Fatty Acid Signaling in the β-Cell and Insulin Secretion

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Fatty acids (FAs) and other lipid molecules are important for many cellular functions, including vesicle exocytosis. For the pancreatic β-cell, while the presence of some FAs is essential for glucose-stimulated insulin secretion, FAs have enormous capacity to amplify glucose-stimulated insulin secretion, which is particularly operative in situations of β-cell compensation for insulin resistance. In this review, we propose that FAs do this via three interdependent processes, which we have assigned to a "trident model" of β-cell lipid signaling. The first two arms of the model implicate intracellular metabolism of FAs, whereas the third is related to membrane free fatty acid receptor (FFAR) activation. The first arm involves the AMP-activated protein kinase/malonyl-CoA/long-chain acyl-CoA (LC-CoA) signaling network in which glucose, together with other anaplerotic fuels, increases cytosolic malonyl-CoA, which inhibits FA partitioning into oxidation, thus increasing the availability of LC-CoA for signaling purposes. The second involves glucose-responsive triglyceride (TG)/free fatty acid (FFA) cycling. In this pathway, glucose promotes LC-CoA esterification to complex lipids such as TG and diacylglycerol, concomitant with glucose stimulation of lipolysis of the esterification products, with renewal of the intracellular FFA pool for reactivation to LC-CoA. The third arm involves FFA stimulation of the G-protein–coupled receptor GPR40/FFAR1, which results in enhancement of glucose-stimulated accumulation of cytosolic Ca^{2+} and consequently insulin secretion. It is possible that FFA released by the lipolysis arm of TG/FFA cycling is partly "secreted" and, via an autocrine/paracrine mechanism, is additive to exogenous FFAs in activating the FFAR1 pathway. Glucose-stimulated release of arachidonic acid from phospholipids by calcium-independent phospholipase A_2 and/or from TG/FFA cycling may also be involved. Improved knowledge of lipid signaling in the β-cell will allow a better understanding of the mechanisms of β-cell compensation and failure in diabetes. *Diabetes* 55 (Suppl. 2): S16–S23, 2006

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AA, arachidonic acid; AMPK, AMP-activated protein kinase; CPT, carnitine palmitoyl-transferase; DAG, diacylglycerol; FA, fatty acid; FFA, free fatty acid; FFAR, free fatty acid receptor; GSIS, glucose-stimulated insulin secretion; HSL, hormone-sensitive lipase; $iPLA_2$, calcium-independent ATP-stimulated phospholipase A_2; K_{ATP} channel, ATP-sensitive K^+ channel; LC-CoA, long-chain acyl-CoA; MCD, malonyl-CoA decarboxylase; RNAi, RNA interference; TG, triglyceride.

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Free fatty acids (FFAs) are important to the pancreatic β-cell for its normal function, its capacity to compensate for insulin resistance, and its failure in type 2 diabetes (1–3). Fatty acid (FA) deprivation of islet tissue causes loss of glucose-stimulated insulin secretion (GSIS), a process rapidly reversible by replacement with exogenous FFAs (4). In contrast, elevated FFA supply augments GSIS (5,6); however, if chronically in excess, particularly in association with elevated glucose (7), saturated FFAs can reduce insulin biosynthesis (8) and secretion (3) and induce β-cell apoptosis (2,3,7,9).

In this review, we consider the lipid signaling pathways involved in the FFA modulation of GSIS in healthy β-cells. By the term "lipid signaling," we refer to mechanisms by which lipid molecules, including FFA themselves, send messages to effector pathways in the cell, which in this case alter insulin vesicle exocytosis. While FFAs can signal directly via a recently discovered FFA receptor, GPR40, which is also known as free fatty acid receptor (FFAR)-1 (10,11), it is also evident that intracellular metabolism of FFAs resulting in the synthesis of lipid signaling molecules such as long-chain acyl-CoA (LC-CoA) (3) and diacylglycerol (DAG) (3,12) is important. As discussed, the latter involves glucose/nutrient regulation of LC-CoA partitioning within the β-cell via the AMP-activated protein kinase (AMPK)/malonyl-CoA signaling network (3,5,13). This network is linked to the promotion of both intracellular FA esterification (5) and lipolysis processes (14), which together form a pathway of triglyceride (TG)/FFA cycling in the β-cell. In addition to having an important role in nutrient-secretion coupling, TG/FFA cycling may have a key role in the prevention of β-cell failure in situations of nutrient excess by preventing β-cell steatosis.

