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The Role of Rapid Lipogenesis in Insulin Secretion: Insulin Secretagogues Acutely Alter Lipid Composition of INS-1 832/13 Cells

Michael J. MacDonald¹, Agnieszka Dobrzyn^{2,3}, James Ntambi², and Scott W. Stoker¹

¹Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, College of Agriculture and Life Sciences, University of Wisconsin, Madison, 53706

²Department of Biochemistry and Department of Nutritional Sciences, College of Agriculture and Life Sciences, University of Wisconsin, Madison, 53706

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 C₂ 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 C₁₄-C₂₄ 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

Introduction

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

Address for correspondence and reprints: Michael J. MacDonald, Madison, WI 53706, Email: mjmacdon@wisc.edu.

³Current Address: Laboratory of Cell Signaling and Metabolic Disorders, Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland

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[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]. ¹³C-NMR isotopomer 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.

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 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.

Experimental Procedures

Materials

Anti-mouse fatty acid synthase antibody (catalog number 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 number 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 units/ml) and gentamycin (100 $\mu\text{g}/\text{ml}$) [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 hour 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, Carlsbad, CA) and further purified on an RNeasy Mini column (Qiagen, CA). RNase-free DNase was applied to the column to digest any contaminating DNA. cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA) and oligo (dT) primers (Promega, Madison, WI). A companion reaction that contained no reverse transcriptase was run as a control. The PCR was run with the cDNA (0.5 μl out of 20 μl in a volume of 50 μl using gene-specific primer pairs

and 25 cycles for amplification. PCR products were stained with ethidium bromide, separated on a 1% agarose gel, and visualized under UV light. The primers used were 5'-AGTGTCTCAGCGATGTTCTGTTGGA-3' and 5'-TCAAACCTATCCCTTGCTCGGA-3' for rat acetyl-CoA carboxylase 1 and 5'-CGTTCAGAGCGAGAGATGAGTTTG-3' and 5'-CTGGAGTCGGTCACTTCTTTGTATA-3' for rat acetyl-CoA carboxylase 2 and 5'-AACGAGGAGCAGAAGCGGAA-3' and 5'-ATGCCAGGACCAATAAAACCCT-3' for rat glutamate dehydrogenase.

Subcellular fractionation

Pancreatic islets and INS-1 cells were homogenized in 220 mM mannitol, 70 mM sucrose and 5 mM potassium Hepes buffer, pH 7.5, and fractionated as previously described [2,10,13]. Cytosol was the $20,000 \times g \times 10$ min postmitochondrial supernatant fraction.

Detection of acetyl-CoA carboxylase with streptavidin

Proteins were separated on 7.5% polyacrylamide gels and electrotransferred to nitrocellulose membranes. The membranes were blocked with 2% gelatin in Tris buffered saline tween-20 (TBST) for 1 hour at room temperature. Membranes were incubated for 1 hour in streptavidin-horse radish peroxidase (Pierce, Rockford, IL) diluted 1:40,000 in 2% gelatin in TBST. The proteins were visualized using a chemiluminescence kit (Pierce) and then the membranes were re-probed with anti- β -actin antibody.

Fatty acid synthase

Fatty acid synthase protein was detected by immunoblotting [13]. The activity of the enzyme was measured in a reaction mixture containing 60 μ M acetyl-CoA, 0.15 mM NADPH, 2.5 mM EDTA, 60 μ g/ml bovine serum albumin, in 100 mM potassium phosphate buffer, pH 6.5 and whole cell homogenate or cell cytosol (a $20,000 \times g \times 10$ min supernatant fraction) by monitoring NADPH oxidation spectrophotometrically at 340 nm and 37° [25]. After a background rate was obtained, the enzyme reaction was started by adding 70 μ M malonyl-CoA. The background rate was subtracted from the rate with malonyl-CoA to give the rate attributable to the enzyme.

Lipid composition

INS-1 832/13 cells (passage number 10–15) were maintained on 100 mm plates for 22 h in INS-1 tissue culture medium containing 5 mM glucose as described above. Plates were washed twice with warm phosphate-buffered saline and cells were incubated for 30 min in the presence of the modified Krebs Ringer bicarbonate Hepes buffer, pH 7.3, containing either no addition, 16.7 mM glucose or 2 mM α -ketoisocaproic acid (KIC) plus 10 mM succinate monomethyl ester (MMS). After 30 min, the medium was quickly removed, 1 ml of water was spread evenly on the plates and they were instantly placed at -70° . After 5–10 min at -70° , the plates were quickly warmed to room temperature and cells were scraped off the plates into test tubes and 60 μ l of the suspension was removed and saved for estimation of total protein. Lipids were extracted by the method of Bligh and Dyer [26] and measured as previously described [27, 28]. Four ml of chloroform/methanol (2:1) containing 0.01% butylated hydroxytoluene was added and cells were vortexed vigorously for 30 min and the mixture allowed to set for 22 h in capped tubes. The lipids were separated into cholesterol esters, triglycerides, free fatty acids and phospholipids by thin layer chromatography on silica gel-60 plates (Merck) in heptane/isopropyl ether/glacial acetic acid (60/40/4, v/v/v) with authentic standards. Additionally, phospholipid classes were separated in chloroform/methanol/glacial acetic acid/water (50/37.5/3.5/2, v/v/v/v). The bands corresponding to standards were scraped off the plate and transferred to screw cap glass tubes containing methylpentadecanoic acid as an internal standard. Fatty acids were then transmethylated in the presence of 14% boron trifluoride in

