargeting insert without inhibiting glucose- or pyruvate-stimulated insulin release in four separate insulin release studies spread out over 1 year (data not shown). The activities of control enzymes, aspartate aminotransferase, malic enzyme, isocitrate dehydrogenase, and acetoacetyl-CoA synthetase were not lowered in the ATP citrate lyase-deficient clone. These results are consistent with the idea that molecule(s) other than citrate can exit mitochondria and be converted to acyl-CoAs in the cytosol, most likely (20 μg of protein/lane) of a human islet whole cell homogenate (50 μg of protein/lane) and rat liver cytosol (25 μg of protein/lane) as a positive control were probed with anti-rat acetyl-CoA acyltransferase antibody. The antibody made against the rat peptide sequence KSQLGTYETAN does not react well against the human protein; therefore, the level of the protein in human islets may be higher than the relative density of the band in the blot indicates.
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TABLE 1
Intracellular location of enzyme activities that influence short chain acyl-CoA metabolism in rat pancreatic islets and in INS-1 832/13 cells

Enzyme activities were measured in mitochondria and cytosol (the extramitochondrial fraction) of both INS-1 832/13 cells and rat pancreatic islets. The percentage of total enzyme activity in a subcellular fraction was determined by multiplying the activity in the fraction assayed by the ratio of total volume of the subcellular fraction to the volume of the fraction assayed. In the case of enzyme activities known to be present in both mitochondria and cytosol in non-islet tissues, glutamate dehydrogenase activity was measured to give an estimate of mitochondrial matrix enzymes from mitochondrial damage contributing to cytosolic enzyme activities. The percentage of an enzyme activity in the cytosol attributable to mitochondria was then subtracted from the total enzyme activity to give the activity attributable to the cytosolic enzyme. Results are the means ± S.E. of duplicate measurements from three or more preparations of islets or INS-1 832/13 cells.

| Enzyme activity                        | Specific enzyme activity | Percentage of total enzyme activity in cytosol |
|----------------------------------------|--------------------------|-----------------------------------------------|
| Succinyl-CoA:3-ketocaid CoA transferase |                           |                                               |
| INS-1 mitochondria                     | 12.1 ± 1.9               | <5a                                           |
| INS-1 cytosol                          | 0.9 ± 0.8                |                                               |
| Islet mitochondria                     | 14.3 ± 1.7               |                                               |
| Islet cytosol                          | 2.3 ± 1.0                |                                               |

| Acetyl-CoA acetyltransferase<sup>a</sup> |                           |                                               |
| INS-1 mitochondria                     | 47 ± 2.9                 |                                               |
| INS-1 cytosol                          | 13 ± 2.3                 |                                               |
| Islet mitochondria                     | 48 ± 5.1                 | 40                                            |
| Islet cytosol                          | 32 ± 4.6                 | 40                                            |

| Acetoacetyl-CoA synthetase             |                           |                                               |
| INS-1 cytosol                          | 2.5 ± 0.2                 | 100                                           |
| Islet cytosol                          | 1.1 ± 0.2                 | 100                                           |

| ATP citrate lyase                      |                           |                                               |
| INS-1 cytosol                          | 113 ± 8                   | 100                                           |
| Islet cytosol                          | 99 ± 6                    | 100                                           |

<sup>a</sup> Total activity in cytosol can be explained by mitochondrial breakage.
<sup>b</sup>This may include acetyl-CoA acetyltransferase activity.

from exported acetooacetate (Fig. 1, reactions 8–11 and 13). In support of this idea, acetooacetyl-CoA synthetase enzyme activity was lowered 29–80% with shRNA constructs in three different INS-1 832/13 cell lines versus the parent INS-1 832/13 cell line or a control cell line transfected with a nontargeting shRNA sequence. This caused proportional 30–57% decreases in glucose-stimulated insulin release in the enzyme-deficient cell lines versus the control cell lines (Fig. 7). Enzyme activities of the enzymes ATP citrate lyase, pyruvate carboxylase, aspartate aminotransferase, malic enzyme, and isocitrate dehydrogenase, measured as controls, were not lowered in the cells with deficient acetooacetyl-CoA synthetase.