The FA supply to the β-cell can be from exogenous sources such as plasma FFAs and lipoproteins or endogenous sources such as intracellular TG and phospholipid stores. It is worth noting that islet tissue expresses lipoprotein lipase (15,16). The islet, therefore, can access plasma TG as a source of FFAs, such that the FFA concentration in the immediate vicinity of β-cells is likely to be higher than that measured in plasma. The actual concentration of FFAs that β-cells are normally exposed to, however, is not known. The importance of regulated access to endogenous lipids for normal β-cell function is increasingly being realized, with particular interest currently being directed to the lipase enzymes that may be involved.

NUTRIENT-SECRETION COUPLING: TRIGGERING AND AMPLIFICATION PATHWAYS

Islet β-cell glucose metabolism is essential for the coupling of glucose sensing to insulin release (12,17,18). It is well accepted that its metabolism through pyruvate to

acetyl-CoA with subsequent mitochondrial oxidation increases the ATP/ADP ratio, which results in closure of ATP-sensitive K^+ (K_{ATP}) channels, depolarization of the plasma membrane, opening of voltage-dependent Ca^{2+} channels, and Ca^{2+} triggering of insulin granule exocytosis (12,19). This pathway, often termed the K_{ATP} channel–dependent pathway, is considered to be the major triggering pathway for GSIS (12,19) (Fig. 1). In addition, pyruvate from glucose can be channeled via pyruvate carboxylase into the anaplerosis pathway, which can affect insulin secretion by increasing the levels of metabolism-derived signaling molecules such as NADPH from the malate-pyruvate shuttle (20–22), citrate cataplerosis (21,23,24), glutamate (25), and lipid signaling molecules from the malonyl-CoA/LC-CoA pathway (3,5,26,27). These latter pathways can be considered to be amplification pathways (Fig. 1). Of particular interest in this review that focuses on lipid signaling is the role of the malonyl-CoA/LC-CoA pathway, which is considered in the next section.

MALONYL-COA/LC-COA PATHWAY OF LIPID SIGNALING

The malonyl-CoA/LC-CoA model of GSIS predicts that malonyl-CoA, derived from glucose metabolism via anaplerosis/cataplerosis (5,27,28), inhibits FA oxidation by allosteric inhibition of carnitine palmitoyl-transferase (CPT)-1 (29), thereby increasing the availability of LC-CoA for lipid signaling to cellular processes involved in exocytosis. In this model (Fig. 1), the effectiveness of malonyl-CoA to promote insulin release depends on both a cytosolic Ca^{2+} rise caused by the K_{ATP}-dependent pathway and the prevailing availability of FAs to the β-cell. Malonyl-CoA levels are also influenced by the activity of AMPK (13). AMPK senses cellular energy status and is activated by an increase in the AMP/ATP ratio brought on by fuel deprivation, fasting or exercise (13,30). AMPK activates cellular energy production (e.g., glucose oxidation and FA oxidation) and reduces energy consumption (e.g., FA synthesis and esterification) (13,30). Malonyl-CoA, on the other hand, is a "signal of plenty" and promotes nutrient storage, including FA esterification (3,5,13). AMPK phosphorylates acetyl-CoA carboxylase and malonyl-CoA decarboxylase, the enzymes that regulate malonyl-CoA synthesis and degradation, respectively, with the resultant effect of lowering malonyl-CoA (13,30). In addition to conditions of food deprivation and exercise, AMPK can be activated by adipokines, including adiponectin (31,32) and leptin (33), and pharmacological agents such as metformin and thiazolidinediones (33,34).