methanol. The resulting methyl esters were extracted with hexane and analyzed on a Hewlett-Packard (Palo Alto, CA) 6890 gas chromatograph equipped with a 7683 autoinjector and an HP-5 column (30 m × 0.25 mm, 0.25 mm film thickness) connected to a flame ionization detector set at 275° C. The injector was maintained at 250° C. The column temperature was held at 180° C for 2 min after injection, increased to 200° C at 8° C/min, held at 200° C for 15 min, and then increased to 250° C at 8° C/min. Total contents were calculated from individual fatty acid content in each sample. Measurements were made of lipids with fatty acyl side chains shown in Table 1 and in addition lipids containing the fatty acid chains 18:3(n-3), 20:5, 22:1 and 24:1 which were not detected. When a fatty acid chain was not shown in a table, it was not detected in the lipid class shown in the table.

For the measurement of ¹⁴C incorporation into the lipid classes, cells were prepared and incubated in suspension in the presence of ¹⁴C-labeled glucose or pyruvate (1 µCi ¹⁴C/test tube) as described above, but with no bovine serum albumin. Metabolism was stopped quickly after 30 min by adding chloroform/methanol (2:1). Lipids were then extracted and fractionated by TLC as described above. The radioactivity of each lipid fraction was measured using Instant Imager (Packard).

Statistical Analysis

Statistical significance was calculated with student's t-test.

Results

Incorporation of glucose carbon into lipid

When INS-1 832/13 cells were incubated for 30 min in the presence of a nearly maximal insulin stimulatory concentration of glucose (10 mM), glucose carbon incorporated into an acid-precipitable pellet and a chloroform/methanol soluble fraction (lipid) was increased 15 times the control that was incubated in the presence of a non-insulinotropic concentration of glucose (1 mM). Metabolic inhibitors lowered the carbon incorporation to values similar to those of the cells incubated in the presence of 1 mM glucose (Figure 1).

Lipogenic enzymes in beta cells

Transcripts for the ACC1 isoform gene of acyl-CoA carboxylase were easily demonstrable in both rat pancreatic islets and INS-1 832/13 cells, as judged from RT-PCR, while transcripts for the ACC2 isoform gene were negligible (Figure 2A). A streptavidin blot showed that the ACC1 protein (265 kDa), but not the ACC2 protein (280 kDa), was present in both rat and human pancreatic islets and INS-1 832/13 cells (Figure 2B). As judged from immunoblotting with anti-fatty acid synthase antibodies, INS-1 832/13 cells possess about 50% the concentration of fatty acid synthase as liver and rat pancreatic islets possess about 25% the level of liver (Figure 3). However, as judged from measurements of enzyme activity, islets and INS-1 cells possessed 65% and 46% the level of fatty acid synthase as liver, respectively (1.7 ± 0.1 (4), 1.2 ± 0.1 (4) and 2.6 ± 0.3 (6) nmol malonyl-CoA used min/mg cell protein (means $\pm$ SE (N)). As judged from either method, the level of fatty acid synthase in islets and INS-1 cells is substantial.

Inhibition of insulin release by inhibitors of lipogenesis

CP-640186, an inhibitor of acetyl-CoA carboxylase [21], partially inhibited insulin release from pancreatic islets and INS-1 832/13 cells (Figures 4 and 5). Triclosan is a potent inhibitor of the enoyl-acyl carrier protein reductase enzyme of the prokaryotic type II fatty acid synthase complex. Triclosan has also been reported to inhibit Type I fatty acid synthase in mammalian cells [29,30]. At concentrations shown not to be cytotoxic in preadipocytes (50 µM) [34]

triclosan was a strong inhibitor of insulin release from islet cells (Figure 4) and INS-1 832/13 cells (Figure 5). TOFA, an inhibitor of acetyl-CoA carboxylase [31,32] and cerulenin, another fatty acid synthase inhibitor [33], at concentrations shown not to permanently lower ATP levels in cultured cortical neuron cells [34], also partially inhibited insulin release from islets (Figure 4) and INS-1 832/13 cells (Figure 5).