**DISCUSSION**

**Formation of Short Chain Acyl-CoAs in Mitochondria and Translocation of Acyl Carbon to the Cytosol**—There is not a single product or class of products of anaplerosis that mediates insulin secretion. Measurements of short chain acyl-CoAs in secretagogue-stimulated INS-1 832/13 cells indicated that short chain acyl-CoAs may be some of the anaplerotic products formed from anaplerosis in the beta cell. The measurements showed relative increases in acetooacetyl-CoA ≥ succinyl-CoA > malonyl CoA > acetyl-CoA ~ HMG-CoA (Fig. 2). Fig. 1 shows the intracellular locations of enzymes that can form these short chain acyl-CoAs from various insulin secretagogues in rat and human pancreatic islets and INS-1 cells and was developed from the measurements of activities and immuno blot analyses of relevant enzymes as well as RT-PCR analyses of their cognate mRNAs. These enzyme studies by themselves show it is feasible for carbon from insulin secretagogues to be incorporated into acyl-CoAs in mitochondria and their acyl groups translocated to the cytosol not only as citrate but also as acetooacetate. In the cytosol coenzyme A can be added to the acetate component of citrate or to acetooacetate directly (Fig. 1, reactions 12 or 8), leading to the formation of various short chain acyl-CoAs. The successful shRNA knockdown of ATP citrate lyase (Fig. 1, reaction 12) enzyme activity without a decrease in insulin release indicates that citrate is not the only carrier of acyl groups from mitochondria to the cytosol. The idea that acetooacetate can also transfer acyl groups in insulin secretion was previously suggested by our finding increased acetooacetate levels in pancreatic islets and INS-1 832/13 cells stimulated with insulin secretagogues (24) as well as our finding increased acetooacetate-CoA in secretagogue-stimulated INS-1 832/13 cells in this study (Fig. 2). To test whether acetooacetate could transfer acyl group precursors of acyl-CoAs from the mitochondria to the cytosol, we used shRNA to knock down acetooacetate-CoA synthetase, an enzyme that converts acetooacetate to acetooacetate-CoA in the extramitochondrial space (Fig. 1, reaction 8). The activity of this enzyme was decreased to various extents in three lines of INS-1 cells, and glucose-induced insulin in these lines was partially decreased in proportion to the decreases in enzyme activity (Fig. 7). After acetooacetate is converted to acetooacetate-CoA by acetooacetate-CoA synthetase in the cytosol, acetooacetate-CoA can be converted to acetyl-CoA by either cytosolic acetyl-CoA acetyltransferase (ACAT2) or acetyl-CoA acetyltransferase (ACAA1) that each catalyze the same reaction (Fig. 1, reaction 9).<sup>3</sup> Malonyl-CoA and HMG-CoA can each be formed from acetyl-CoA in the cytosol via reactions 13 and 10, respectively (Fig. 1). When only acetooacetate-CoA synthetase (Fig. 1, reaction 9) is deficient, ATP citrate lyase (Fig. 1, reaction 12) should still be able to form acetyl-CoA, which is a substrate for reactions 10 and 13. Therefore, the partial inhibition of insulin release is somewhat surprising and apparently means that acetooacetate-CoA synthase is very important for insulin secretion.
Pathways of Acyl-CoA Formation from Physiologic Insulin Secretagogues—Fig. 1 shows how carbon derived from various insulin secretagogues, such as from the three major secretagogues that stimulate insulin in vivo, glucose, leucine alone, and glutamine in the presence of leucine, can be incorporated into the acyl components of short chain acyl-CoAs in mitochondria and their acyl groups translocated to the cytosol and converted to short chain acyl-CoAs. (Glutamine only stimulates insulin secretion when leucine is present to activate glutamate dehydrogenase, which enhances metabolism of glutamine-derived glutamate (34, 37, 38).) As depicted in Fig. 1, upper left corner, inside the mitochondrion carbon from glucose-derived pyruvate can be decarboxylated to acetyl-CoA catalyzed by pyruvate dehydrogenase and carboxylated to oxaloacetate, catalyzed by pyruvate carboxylase. The acetyl-CoA can be converted to acetoacetyl-CoA catalyzed by either acetyl-CoA acetyltransferase or acetyl-CoA acyltransferase, which each catalyze the same reaction (Fig. 1, reaction 2). The acetoacetyl-CoA can be converted to acetoacetate by succinyl-CoA:3-ketoadhisiser CoA transferase (Fig. 1, reaction 3). Thus, carbon in the acetyl group of acetyl-CoA, originally from glucose, can be converted to acetoacetate via the sequential reactions 2, 3, 8–10, and 13 shown in Fig. 1. Alternatively, the acetyl-CoA can be converted to acetoacetyl-CoA catalyzed by either acetyl-CoA acetyltransferase or acetyl-CoA acyltransferase, which each catalyze the same reaction (Fig. 1, reaction 2). The acetoacetyl-CoA can be converted to acetoacetate by succinyl-CoA:3-ketoadhisiser CoA transferase (Fig. 1, reaction 3). Thus, carbon in the acetyl group of acetyl-CoA, originally from glucose, can be converted to acetoacetyl-CoA synthetase deficient INS-1 cell lines are proportional to the enzyme deficiencies. INS-1 832/13 cells were made deficient in acetoacetyl-CoA synthetase (AACS) enzyme activity with shRNAs targeting the expression of the acetoacetyl-CoA synthetase gene. Relative acetoacetyl-CoA synthetase enzyme activities of the cell lines are expressed as a percent of the untransfected parent cell line (mean ± S.E. of six isolates) and are shown below the abscissa. The parent cell line as well as the control cell line that was transfected with a vector containing a nontargeting shRNA sequence (CHS) and three stable acetoacetyl-CoA synthetase-deficient cell lines with various levels of acetoacetyl-CoA synthetase were incubated for 1 h in the presence or absence of 11.1 mM glucose. There were 85 μg of cellular protein/incubation. Insulin release results are the mean ± S.E. with the number of replicate incubations shown in the boxes inset within the bars showing the insulin release. The p value that confirms the difference between each glucose-stimulated enzyme-deficient cell line, and the controls are shown near each bar.