The evidence supportive of a role for malonyl-CoA/LC-CoA signaling in the regulation of insulin secretion is substantial. Malonyl-CoA levels have been documented to increase in response to glucose, and this precedes GSIS in β-cells (5,24,35,36). Elevated glucose causes inhibition of FA oxidation (5,37,38) and stimulates FA esterification processes (5,28,36). Studies that have used pharmacological and/or molecular biological tools to perturb this pathway have been mostly consistent with its importance (5,23,28,39,40), with few exceptions (41,42). The latter two studies overexpressed malonyl-CoA decarboxylase (MCD), the enzyme that lowers malonyl-CoA levels, and showed no effect on GSIS in islets or insulinoma cell lines. GSIS, however, was assessed mostly in the absence of FFAs in these studies (41,42). Considering that the malonyl-CoA/LC-CoA model proposes that malonyl-CoA signals via promoting the partitioning of FAs away from their oxidation into esterification pathways, we reexamined the effects of overexpression of MCD in the cytosol of INS832/13 β-cells and islets, but with particular focus on FFA augmentation of GSIS (5). Consistent with the earlier negative studies, MCD overexpression, despite causing a dramatic reduction in malonyl-CoA levels, had no effect on GSIS in the absence of FFAs (5). MCD expression, however, markedly diminished FFA augmentation of GSIS (5). Thus, in our opinion, the controversy raised by the above two studies (41,42) has been solved by showing that inhibiting malonyl-CoA signaling by MCD overexpression results in a reduction of GSIS only under conditions in which FFAs are present in the incubation medium (5), a more "physiological" situation. This finding is also completely in keeping with the proposed model (Fig. 1) that malonyl-CoA signals by altering FA partitioning.

Consistent with the view that inhibition of FA oxidation plays a key role in the regulation of insulin secretion are the following: *1*) islets from peroxisome proliferator–activated receptor-α–deficient mice show reduced fat oxidation and enhanced GSIS (43); *2*) overexpression of peroxisome proliferator–activated receptor-α in the β-cell causes reduction in GSIS (44); *3*) hyperinsulinemic hypoglycemia has been observed in subjects with short-chain L-3-hydroxyacyl-CoA dehydrogenase deficiency that causes impaired FA oxidation (45); *4*) the FA oxidation inhibitor 2-bromopalmitate restores GSIS in fasted rat islets in which fat oxidation is known to be increased (12); and *5*) overexpression of a malonyl-CoA–insensitive CPT-1 mutant in INS832/13 cells and rat islets, which predictably caused failure of glucose to inhibit FA oxidation, curtailed GSIS (39).

Another important finding from our study of cytosolic MCD overexpression (5) was that FFAs markedly amplified insulin secretion in response to all fuel and non-fuel stimuli and that this was dependent on a normal malonyl-CoA/LC-CoA signaling pathway. It should be underscored that in vitro islet experiments have traditionally been carried out in the absence of exogenous FFAs, a situation that may lead to a reduction or suppression of normal β-cell signaling processes, in particular those involving lipid signaling. Thus, we recommend that insulin secretion studies be performed in vitro with FFAs present.

TG/FFA CYCLING AND INSULIN SECRETION

In addition to FA oxidation and esterification, lipolysis is the third major pathway of intracellular FA partitioning. Lipolysis of intracellular TG refers to the hydrolytic removal of the fatty-acyl chains from the glycerol backbone by lipase enzymes. Of particular interest to this discussion on FA signaling is the recent finding that both FA esterification and lipolysis processes are glucose-responsive in the β-cell (Fig. 1). We recently demonstrated that glucose increased lipolysis, as determined by glycerol release, in islets from both wild-type and hormone-sensitive lipase (HSL) null mice (14). Another group similarly demonstrated glucose stimulation of lipolysis in isolated islets from rats (46). Furthermore, we recently showed that glucose-responsive FA esterification and lipolysis processes are increased by approximately threefold in islets of the nondiabetic severely insulin-resistant Zucker fatty rat, which, unlike the Zucker diabetic fatty rat, maintains normoglycemia by sustained β-cell compensation with insulin hypersecretion (1). These findings are consistent with the presence of glucose-responsive TG/FFA cycling in