Measurement of lipids

As judged from gas chromatography measurements, the most abundant class of lipid in the INS-1 832/13 cells was cholesterol esters (CE) followed by phospholipids (PL) and free fatty acids (FFA). Among the PL class phosphatidylcholine (PC) was the most abundant (60%), followed by phosphatidylethanolamine (PE) (24%), phosphatidylinositol (PI) (11%) and phosphatidylserine (PS) (4%). Palmitate (16:0) was the most abundant fatty acid side chain among all lipid classes and PL subclasses. In CE; myristic acid (14:0) was the second most abundant fatty acid side chain followed by palmitoleate (16:1), stearate (18:0), oleate (18:1 (n-9)), and vaccenate (18:1 (n-7)) and linoleate (18:2). The relative abundances among the PL were similar, except that the myristate (14:0) side chain ranked sixth in abundance and the phospholipids contained a relatively high amount of arachidonate (20:4) (Table 1). Triglycerides (TG) and FFA were present in lower amounts than the other lipid classes in the INS-1 cells. A concentration of glucose that stimulates maximal insulin release from these cells, as well as 2 mM KIC plus 10 mM monomethyl succinate, which also releases insulin from these cells [12], increased the levels of various individual PL, CE and TG with various acyl side chain lengths after 30 min (Tables 2 and 3). Methyl succinate by itself does not stimulate insulin release from INS-1 cells but it caused increases in some lipids (Supplemental Tables 1–8 Online)¹. This may be because it provides NADPH [2,12–15] which is used for fatty acid elongation and HMG-CoA synthesis. The relative increase in total CE induced by glucose was 17% and that induced by MMS plus KIC was 21% (Tables 2 and 3 and Supplemental Table 2). Among the individual PLs and CEs, glucose and KIC plus MMS stimulated the largest increase in those with myristate (14:0), palmitate (16:0), palmitoleate (16:1), stearate (18:0), oleate (18:1, n-9) and vaccenate (18:1, n-11) side chains. The average relative increase in total PL was 20% in the presence of high glucose and 23% in the presence of MMS plus KIC (Tables 2 and 3 and Supplemental Table 1). Glucose and MMS plus KIC increased PC, which was the major component of the PL class, 20% and 32% respectively (Tables 2 and 3 and Supplemental Table 5) and PS, a very minor component of this class, 73% and 172% respectively (Tables 2 and 3 and Supplemental Table 6). Glucose decreased PI 43%, in agreement with previous data [35,36] and MMS plus KIC decreased this subclass by 30% (Tables 2 and 3 and Supplemental Table 7). Glucose lowered PE, the second largest subclass of PL, by 17%, and MMS plus KIC had no effect on PE levels (Tables 2 and 3 and Supplemental Table 8). Glucose and MMS plus KIC lowered PC, PI, and PE containing arachidonate (20:4) (Tables 2 and 3 and Supplemental Tables 5, 7 and 8). Total FFA levels were increased by 10% in the presence of glucose and 4% in the presence of MMS plus KIC (Tables 2 and 3 and Supplemental Table 3). The levels of TG were very low and glucose increased TG 23% and MMS plus KIC increased TG 30%. Obviously, when an individual lipid or a class of lipids is present at a very low concentration, less weight can be placed on large relative changes and these changes should be interpreted in that light. All values were included for completeness.

INS-1 832/13 cells were also stimulated with 10 mM ¹⁴C-labeled glucose or 1 mM or 5 mM ¹⁴C-labeled pyruvate for 30 min. Incorporation of ¹⁴C from glucose into CE, PL, including PC, PE and PI, TG and sphingomyelins was dramatically increased. ¹⁴C incorporation into FFA and diglycerides was increased to a smaller extent. Pyruvate is a potent stimulant of insulin release in INS-1 cells [12,13,37,38]. ¹⁴C incorporation into lipids in the presence of both

¹More detailed data, including control data, can be seen in Supplemental Tables 1–8 on the BBA website.

concentrations of pyruvate was also increased, but not quite to the extent of ^{14}C incorporation from glucose (Table 4). These data show that there is acute de novo anaplerotic input of secretagogue carbon into lipids.