FIGURE 6. Transcripts of the cytosolic acetyl-CoA acetyltransferase (ACAT2) and peroxisomal (ACAT1) and mitochondrial (ACAT2) acetyl-CoA acyltransferases are present in pancreatic islets and INS-1 cells. Mitochondrial acetyl-CoA acyltransferase (ACAT1) transcripts are present in rat and human pancreatic islets but not in INS-1 cells. The figure shows RT-PCR experiments in which cDNA from rat pancreatic islets, INS-1 832/13 cells grown at various concentrations of glucose, as well as rat liver as a positive control, was tested for both ACAT1 and ACAT2. Two sets of primers that give PCR products of 445 bp (A) and 573 bp (B) from the rat mitochondrial acetyltransferase (ACAT1) cDNA and 399 bp from the rat cytosolic acetyltransferase (ACAT2) cDNA (A) were used. C shows that the transcripts for the mitochondrial (ACAT1) and cytosolic (ACAT2) acetyltransferases are present in human pancreatic islets from three individuals. D shows that transcripts for both the mitochondrial (Acaa1) (431 bp) and the cytosolic (Acaal) (315 bp) acetyl-CoA acyltransferase are present in rat pancreatic islets and INS-1 cells.

FIGURE 7. The decreases of glucose-induced insulin release in acetoacetyl-CoA synthetase-deficient INS-1 cell lines are proportional to the enzyme deficiencies. INS-1 832/13 cells were made deficient in acetoacetyl-CoA synthetase (AACS) enzyme activity with shRNAs targeting the expression of the acetoacetyl-CoA synthetase gene. Relative acetoacetyl-CoA synthetase enzyme activities of the cell lines are expressed as a percent of the untransfected parent cell line (mean ± S.E. of six isolates) and are shown below the abscissa. The parent cell line as well as the control cell line that was transfected with a vector containing a nontargeting shRNA sequence (CHS) and three stable acetoacetyl-CoA synthetase-deficient cell lines with various levels of acetoacetyl-CoA synthetase were incubated for 1 h in the presence or absence of 11.1 mM glucose. There were 85 μg of cellular protein/incubation. Insulin release results are the mean ± S.E. with the number of replicate incubations shown in the boxes inset within the bars showing the insulin release. The p value that confirms the difference between each glucose-stimulated enzyme-deficient cell line, and the controls are shown near each bar.
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Synthase (Fig. 3). Several pathways can export carbon from leucine and α-ketoisocaproate from mitochondria to the cytosol. Carbon from α-ketoisocaproate can form both acetoacetate and acetyl-CoA in the mitochondria catalyzed by HMG-CoA lyase (Fig. 1, reaction 4). The acetoacetate can be directly exported to the cytosol to form short chain acyl-CoAs via reactions 8–10, and 13 in Fig. 1. In addition, the acetyl group of HMG-CoA-derived acetyl-CoA can be converted to acetoacetate in the mitochondria via reactions 4, 2 and 3 shown in Fig. 1, and the acetoacetate exported to the cytosol or the acetyl group can be exported from mitochondria to the cytosol as citrate. The acetoacetate formed by HMG-CoA lyase (Fig. 1, reaction 4) could also be converted to acetyl-CoA and then citrate in the mitochondria via reactions 3, 2 and 1 shown in Fig. 1 and the citrate exported to the extramitochondrial space.

