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Archives of Biochemistry and Biophysics 470 (2008) 153–162
ABB
www.elsevier.com/locate/yabbi

The role of rapid lipogenesis in insulin secretion: Insulin secretagogues acutely alter lipid composition of INS-1 832/13 cells

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Received 2 November 2007, and in revised form 20 November 2007
Available online 3 December 2007

Abstract

Pancreatic beta cell mitochondria convert insulin secretagogues into products that support insulin exocytosis. We explored the idea that lipids are some of these products formed from acyl group transfer out of mitochondria to the cytosol, the site of lipid synthesis. There are two isoforms of acetyl-CoA carboxylase, the enzyme that forms malonyl-CoA from which C_2 units for lipid synthesis are formed. We found that ACC1, the isoform seen in lipogenic tissues, is the only isoform present in human and rat pancreatic islets and INS-1 832/13 cells. Inhibitors of ACC and fatty acid synthase inhibited insulin release in islets and INS-1 cells. Carbon from glucose and pyruvate were rapidly incorporated into many lipid classes in INS-1 cells. Glucose and other insulin secretagogues acutely increased many lipids with C_{14}–C_{24} chains including individual cholesterol esters, phospholipids and fatty acids. Many phosphatidylcholines and phosphatidylserines were increased and many phosphatidylinositols and several phosphatidylethanolamines were decreased. The results suggest that lipid remodeling and rapid lipogenesis from secretagogue carbon support insulin secretion.

Keywords: Pancreatic islets; INS-1 832/13 cells; Anaplerosis; Acetyl-CoA carboxylase; Fatty acid synthase; Cholesterol esters; Phospholipids; Lipogenesis; Lipid remodeling

There is a great deal of evidence to indicate that insulin secretagogues stimulate insulin secretion via their metabolism in mitochondria. In addition to mitochondria providing ATP to power cellular processes and activate insulin exocytosis via ATP acting on the ATP-dependent potassium channel, it is clear that the net synthesis of citric acid cycle intermediates by mitochondria (anaplerosis) [1] is involved in insulin secretion. The evidence for anaplerosis is the high level of the anaplerotic enzyme pyruvate carboxylase in the pancreatic islet beta cell [2,3] that enables about 50% of pyruvate derived from glucose, the most potent insulin secretagogue, to be carboxylated to oxaloacetate [2,4–7]. This permits the net synthesis of any citric acid cycle intermediate in beta cell mitochondria and indicates that secretagogue carbon is used for anaplerosis. The rate of pyruvate carboxylation correlates with the glucose concentration applied to pancreatic islets and thus is correlated with the rate of insulin secretion [5]. ^{13}C-NMR isoprotomer studies of glucose metabolism in clonal cell lines have also shown a correlation between insulin secretion and pyruvate flux through the pyruvate carboxylase reaction [8,9]. The purpose of anaplerosis in the beta cell is different from that in many other tissues which possess a high level of pyruvate carboxylase. The level of this enzyme in the beta cell is as high as in gluconeogenic tissues, liver and kidney. However, the normal beta cell is incapable of gluconeogenesis because it lacks all gluconeogenic enzymes [10,11] except pyruvate carboxylase.

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doi:10.1016/j.abb.2007.11.017

Despite the firm evidence that secretagogue carbon is used for anaplerosis in the beta cell, very little is known about the identities of the products of anaplerosis. In a search for products of anaplerosis, we uncovered preliminary evidence to suggest that lipids are one of the numerous possible products of secretagogue metabolism in the beta cell. This idea came in part from the fact that certain lipid precursors, that is various short chain acyl-CoAs, are increased by insulin secretagogues in beta cells and the beta cell possesses several pathways for the transfer of acyl groups from the mitochondria to the cytosol, where lipid synthesis would take place [12,13]. It has also been demonstrated that glucose carbon in acutely stimulated beta cells is incorporated into material extractable with organic solvents [14,15], consistent with the idea that secretagogue carbon is incorporated into lipid. One of the short chain acyl-CoAs that has been frequently shown to be increased by insulin secretagogues is malonyl-CoA [12–17]. Malonyl-CoA supplies the two-carbon units for fatty acid synthesis. In some studies, HMG-CoA was increased by insulin secretagogues [12,13] and HMG-CoA is a precursor of cholesterol.