Discussion

The beta cell as a lipogenic tissue

Data reported here, as well as previously available data, are consistent with pancreatic beta cells more closely resembling a tissue that uses anaplerosis to synthesize lipids than a tissue that oxidizes lipids for fuel. Figure 1 shows that glucose carbon is rapidly incorporated into lipid in INS-1 cells indicating the beta cell's capacity for anaplerosis enables the de novo synthesis of lipids from the carbon of an insulin secretagogue. In lipogenic tissues malonyl-CoA is the C_2 -unit donor for the de novo synthesis of long chain fatty acids catalyzed by fatty acid synthase and for the chain elongation of fatty acids to very long chain fatty acids. Malonyl-CoA is formed by the carboxylation of acetyl-CoA catalyzed by acetyl-CoA carboxylase (ACC). There are two isoforms of ACC encoded by two separate genes in animals including humans; ACC1 ($M_r = 265,000$) which is abundant in the cytosol of lipogenic tissues, such as liver and adipose tissue, and ACC2 ($M_r = 280,000$) which is abundant in oxidative tissues, such as heart and skeletal muscle, and is present to a small degree in the liver [18–20]. (In line with this correlation is the fact that cardiac muscle possesses little or no pyruvate carboxylase activity, unlike islets and liver, and is capable of little or no anaplerosis [2,39].) Malonyl-CoA is also a key regulator of fatty acid oxidation because it is an allosteric inhibitor of carnitine palmitoyltransferase-1 (CPT-1), localized in the outer surface of the inner mitochondrial membrane, where CPT-1 is involved in the transport of long chain acyl-CoAs acids into mitochondria for oxidation. Work from the Wakil group has shown that ACC2 is localized at the mitochondrial membrane, situated so that the malonyl-CoA it synthesizes controls the transport of long chain fatty acyl-CoA by the membrane bound CPT-1 [18,19]. Wakil's data are consistent with ACC1 producing malonyl-CoA for lipogenesis and ACC2 producing malonyl-CoA for inhibition of long chain acyl-CoA uptake and their oxidation into mitochondria [18,20]. The finding of the current study (Figure 2) that ACC1 is the predominant, or only, ACC isoform in pancreatic islets and INS-1 832/13 cells, and a previous observation that the 265 kDa isoform of ACC is the major isoform present in rat islets and INS-1 cells [40], are consistent with the idea that the beta cell is a lipogenic tissue. The involvement of ACC in insulin release is further supported by the work of Zhang and Kim [41] who developed a stable INS-1 transfectant cell line expressing ACC antisense RNA that showed decreased insulin release to glucose and other nutrient secretagogues.

We also found significant levels of fatty acid synthase protein as judged from immunoblotting, as well as fatty acid synthase enzyme activity, in both pancreatic islets and INS-1 832/13 cells (Figure 3). The levels of enzyme were quite high and ranged from 25% to 75% those in liver. The acute involvement of acetoacetyl-CoA carboxylase and fatty acid synthase in insulin secretion was demonstrated with inhibitors of these enzymes. Consistent with the idea that fatty acid synthesis is important for insulin release, inhibitors of acetyl-CoA carboxylase such as TOFA and CP-640186, and of fatty acid synthase, such as triclosan and cerulenin, lowered insulin release from 50% to 90% in both rat pancreatic islets and INS-1 832/13 cells (Figures 4 and 5). Cerulenin has previously been reported to inhibit insulin release and protein acylation in rat pancreatic islets [42] while only slightly decreasing ATP levels of the islets (15% to 37%) [43].

Lipid composition in beta cells

Previous reports of the lipid composition in pancreatic islets usually focused on phospholipids and less often on all lipid classes, and our findings in clonal beta cells resemble the aggregate

results of many of these studies. However, the current study is perhaps the most extensive and detailed report to date of measurements of numerous individual lipids in unstimulated or secretagogue-stimulated pancreatic beta cells. Our current study analyzed fatty acid compositions of four classes of lipids: PLs, CEs, TGs and FFAs, as well as the PL subclasses PC, PE, PS and PI. We observed that the predominant lipids were CEs which were present in higher levels in beta cells than in many other tissues of the body [44], such as the liver, followed by phospholipids. These results are similar to those of Diaz et al [45] who performed studies of the lipid composition of freshly isolated unstimulated islets from normal male rats. They observed that neutral lipids and PLs represented 86% and 14%, respectively, of islet lipids. Similar to our results, they observed that esterified cholesterol (39%), cholesterol (20%) and FFA (24%) were the major components of the neutral lipid fraction. Our phospholipid results in INS-1 832/13 cells also were similar to those reported in several studies of islets. The relative abundances of PC, PE, PI and PS that we found in INS-1 832/13 cells (60%, 24%, 11% and 4%) very closely resemble those reported by Hallberg (64%, 26%, 5.1% and 4.9%) who studied NMRI mouse islets [46] and by Turk et al (63%, 23%, 6.7% and 4.9%) who studied overnight-cultured rat islets [47]. Diaz et al [45] in their studies of fresh islets found that PC (46%) and PE (21%) were the major components of the PL fraction and PI was a minor component (9%). Both Diaz et al [45] and Turk et al [47] observed that palmitate and stearate were the predominant fatty acids in PLs. We found that palmitate (36%), oleate (18:1 (n-9)) (21%), palmitoleate (16:1) (12%) and stearate (18:0) (12%) were the major classes of PLs in INS-1 832/13 cells, followed by myristate (14:0) (5%), vaccenate (18:1 (n-7)) (5%), arachidinate (20:4) (3.6%) and linoleate (18:2) (2.7%) (Table 1).