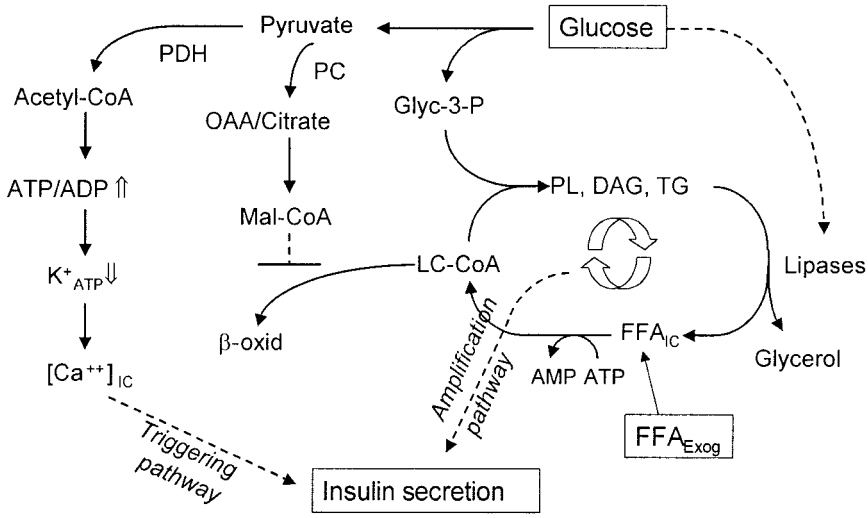

FIG. 1. Interactions between glucose and fatty acid metabolism in nutrient-secretion coupling. This model illustrates the synergistic interaction between glucose and FA metabolism and the generation of lipid signaling molecules that augment GSIS. Glucose gives rise to pyruvate, which, when channeled through pyruvate dehydrogenase (PDH), contributes to induction of insulin secretion via ATP production and the K_{ATP}-dependent triggering pathway. Pyruvate alternatively can be channeled via pyruvate carboxylase (PC) into the anaplerosis/cataplerosis pathway, which contributes to increases in cytosolic oxaloacetate (OAA) and citrate. Glucose increases malonyl-CoA (Mal-CoA), which blocks FA oxidation by inhibiting CPT-1. Inhibition of FA oxidation allows LC-CoA esters to accumulate in the cytosol. LC-CoAs are formed from FFAs supplied externally or produced by the lipolysis of endogenous TG. LC-CoAs can be esterified with glycerol-3-phosphate (Glyc-3-P) to form complex lipids such as TG, DAG, and phospholipids (PL). Glucose also activates lipolysis, which favors TG/FFA cycling from endogenous lipids. Exogenous FFAs (FFA_{Exog} contribute by amplifying the levels of cycle intermediates (LC-CoAs, TG, DAG, PL, and intracellular FFAs [FFA_{IC} themselves), all of which may be implicated in lipid signaling effector pathways. β-oxid, β-oxidation; $[Ca^{++}]_{IC}$, intracellular calcium.

pancreatic β-cells. To verify that TG/FFA cycling exists in insulin-secreting cells, we performed a series of radioactive tracer pulse-chase experiments in INS832/13 β-cells in which either the acyl chains or the glycerol backbone were labeled. During the chase period, label from the acyl moieties of TG fell at a substantially slower rate (41% in 20 min) than labeling of the glycerol moiety (60% in 20 min), consistent with acyl chain recycling (C.J.N., M.P., unpublished data).