In the current study, we directly explored the idea that lipids are some of the products of anaplerosis in the beta cell by estimating the levels of two lipogenic enzymes in pancreatic islets and INS-1 cells and correlating inhibition of these enzymes with inhibition of insulin release and by measuring lipids in secretagogue-stimulated INS-1 cells. This showed that of the two isoforms of acetyl-CoA carboxylase, the enzyme that converts acetyl-CoA into malonyl-CoA, ACC1[2] is the major, or only, isoform present in the beta cell. ACC1 is the predominant isoform of the enzyme in lipogenic tissues, such as liver and adipose tissue, whereas the other isoform, ACC2, is found in oxidative tissues, such as cardiac muscle and skeletal muscle [18–20]. We also observed that rat pancreatic islets and INS-1 832/13 cells possess a fairly high level of fatty acid synthase. We found that inhibitors of fatty acid synthase and acetyl-CoA carboxylase inhibited insulin release from rat pancreatic islets and INS-1 832/13 cells. Glucose and other insulin secretagogues acutely increased individual lipids with various fatty acid compositions in INS-1 832/13 cells and carbon from glucose and pyruvate was incorporated into various lipid classes as judged from gas chromatography analysis. These results suggest that lipids are some of the products of acute anaplerosis in the beta cell and that alterations in the cellular lipid composition, especially individual phospholipids and cholesterol esters, which showed the largest increases in secretagogue-stimulated cells, may be products of anaplerosis which support insulin secretion.

[2] *Abbreviations used*: ACC, acetyl-CoA carboxylase; CE, cholesterol ester; FFA, free fatty acid; KIC; α-ketoisocaproate; MMS, monomethylsuccinate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipid; PS, phosphatidylserine; TG, triglycerides.

Experimental procedures

Materials

Anti-mouse fatty acid synthase antibody (Catalog No. 610962) was from BD Biosciences. TOFA (5-(tetradecyloxy)-2-furoic acid) was from Alexis Biochemicals. CP-640186 was from H. James Harwood at Pfizer [21]. Triclosan (5-chloro-2-(2,4-dichlorophenoxy)-phenol) was from Fluka BioChemika. Anti-actin (20–33) antibody (catalog number A5060) and other chemicals were from Sigma. INS-1 832/13 cells were from Chris Newgard [22]. Rat pancreatic islets were isolated by collagenase digestion of pancreases of 250 g fed rats [2,4–6].

Measurement of ^{14}C incorporation from glucose

INS-1 832/13 cells (Passage Nos. 10–15) were cultivated as monolayers on 150 mm tissue culture plates in INS-1 medium (RPMI tissue culture medium (contains 11.1 mM glucose) supplemented with 10% fetal bovine serum, 10 mM Hepes buffer, 1 mM pyruvate and 50 μM β-mercaptoethanol) [23] and penicillin (100 U/ml) and gentamycin (100 μg/ml) [12,13]. Twenty-two hours before experiments were performed the glucose concentration in the tissue culture medium was reduced to 5 mM. On the day of an experiment the monolayers were washed once with phosphate-buffered saline, cells were trypsinized and the trypsin was neutralized with tissue culture medium containing 10% fetal calf serum and no glucose. Cells were collected from the plate and centrifuged and the cell pellet was washed twice with Krebs Ringer bicarbonate buffer. Cells (about 0.1 ml of loosely packed cells) were incubated in 1.5 ml of Krebs Ringer bicarbonate solution modified to contain 15 mM $NaHCO_3$ and 15 mM Hepes buffer (with the NaCl concentration adjusted to maintain osmolarity at 300–310 mM), pH 7.3 and 0.5% bovine serum albumin and radiolabeled glucose. After 30 min, metabolism was stopped by adding 1 ml of 10% trichloroacetic acid (TCA). Cells were washed six to nine more times until the radioactivity in the supernatant fraction equaled the background level. The acid pellet was taken up in 1 ml of water and 0.5 ml of the suspension was used to measure radioactivity by liquid scintillation spectrometry. Water (0.6 ml) was added to the remaining suspension and it was extracted with 2.5 ml of chloroform/methanol (3:1). Radioactivity in the aqueous and organic layers was measured by liquid scintillation spectrometry. Radioactivity and protein values due to bovine serum albumin in blank test tubes that contained all ingredients except cells were subtracted from test tubes containing cells to calculate values attributable to metabolism.

Insulin release

Insulin release from freshly isolated rat pancreatic islets incubated in Krebs Ringer bicarbonate buffer was studied as previously prescribed [12,24]. INS-1 832/13 cells were cultivated as monolayers in INS-1 medium in 24-well tissue culture plates in the tissue culture medium described above. Twenty-two hours before an insulin release experiment was to be performed, the glucose concentration in the medium was reduced to 5 mM. For two hours before the insulin release study, the cells were maintained in Krebs Ringer bicarbonate buffer containing 15 mM sodium Hepes and 15 mM $NaHCO_3$ buffers (with the NaCl concentration adjusted to maintain the osmolarity at 300–310 mM), 0.5% bovine serum albumin and 3 mM glucose [12,26]. The plates were washed and 1 ml of the modified Krebs Ringer solution containing no secretagogue as a control or secretagogue with or without inhibitor was added to each well. After 1 h samples of incubation medium were collected and analyzed for insulin as previously described [12]. As described in individual figures, some inhibitors were incubated on the cells overnight and/or were added 15 min before a secretagogue was added to the insulin release test solution.

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,