Secretagogue effects on beta cell lipids

The current study shows that insulin secretagogues rapidly increase various individual lipid levels by 20% or more, especially certain cholesterol esters, fatty acids and PLs, and decrease certain PLs in beta cells suggesting that lipid remodeling might be part of the exocytosis mechanism. Within the PL classes, individual PCs which were quantitatively the major subclass of PLs, and also PSs, were increased, while most PIs and PEs were decreased. The incorporation of carbon from glucose and other secretagogues into individual lipids, especially CEs, PLs, TGs and sphingomyelin (Table 4), indicates that rapid de novo lipid synthesis correlates with the time course of insulin secretion.

Our observation that insulin secretagogues acutely increase the levels of certain lipids and decrease other lipids in INS-1 cells are in agreement with many, but not all, previous studies with pancreatic islets. In the late 1970s and early 1980s Berne and Andersson [48,49] reported that the incorporation of ^{14}C -labeled glucose into triglycerides and PLs in pancreatic islets from normal NMRI mice and obese-hyperglycemic mice was increased in response to an acute increase in the glucose concentration. Hallberg [46] found that PL content of the NMRI mouse islets increased when the islets were maintained in the presence of a high concentration of glucose in long-term tissue culture and that in short term culture (two hours) glucose carbon was incorporated into PC, PE, PI and PS in a dose dependent manner [50]. Vara and Tamarit-Rodriguez [51] reported that glucose-stimulated insulin secretion in rat islets was accompanied by decreased palmitate oxidation and increased ^{14}C -labeled glucose incorporation into di- and triacylglycerols, predominantly into PLs. Martins et al [52] recently exposed rat pancreatic islets to a high concentration of glucose for one hour. They extracted and saponified lipids and found glucose increased the amounts of lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0) and arachidonic acid (20:4) 93%, 103%, 575%, 25%, 124%, and 13%, respectively, as judged by HPLC.

Our data showed convincing secretagogue-induced increases in the masses of many individual PLs and total PL subclasses, especially in PC which comprises the majority of the mass of PLs.

These were PLs and PCs with saturated and unsaturated side chains (Tables 2 and 3). Glucose also increased the level of PS containing these fatty acid chains (Table 2), but PS comprised only 3.7% of the total PLs. Phospholipase C-mediated hydrolysis of PI catalyzes the formation of IP₃ and PIP₂ which have signaling roles in numerous types of cells including the pancreatic beta cell. Our observation that glucose decreased the amounts of individual and total PIs (Table 2) is consistent with results of studies of pancreatic islets with radioactive tracers, such as [³H]myoinositol or ³²Pi, used to prelabel PLs in islets. These studies showed that glucose acutely lowered radioactivity in PI indicating PIs were rapidly broken down in the insulin release process [35,36]. In addition, our results showed that glucose lowered the levels of the most abundant PEs (Table 2), indicating PE hydrolysis may contribute to remodeling of cellular membranes during insulin exocytosis.

Turk [47] performed a thorough study of PL changes in rat pancreatic islets and detected slight acute decreases in the mole percent of PCs in rat islets stimulated for 30 min with 28 mM glucose. Their major finding was that glucose caused a decrease in the arachidonate in PC. They also showed that glucose-stimulated islets incorporated [³H]arachidonate into PC with a higher specific radioactivity than that of any other major PL and that glucose acutely lowered the radioactivity in PC in islets that were prelabeled with [³H]arachidonate. These data are consistent with a phospholipase A₂ hydrolysis of PLs, primarily of PC, for generating arachidonate which may have signaling properties in insulin secretion. In respect to the relatively high arachidonate content of PLs, the current data on INS-1 832/13 cells and also our earlier findings, which showed that glucose stimulated the prelabeling of islets incubated with [³H]arachidonate and also acutely decreased levels of this label in PC [35], agree with Turk's observations. In the current study, arachidonate (20:4) was also a major component of the total PL fraction of INS-1 832/13 cells (Table 1); and glucose as well as KIC plus MMS stimulated decreases in arachidonate in PC, PE and PI (Tables 2 and 3).

Conclusion

In conclusion, we believe that, as suggested previously [53], modifications of lipids in cellular membranes, including the plasma membrane, are necessary in secretagogue-stimulated beta cells as insulin is synthesized in the endoplasmic reticulum, traverses intracellular membranes and cisternae, including the Golgi apparatus, and is packaged into granules which fuse with the plasma membrane as insulin is secreted into the blood stream. This may be achieved through lipids synthesized in part from secretagogue carbon via anaplerosis and lipid modifications, such as changes in lipid desaturation, also as previously suggested [54].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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

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Figure 1. Increased incorporation of carbon from stimulatory glucose into acid precipitable and lipid products INS-1 cells

INS-1 832/13 cells were incubated for 30 min in the presence of 2 μ Ci of [U- ^{14} C]glucose in Krebs Ringer bicarbonate containing 1 mM or 10 mM glucose or 10 mM glucose plus 10 μ M rotenone and 10 mM KCN to inhibit metabolism. ^{14} C incorporated into an acid washed cell pellet and a lipid fraction were measured as described under Experimental Procedures. Results are the mean $\pm$ SE of six replicate incubations. The difference between 10 mM glucose and 1 mM glucose was significant with $p < 0.005$.

