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Knockdown of pyruvate carboxylase or fatty acid synthase lowers numerous lipids and glucose-stimulated insulin release in insulinoma cells

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ABSTRACT

We previously showed that knockdown of the anaplerotic enzyme pyruvate carboxylase in the INS-1 832/13 insulinoma cell line inhibited glucose-stimulated insulin release and glucose carbon incorporation into lipids. We now show that knockdown of fatty acid synthase (FAS) mRNA and protein also inhibits glucose-stimulated insulin release in this cell line. Levels of numerous phospholipids, cholesterol esters, diacylglycerol, triglycerides and individual fatty acids with C_{14}–C_{24} side chains were acutely lowered about 20% in glucose-stimulated pyruvate carboxylase knockdown cells over a time course that coincides with insulin secretion. In FAS knockdown cells glucose carbon incorporation into lipids and the levels of the subclasses of phospholipids and cholesterol ester species were lower by 20–30% without inhibition of glucose oxidation. These studies suggest that rapid lipid modification is essential for normal glucose-stimulated insulin secretion.

Introduction

Recent studies of pancreatic beta cells suggested that rapid lipid remodeling of cellular lipids might be important for insulin exocytosis. We noticed that stimulation of INS-1 832/13 insulinoma cells with glucose and other insulin secretagogues acutely increased the level of many lipids with C_{14}–C_{24} chains, including phospholipids (PLs)[1], cholesterol esters (CEs), triglycerides (TGs) and free fatty acids (FFAs), by about 20% [1]. Others [2–7] and we [1] have observed that glucose carbon is rapidly incorporated into lipids in pancreatic islets and in insulin cell lines indicating de novo lipid synthesis from glucose carbon occurs over a time course that coincides with insulin secretion. In addition, the enzyme patterns in pancreatic islets and pancreatic beta cell lines suggest they are a lipogenic tissue. Acetyl-CoA carboxylase is a cytosolic enzyme that catalyzes the formation of malonyl-CoA that cells use for fatty acid synthesis as well as possibly for signaling purposes [8,9]. Of the two isoforms of acetyl-CoA carboxylase (ACC1 or 2) the one that is present in pancreatic islets of humans and rats, as well as the insulinoma INS-1 832/13 cell line, is ACC1 [1] which is the isoform found in lipogenic tissues. In addition, the level of fatty acid synthase is quite high in human pancreatic islets [10] and in the INS-1 832/13 cell line [10].

Rodent pancreatic islets [10–13] and various insulin cell lines, such as the INS-1 832/13 cell line [10], contain a high level of the anaplerotic enzyme pyruvate carboxylase. This allows the mitochondria of these cells to synthesize lipid precursors from pyruvate to form malate and citrate. Citrate can be exported from the mitochondria to the cytosol of the beta cell where ATP citrate lyase, which is also abundant in the beta cell [14,15], can catalyze the conversion of citrate to oxaloacetate and acetyl-CoA. The acetyl-CoA can be converted to malonyl-CoA catalyzed by ACC1 and the acetyl-CoA and malonyl-CoA can both be used for lipid synthesis as shown in Fig. 1. Our research [10,16–20] has provided extensive evidence to suggest that in addition to this classical pathway that provides short chain acyl-CoA precursors to the cytosol for lipid synthesis, pancreatic islets (especially pancreatic islets of humans [10]) possess enzymes for another pathway for the synthesis of short chain acyl-CoA lipid precursors. This pathway also begins in mitochondria, but with acetyl-CoA formed in the reaction catalyzed by the pyruvate dehydrogenase complex. Within the

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[1] *Abbreviations used:* AACS, acetoacetyl-CoA synthetase; ACAA1 or 2, acetyl-CoA acyltransferase 1 or 2; ACAT1 or 2, acetyl-CoA acetyltransferase 1 or 2; ACC1 or 2, acetyl-CoA carboxylase 1 or 2; CE, cholesterol ester; DAG, diacylgycerol; FFA, free fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipid; PS, phosphatidylserine; SCOT, succinyl-CoA:3-ketoacid-CoA transferase; TG, triglyceride.

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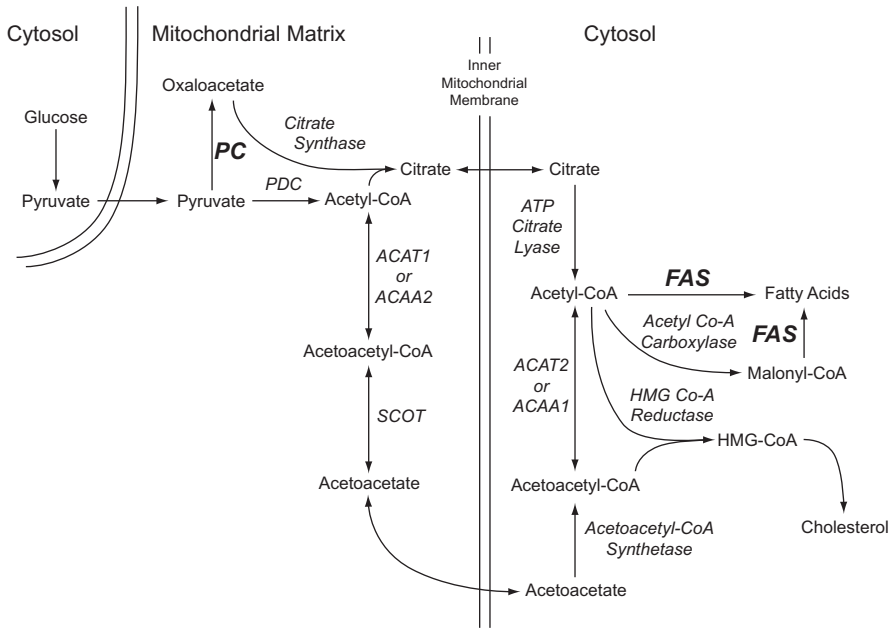