The potential importance of this pathway in lipid signaling for insulin secretion comes from studies in which lipolysis is inhibited. GSIS is markedly curtailed in rat islets by inhibition of lipolysis by the potent inhibitors orlistat (46) and 3,5-dimethylpyrazole (47). We have also shown that FFA augmentation of GSIS in isolated Zucker fatty islets is reduced by 50% by orlistat (1). Unclear, however, is the mechanism by which TG/FFA cycling, including lipolysis, contributes to FFA amplification of GSIS. Elevated glucose, particularly in the presence of exogenous FFAs, will result in increased levels of all lipid moieties within the cycle, including LC-CoA, DAG, phospholipids, and FFAs (Fig. 1). Insulin vesicle exocytosis is a complex process involving many steps, including vesicle movement, docking, priming, and finally fusion with the plasma membrane (48). Some of these steps could be modulated by TG/FFA cycle intermediates acting as lipid signaling molecules. For example, LC-CoA can be used to acylate proteins, such as the synaptosomal-associated protein-25 (49) and synaptogamin (50), which can enhance their association with target membranes. Interestingly, LC-CoA has also been shown to activate lipases from islet tissue (51). As such, a mediator of glucose-induced lipolysis could be elevated LC-CoA themselves. DAG, the levels of which rise in the β-cell in response to glucose (36,52), not only activate protein kinase C, which is implicated in insulin secretion (53), but also bind to the C_1 domain of the synaptic vesicle priming protein Munc-13 (54), which has recently been shown to be important for normal insulin secretion (55,56). Thus, heterozygous Munc-13-$1^{+/-}$ mice show reduced GSIS, both in vivo and in isolated islets (56), such that Munc-13 is a prime candidate as an effector molecule in β-cell lipid signaling. TG/FFA will also affect membrane glycerophospholipid metabolism, which could influence secretion via alteration of membrane physicochemical properties (57). The glycerophospholipids may also have more direct effects (58), as is discussed below in the sections on fatty acid receptor signaling and phospholipase A_2. It may be that the TG/FFA cycle is a means of targeting the delivery of FFAs, and perhaps specific FFAs such as arachidonic acid (AA), to a particular subcellular site within the β-cell. Of relevance to this, HSL has been shown to be colocalized with insulin vesicles (59).

The key regulatory lipase enzyme(s) of the islet TG/FFA cycle is uncertain. The importance of HSL in the islet is unclear, such that there is current interest in searching for alternate lipases. HSL is expressed in the islet (60) and has been shown to be upregulated by persistently elevated glucose levels (61). Zucker fatty rat islets, which compensate for insulin resistance with insulin hypersecretion, also appear to have increased expression of HSL at the mRNA level, which correlates with the enhanced islet TG/FFA cycling in this model (1). Against a significant role for HSL, however, are studies in the HSL null mouse model (14,62). In one study, lipolysis and GSIS was not at all impaired in HSL null mice (62), suggesting that HSL is not important. In another study from our group, GSIS was shown to be considerably impaired, but only in fasted male mice; all female and fed male mice had normal GSIS (14). Furthermore, lipolysis could be still measured in these mice, and as stated above, glucose was shown to enhance it (14), a clear indication for alternate enzymes controlling β-cell lipolysis. These may include the newly described lipases that contain a patatin-like domain such as adipose tissue TG lipase (also called desnutrin) (63–65), adiponutrin

(66), and GS2 (67). Another possibility is carboxylesterase 3, also called TG hydrolase (68). These newly described lipases show much higher activity for TG rather than DAG (63,65,67,68) as compared with HSL, which primarily hydrolyzes DAG, monoacylglycerol, and cholesteryl esters (63,69). Clearly, more work is needed to delineate the roles of lipases in the β-cell.

Another unexpected finding in the studies of Zucker fatty rat islets and INS832/13 cells was that the flux of glucose carbons via the glycerol backbone of glycerolipids accounts for a substantial proportion of total glycolytic flux. At 16 mmol/l glucose, we estimated that 13 and 25% of the total glucose utilization by Zucker lean and Zucker fatty rat islets, respectively, could be accounted for by glycerol released into the media. Because islets do not contain glycerokinase (70), glycerol released from the lipolysis arm of TG/FFA cycling escapes the cell, since it cannot be reactivated to glycerol-3-phosphate. For INS832/13 cells incubated at 2 or 10 mmol/l glucose, 15 and 38%, respectively, of total glucose carbon flux was via this pathway (C.J.N., M.P., unpublished data). The TG/FFA cycle of β-cells, therefore, appears to be a major and hitherto overlooked pathway for both glucose and FFA metabolism.