A. RT-PCR

Size (bp)

B. Streptavidin blot

Size (kDa)

Figure 2. The high level of acetyl-CoA carboxylase 1 in human and rat pancreatic islets and INS-1 cells is consistent with lipogenesis

Panel A shows RT-PCR of RNA from liver, INS-1 832/13 cells and rat pancreatic islets with primers designed for transcripts from the acetyl-CoA carboxylase genes 1 (ACC1) and 2 (ACC2). Liver RNA was used as a positive control for transcripts of both genes. Primers for glutamate dehydrogenase (Glu DH) mRNA were used as a control showing cDNA amounts were equal. More than 99% of ACC expression in islets and INS-1 832/13 cells is that of the ACC1 isomer. Panel B shows a streptavidin-probed blot of proteins from human and rat pancreatic islets and INS-1 832/13 cells with rat liver as control representing a tissue possessing predominantly ACC1 protein (265 kDa) and rat heart as a tissue possessing exclusively ACC2 (280 kDa). The islets and INS-1 cells show only the 265 kDa band which represents the isoform present in lipogenic tissues as compared to oxidative tissues which possess the 280 kDa band.

FA Synthase

Figure 3. Measurements of enzyme activity and an immunoblot show fatty acid synthase is present in rat pancreatic islets and INS-1 832/13 cells

Enzyme activity (nmol malonyl-CoA used/min/mg cell protein) is the mean $\pm$ SE measured on 4 to 6 preparations of cytosol from each tissue. ND indicates not done. For immunoblotting, liver cytosol (8 μ g protein) per lane from fed and 24-h starved rats is shown as controls. There was 40 μ g of islet cytosol protein and 20 μ g INS-1 832/13 cell cytosol protein per lane.

Figure 4. Inhibitors of fatty acid synthase and acetyl-CoA carboxylase inhibit glucose-induced insulin release from rat pancreatic islets

Islets were incubated with inhibitors for 10 – 15 min before glucose was added and for 1 h in the presence of 16.7 mM glucose. Inhibitors were dissolved in DMSO to give a final concentration of DMSO of 0.2% to 1%. The numbers under the abscissa show the concentrations of inhibitors. Inhibition of glucose-induced insulin release by each inhibitor concentration was significantly different versus glucose alone or glucose plus DMSO with $p < 0.001$. Results are the mean $\pm$ SE with 6–20 replicate incubations for each condition.

Figure 5. Inhibitors of fatty acid synthase and acetyl-CoA carboxylase inhibit glucose-induced insulin release from INS-1 832/13 cells

In Experiment 1 cells were maintained overnight (about 16 h) in tissue culture medium in the presence of 5 mM glucose and an inhibitor in 24-well plates. To study insulin release cells were incubated for 1 h with glucose (11.1 mM) plus the same inhibitor that was present in overnight culture. In Experiment 2 inhibitors were added to the incubation mixture 15 min before the glucose was added and incubated for 1 h. Inhibitors were dissolved in dimethyl sulfoxide (DMSO) and added to give a final concentration of 0.2% to 1% DMSO. There was about 100 μg of total cell protein per well. The numbers under the abscissa show the concentrations of inhibitors. Inhibition of glucose-induced insulin release by each concentration of inhibitor was significantly different versus glucose alone or glucose plus DMSO with $p < 0.001$. Results are the mean $\pm$ SE of 4–9 replicate incubations for each condition.

Table 1

Lipid composition of unstimulated INS-1 832/13 cells

Results are the mean of six measurements for each lipid class and three measurements for phospholipids subclasses. Standard errors are not included in the table to save space and improve readability and can be found in Supplemental Tables 1–8 on the BBA website. ND indicates a species was not detected.