Fig. 1. Pathways of formation of lipid from glucose-derived pyruvate in the pancreatic beta cell. Abbreviations: ACAA1 or 2, acetyl-CoA acyltransferase 1 or 2; ACAT1 or 2, acetyl-CoA acetyltransferase 1 or 2; FAS, fatty acid synthase; PC, pyruvate carboxylase; PDC, pyruvate dehydrogenase complex; SCOT, succinyl-CoA:3-ketoacid-CoA transferase.

mitochondria the acetyl-CoA can be converted to acetoacetyl-CoA by either acetyl-CoA acetyltransferase 1 (ACAT1) or acetyl-CoA acyltransferase 2 (ACAA2) and then to acetoacetate catalyzed by succinyl-CoA:3-ketoacid-CoA transferase (SCOT). The acetoacetate can then be exported from the mitochondria to the cytosol where, via the reactions that begin with acetoacetyl-CoA synthetase (AACS), it can be converted into acetyl-CoA and malonyl-CoA for lipid synthesis (Fig. 1) [10,16–20].

Human pancreatic islets and the INS-1 832/13 insulinoma cell line, but less so islets of rats, possess a high level of fatty acid synthase [10,21]. We have found that application of small molecule inhibitors of either acetyl-CoA carboxylase or fatty acid synthase to rat pancreatic islets and INS-1 832-13 insulinoma cells lowers insulin release [1] suggesting rapid lipid synthesis is important for insulin secretion. In contradiction to the idea that lipid synthesis is necessary for insulin secretion, Joseph et al. [22] knocked down fatty acid synthase mRNA levels 81% in the INS-1 832/13 cell line and observed a 59% decrease in [U-^{14}C]glucose incorporation into lipid, but did not see a decrease in glucose-stimulated insulin release. They also knocked down fatty acid synthase mRNA levels 52% in rat pancreatic islets without observing a decrease in glucose stimulated insulin release [22]. Neither the Newgard group with adenoviral delivery of siRNA that targeted ATP citrate lyase [21], nor our laboratory with stable transfection of multiple different shRNAs that knocked down ATP citrate lyase, has detected a lowering of glucose-stimulated insulin release in INS-1 832/13 cells [19]. Guay et al. [23] did observe inhibition of insulin release with siRNA that targeted ATP citrate lyase mRNA in this cell line. The severe knockdown of ATP citrate lyase activity without inhibition of insulin release suggests that the pathway that uses ATP citrate lyase is redundant with another pathway that exports short chain acyl-CoAs from the mitochondria to the cytosol. The pathway that uses SCOT and AACS is likely the redundant pathway (Fig. 1). In further support of this idea, the cell lines we have generated from the INS-1 832/13 cell line with either knocked down SCOT [17] or AACS [19] show decreased glucose-stimulated insulin release.

To further study the idea that rapid lipid synthesis could be important for insulin secretion we measured the levels of glucose-stimulated individual lipids in our previously generated cell lines with knocked down pyruvate carboxylase that were shown to have severely inhibited glucose-stimulated insulin release [24]. Pyruvate carboxylase catalyzes a reaction near the beginning of pathways for lipid synthesis (Fig. 1). Inhibition of insulin secretion via knockdown of pyruvate carboxylase could affect processes intermediate between its reaction and the final stages of lipid synthesis. Therefore, to study the effect of knockdown of an enzyme that catalyzes a reaction closer to the end of the pathways for synthesis of individual lipids, we also generated a series of cell lines from INS-1 832/13 cells using stably integrated shRNA constructs that target the fatty acid synthase gene. We studied insulin release and measured insulin content in the cell lines with knocked down fatty acid synthase and measured the levels of lipids with C_{14}–C_{24} side chains, such as PLs and CE, as well as the rate of incorporation of glucose carbon into lipid. The results show that the secretagogue-stimulated insulin release and levels of numerous lipids were knocked down proportionate to fatty acid synthase knockdown without a decrease in glucose oxidation. The levels of many lipid species were also lowered proportionate to knockdown of pyruvate carboxylase in the cell lines in which the pyruvate carboxylase gene was targeted. The results suggest rapid remodeling of cellular lipids occurs during insulin exocytosis.

Experimental procedures

Materials and methods

pSilencer™ hygro was from Ambion (Austin TX). The polyclonal antibody against fatty acid synthase (H-300) was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). The INS-1 832/13 cell line [25] was a gift from Chris Newgard. Lipid mixture 1 (Catalog No. L0288) was from Sigma–Aldrich (St. Louis, MO). All other chemicals, in the highest purity available, were from Sigma–Aldrich (St. Louis, MO).

Generation of fatty acid synthase knockdown and control cell lines

The control CHS cell line contained a nontargeting DNA sequence and was described previously [24]. All target DNA