Stimulation of insulin release by leucine plus glutamine is about as potent as glucose-stimulated insulin release in islets and INS-1 cells, and this combination of amino acids caused large increases in the short chain acyl-CoAs (Fig. 2). Mitochondrial formation of short chain acyl-CoAs from the carbon of leucine plus glutamine and the translocation of acyl carbon as acetate and/or citrate to the cytosol and the re-formation of short chain acyl-CoAs in the cytosol can occur via numerous interconnected pathways (Fig. 1). Leucine can be converted to α-ketoisocaproate and α-ketoisocaproate converted to HMG-CoA in the mitochondrion, and then HMG-CoA can be metabolized to acetyl-CoA and acetoacetate (Fig. 1, reaction 4), which can be converted to other acyl-CoAs as described above. Leucine also allosterically activates glutamate dehydrogenase, which will increase the rate of conversion of glutamate to α-ketogluarate. The α-ketoglutarate can be converted to succinyl-CoA via α-ketoglutarate dehydrogenase (Fig. 1, reaction 5) and then to other short chain acyl-CoAs when acetoacetate derived from α-ketoisocaproate combines with the succinyl-CoA (Fig. 1, reactions 4, 3, and 2). In addition, α-ketoglutarate can be converted to malate via reactions of the citric acid cycle. Malate can be converted to pyruvate by malic enzyme in the cytosol and then pyruvate can enter the mitochondria where it can be converted to acetyl-CoA by pyruvate dehydrogenase (4, 9). The acetyl groups can be exported to the cytosol as acetoacetate and citrate as described in the previous paragraph.

Short Chain Acyl-CoAs Are Only Some of the Products of Anaplerosis in the Beta Cell—The data in Fig. 2 show that although multiple short chain acyl-CoAs might be involved in insulin secretion, they are not sufficient to support insulin secretion. Both methyl succinate and α-ketoisocaproate by themselves are potent insulin secretagogues in pancreatic islets, but by themselves do not stimulate insulin release from INS-1 cells (24). The reason for this is not known. However, because α-ketoisocaproate alone does not stimulate insulin release in INS-1 cells, but it increases all short chain acyl-CoAs and methyl succinate increases succinyl-CoA in INS-1 cells, this indicates that other factors besides generation of short chain acyl-CoAs are necessary for insulin secretion. One of these factors, as suggested in the original succinate mechanism scheme (14), may be the generation of NADPH equivalents by mitochondria and their export to the cytosol (6, 10, 16, 17), and this may be one of the factors that methyl succinate contributes that allows it to synergize with α-ketoisocaproate to stimulate nearly as much insulin release as glucose does in INS-1 cells (24). Methyl succinate increases malate up to 30-fold in islets or INS-1 cells (6, 40), and malate will increase cytosolic NADPH via the malic enzyme reaction (2, 6, 10, 41, 42). Another mechanism by which methyl succinate and α-ketoisocaproate may interact synergistically in INS-1 cells is via succinate derived from methyl succinate combining with acetoacetyl-CoA derived from α-ketoisocaproate to increase succinyl-CoA and acetocacetate in the succinyl-CoA:3-ketoacid CoA transferase reaction (Fig. 1, reaction 3). The acetocacetate can be transported out of mitochondria to form short chain acyl-CoAs as described above.

Conclusion—The increases in the beta cell concentrations of several short chain acyl-CoAs by insulin secretagogues suggest a scheme in which multiple short chain acyl-CoAs might perform important functions in insulin secretion. In this study we found that rat and human pancreatic islets and INS-1 832/13 cells possess enzymes for at least two redundant pathways for the translocation of short chain acyl carbon from the mitochondria to cytosol. One involves acetocacetate and the other from the citrate. In this respect, the beta cell may resemble the liver where mitochondrially derived carbon used for lipogenesis is transferred to the cytosol as both acetocacetate and citrate (43). The use of shRNA to knock down the cytosolic enzymes ATP citrate lyase, which does not decrease insulin release, and acetocetyl-CoA synthetase, which does cause a concomitant decrease in insulin release, supports the idea that acetocacetate may be very important as a carrier of short chain acyl groups from the mitochondria to the cytosol in the beta cell. Our multiple short chain
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acyl-CoA mechanism additionally hypothesizes that short chain acyl-CoA carbon is derived from insulin secretagogue carbon via anaplerosis and that malonyl-CoA might be more important in supplying its carbon for lipid synthesis than its being an inhibitor of the transport of long chain fatty acids into mitochondria. Possible roles of short chain CoAs are protein acylation (44, 45), including protein isoprenylation (46), and lipid synthesis (47–49) that might modify the lipid composition of intracellular membranes that could influence the trafficking of insulin secretory granules (2, 50).

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