Fatty Acid Group	PL	CE	Level of Individual Lipid in Lipid Class (nmol lipid/mg protein)					PI	PE
			FFA	TG	PC	PS			
14:0	6	88	11	0.2	4.9	0.1	0.2	1.5	
16:0	40	134	47	2.3	26	0.8	2.5	7.5	
16:1	13	60	0.4	0.4	8.8	0.4	0.6	0.6	
18:0	13	35	38	0.8	2.4	1.4	4.2	5.6	
18:1 (n-9)	24	26	0.8	0.6	13	0.8	2.0	5.3	
18:1 (n-7)	6	11	0.3	0.3	5.7	0.3	0.9	1.8	
18:2	3	2	0.1	0.01	0.9	0.1	0.1	0.8	
20:0	0.2	ND	0.1	ND	ND	ND	ND	ND	
20:1 (11-cis)	0.5	ND	ND	ND	0.3	ND	ND	0.2	
20:1 (13-cis)	0.4	ND	ND	ND	ND	ND	ND	ND	
20:3	0.7	ND	ND	ND	0.04	0.02	0.03	0.03	
20:4	4	ND	0.3	0.4	0.3	0.3	0.9	1.5	
22:0	0.1	ND	ND	ND	ND	ND	ND	ND	
22:6	1	ND	ND	ND	ND	ND	ND	0.3	
24:0	0.3	ND	ND	ND	ND	ND	ND	ND	
Total	112	361	98	4.9	62	4.1	11.5	25	

Table 2

Glucose changes the levels of various lipids in INS-1 832/13 cells

Cells were incubated with 16.7 mM glucose and other conditions, including non-stimulatory conditions as controls, for 30 min after which metabolism was stopped and lipids were analyzed as described under Experimental Procedures. Only the results for glucose are shown and are the mean $\pm$ SE of six replicate incubations for all except individual PC, PS, PI and PE, where N = 3. Results are shown as the percentage increase or decrease versus the no addition control value. Statistical significance was calculated with the raw data. Large percentage changes that are not significant are likely associated with large SEs and/or very low levels of the lipids. Complete sets of data with lipid contents of the unstimulated control and percent changes can be viewed in Supplemental Tables 1–8 at the BBA website. ND indicates not detected.

Fatty Acid Group	Lipid Class					
	PL	CE	FA	TG	PC	PS
14:0	26 $\pm$ 3 ^a	16 $\pm$ 15	26 $\pm$ 9	175 $\pm$ 46	22 $\pm$ 18	64 $\pm$ 32
16:0	22 $\pm$ 3 ^a	18 $\pm$ 10	9 $\pm$ 4	20 $\pm$ 12	28 $\pm$ 8 ^a	41 $\pm$ 32
16:1	18 $\pm$ 6	9 $\pm$ 18	14 $\pm$ 9	10 $\pm$ 12	11 $\pm$ 14	93 $\pm$ 40
18:0	19 $\pm$ 3 ^a	32 $\pm$ 10 ^a	8 $\pm$ 4	10 $\pm$ 11	15 $\pm$ 16	108 $\pm$ 24
18:1 (n-9)	19 $\pm$ 5	9 $\pm$ 12	-11 $\pm$ 10	30 $\pm$ 14	12 $\pm$ 15	68 $\pm$ 21
18:1 (n-7)	21 $\pm$ 4	17 $\pm$ 15	-15 $\pm$ 17	17 $\pm$ 13	14 $\pm$ 15	50 $\pm$ 22
18:2	-4 $\pm$ 11	23 $\pm$ 17	9 $\pm$ 15	226 $\pm$ 88	7 $\pm$ 12	55 $\pm$ 15
20:0	3 $\pm$ 15	ND	126 $\pm$ 51	ND	ND	ND
20:1 (11-cis)	-1 $\pm$ 17	ND	ND	ND	345 $\pm$ 41 ^b	ND
20:1 (13-cis)	-53 $\pm$ 5 ^b	ND	ND	ND	ND	ND
20:3	10 $\pm$ 6	ND	ND	ND	48 $\pm$ 17	26 $\pm$ 6
20:4	8 $\pm$ 5	ND	15 $\pm$ 5	-12 $\pm$ 4	-37 $\pm$ 15	50 $\pm$ 5
22:0	10 $\pm$ 4	ND	ND	ND	ND	ND
22:6	11 $\pm$ 4	ND	ND	ND	ND	ND
24:0	23 $\pm$ 15	ND	ND	ND	ND	ND
Total	20 $\pm$ 4 ^b	17 $\pm$ 6	10 $\pm$ 5	23 $\pm$ 8	20 $\pm$ 7	73 $\pm$ 14 ^a

^a p $\leq$ 0.05 or

^b p $\leq$ 0.01 vs no addition control

Table 3

Methyl succinate plus KIC changes the levels of various lipids in INS-1 832/13 cells

Cells were incubated with 2 mM α -ketoisocaproate (KIC) plus 10 mM monomethyl succinate (MMS) which stimulates insulin from INS-1 832/13 cells [12 and MJM, unpublished data], and other conditions, including non-stimulatory conditions as controls, for 30 min after which metabolism was stopped and lipids were analyzed as described under Experimental Procedures. Only the results for MMS plus KIC are shown. This experiment was part of the experiment shown in Table 2 and data were calculated the same as in Table 2 (See legend of Table 2 and Supplemental Tables 1–8 at the BBA website.). ND indicates not detected.