There are two conceivable advantages for the β-cell to use glucose in this way. First, glucose usage via the TG/FFA cycle provides an alternative pathway by which glucose metabolism may be coupled to insulin secretion, which, via the provision of lipid-signaling molecules, allows amplification of the triggering pathway for exocytosis. The second is related to the preservation of β-cell mass in the face of fuel surfeit as occurs in obesity and diabetes. Thus, nutrient toxicity to cells occurs when the flux of metabolites through mitochondrial oxidation is high, since this causes the production of damaging superoxides and reactive oxygen species (71–73). Thus, glucose flux through TG/FFA cycling is a means of nutrient-secretion coupling that bypasses the need for oxidation to cause secretion and allows for detoxification of glucose carbons in the form of glycerol that is released from the β-cell, as discussed above. Furthermore, use of ATP by the cycle will reduce the mitochondrial membrane potential and, in this way, lessen the rate of reactive oxygen species production (74).

AA SIGNALING AND PHOSPHOLIPASE A_2

AA is a major acyl component of glycerolipids, constituting >30% of the glycerolipid fatty acid mass in rodent islets (75). Furthermore, glucose stimulation of β-cells is accompanied by release of free AA from glycerolipids, and inhibition of AA release impairs GSIS (75,76). The exact relationship between AA and insulin secretion, however, has been a matter of speculation. AA release depends on β-cell metabolism of glucose (76), and it has been shown that exogenous AA is capable of causing a rise in cytosolic Ca^{2+} (77). Importantly, AA is substrate for the synthesis of eicosanoid signaling molecules (78). Lysophosphatidic acids, produced in addition to AA by the action of phospholipase A isoforms on membrane phospholipids, also have signaling actions (78). Although it was thought that glucose-stimulated AA release is from hydrolysis of membrane phospholipids (75–79), another possibility is that it is from hydrolysis of TG within the TG/FFA cycling pathway. It is noteworthy that in many cell types (80), and in INS832/13 cells (M.S.R.M., M.P., unpublished data), externally added AA is incorporated into both TG and phospholipids. Also, AA is predominantly incorporated into TG first, whereas AA for phospholipid biosynthesis and remodeling of membrane phospholipids is from TG lipolysis and TG/FFA cycling (80–82).

Calcium-independent ATP-stimulated phospholipase A_2 ($iPLA_2$) isoenzymes have been implicated in glucose-stimulated AA release (76,77), since glucose activates AA release in the absence of Ca^{2+} (83), and the pharmacological $iPLA_{2\beta}$ inhibitor, bromoenol lactone suicide substrate, inhibits AA release and GSIS in vitro (76,77) and insulin secretion and glucose tolerance in vivo (77). While the effect of bromoenol lactone suicide substrate was taken as evidence that $iPLA_{2\beta}$ was the responsible enzyme increasing AA release, bromoenol lactone suicide substrate also has an inhibitory effect on desnutrin/adipose tissue TG lipase, adiponutrin, and GS2, which are able to hydrolyze TG (84). These newly described lipases have an $iPLA_2$ signature motif and are also known as $iPLA_{2\varepsilon}$, $iPLA_{2\eta}$, and $iPLA_{2\varsigma}$, respectively (84), but their activity on hydrolysis of phospholipid is 100- to 500-fold lower than that of their TG lipase activity (84). Furthermore, bromoenol lactone suicide substrate also inhibits phosphatidate phosphohydrolase (85), which is an important enzyme in TG synthesis. It therefore cannot be excluded that bromoenol lactone suicide substrate may act by inhibition of these alternative lipases and release of AA via TG/FFA cycling.