Fatty Acid Group	PL	CE	Lipid Class					PS	PI	PE
			Percentage Change in Lipid Induced by MMS Plus KIC							
			FA	TG	PC	MMS Plus KIC				
						PC	PE			
14:0	27 ± 12	11 ± 9	18 ± 13	198 ± 53	34 ± 6	52 ± 8	-16 ± 8	-69 ± 4 ^a		
16:0	27 ± 9 ^a	34 ± 16	3 ± 10	22 ± 16	28 ± 6	63 ± 3	-30 ± 7 ^a	-27 ± 11		
16:1	14 ± 17	3 ± 4	64 ± 16 ^b	27 ± 23	34 ± 5	41 ± 7 ^a	-34 ± 7 ^a	35 ± 22		
18:0	25 ± 9 ^a	59 ± 17 ^a	-1 ± 10	13 ± 14	27 ± 5	289 ± 19 ^b	-39 ± 7 ^a	-3 ± 18		
18:1 (n-9)	19 ± 13	-16 ± 8	61 ± 11 ^b	30 ± 21	29 ± 5	243 ± 18 ^b	-43 ± 1 ^b	44 ± 20		
18:1 (n-7)	26 ± 12	24 ± 6 ^a	127 ± 66	117 ± 75	50 ± 20	209 ± 17 ^b	-33 ± 6 ^b	20 ± 17		
18:2	10 ± 11	-47 ± 12 ^a	3 ± 14	100 ± 181	30 ± 7	91 ± 11	-54 ± 13 ^b	21 ± 17		
20:0	36 ± 7 ^b	ND	126 ± 59	ND	ND	ND	ND	ND		
20:1 (11-cis)	11 ± 12	ND	ND	ND	7 ± 15	ND	ND	76 ± 31		
20:1 (13-cis)	-53 ± 4 ^b	ND	ND	ND	ND	ND	ND	ND		
20:3	13 ± 11	ND	ND	ND	96 ± 24	50 ± 13 ^a	-39 ± 14	45 ± 23		
20:4	5 ± 12	ND	12 ± 18	-17 ± 13	-16 ± 20	57 ± 4 ^b	-12 ± 10	19 ± 17		
22:0	30 ± 4 ^b	ND	ND	ND	ND	ND	ND	ND		
22:6	6 ± 9	ND	ND	ND	ND	ND	ND	-31 ± 6		
24:0	38 ± 25	ND	ND	ND	ND	ND	ND	ND		
Total	23 ± 9 ^a	21 ± 11	4 ± 16	30 ± 14	32 ± 9 ^a	172 ± 4 ^a	-30 ± 9 ^b	2 ± 15		

^a $p \leq 0.05$ or

^b $p \leq 0.01$ vs no addition control

Table 4

Incorporation of ¹⁴C from glucose or pyruvate into various lipid fractions in INS-1 cells

INS-1 832/13 cells were incubated in suspension in the presence of [2-¹⁴C]pyruvate or [U-¹⁴C]glucose for 30 min. The control condition was 1 mM glucose which is the only condition shown that does not stimulate insulin release from INS-1 832/13 cells. There was 1.5 mg cell protein per test tube. In our laboratory, the relative insulin release in INS-1 832/13 cells caused by 1 mM pyruvate, 5 mM pyruvate and 10 mM glucose is 10–20, 12–25 and 10–25 fold the 1 mM glucose control, respectively [12 and MJM, unpublished data]. Results are the mean ± SE of three replicate incubations for each condition. Incorporation of carbon into phosphatidyl serine was not detected. All values for 5 mM pyruvate and 10 mM glucose were significantly different from the 1 mM glucose control values with $p \leq 0.001$ to $p \leq 0.05$.

Fatty Acid Class	Secretagogues		
	1 mM Glucose	1 mM Pyruvate Pyruvate or Glucose Incorporated [pmol pyruvate or glucose/sample]	5 mM Pyruvate Pyruvate or Glucose Incorporated [pmol pyruvate or glucose/sample]
Cholesterol Ester	5.8 ± 0.3	12 ± 0.7	32 ± 0.4
Triglyceride	13.5 ± 0.6	28 ± 0.6	100 ± 3
Free Fatty Acids	5.4 ± 0.3	52 ± 2.3	210 ± 1.4
Diacylglyceride	3.5 ± 0.2	5.5 ± 0.1	20 ± 0.3
Phospholipids	84 ± 7.3	61 ± 0.10	200 ± 10
Phosphatidyl-choline	50 ± 3.4	45 ± 1.5	181 ± 4
Phosphatidyl-ethanolamine	2.3 ± 0.1	1.2 ± 0.1	6.2 ± 0.3
Phosphatidyl-inositol	4.5 ± 0.2	12 ± 0.7	46 ± 0.9
Sphingomyelin	11 ± 0.3	11 ± 4.8	18 ± 1.7