RNAi-knockdown of $iPLA_{2\beta}$ in INS1 cells resulted in decreased insulin secretion, but without effect on the content or composition of its glycerophospholipids (86). This again raises doubt on the role of $iPLA_2$-mediated phospholipid hydrolysis in the control of GSIS. The role of other types of phospholipase enzymes are also not clear. Overexpression of cytosolic $PLA_{2\alpha}$ has been shown to enhance insulin exocytosis from mouse β-cells, probably via the release of AA (87), but involvement of cytosolic $PLA_{2\alpha}$ in the regulation of insulin secretion has also been questioned (88,89). Excess production of AA by the β-cells may also be detrimental for normal secretory and metabolic functions (89). Clearly, further investigation into the roles of phospholipid, phospholipases, and AA signaling, including interactions between phospholipid and TG metabolism, is warranted.

FATTY ACIDS AND LIPID RECEPTOR SIGNALING

Recently, it was discovered that FFAs were the natural ligands for a small group of previously orphaned G-protein–coupled transmembrane receptors, including GPR40/FFAR1 (11). In one of the first reports, FFAR1 was shown to be highly expressed in rodent pancreatic β-cells (10), and its downregulation by RNAi caused impaired FA augmentation of insulin secretion (10). FFAR1 has also been documented to be highly expressed in human β-cells (90). Activation of FFAR1 by FFAs causes an increase in intracellular Ca^{2+} levels, which is believed to be via activation of the Gαq-phospholipase C pathway with release of Ca^{2+} from the endoplasmic reticulum (91). The capacity for FFAs to increase cytosolic Ca^{2+}, however, also depends on glucose activation of L-type Ca^{2+} channels and the presence of extracellular Ca^{2+} (91). FFAR1 activation, via Ca^{2+} release from the endoplasmic reticulum, may potentiate glucose-induced activity of L-type Ca^{2+} channels (91), with the end result of enhanced Ca^{2+} uptake from the extracellular Ca^{2+} pool, which then affects exocytosis. Studies in GPR40/FFAR1-deficient mice

confirmed a role for this receptor in FFA augmentation of insulin secretion (92). GPR40 deficiency protected the mice from the harmful metabolic effects of high-fat feeding (92). Conversely, overexpression of GPR40, led to liver steatosis, impairment of islet function, and diabetes (92). FFAR1, therefore, may be one of the links between fuel surfeit and β-cell failure in obesity-associated type 2 diabetes in genetically susceptible individuals (92). Whether FFAR1 plays a role in the β-cell compensation processes and the control of β-cell growth remains to be investigated. In relation to FFAR1 and cell growth, we recently showed that FFAR1/GPR40 mediates the action of oleate to promote proliferation of breast cancer cells in vitro (93).

Another G-protein–coupled transmembrane receptor that may have a role in lipid signaling in β-cells is GPR119. This G-protein–coupled transmembrane receptor is expressed particularly in the gastrointestinal tract and pancreas (94), including islet β-cells (58). Its natural ligand is oleoylethanolamide (94); however, lysophosphatidylcholine is also known to have some stimulatory activity (58), including enhancement of GSIS (58). The direct effects on islets of oleoylethanolamide, the true ligand of GPR119, have not been studied so far. Because the formation of oleoylethanolamide and related compounds is coupled to membrane phospholipid metabolism, it is likely that fuel stimuli that alter TG/FFA cycling also influence the levels of oleoylethanolamide, and this may have modulatory effects on insulin secretion.

REFINED MODEL OF LIPID SIGNALING IN NUTRIENT-SECRETION COUPLING

We now have a more advanced, but as yet incomplete understanding of lipid signaling in nutrient-secretion coupling for insulin secretion. FFAs are involved in amplification of GSIS that is triggered by the K_{ATP}-dependent pathway. This involves indirect signaling that requires intracellular FA metabolism, which implicates both the anaplerotic/malonyl-CoA pathway and intracellular TG/FFA cycling, as depicted in Fig. 1. The model needs to be extended, however, to include direct signaling via FFAR1/GPR40. We propose that lipid signaling in the β-cell involves three arms (trident model) that comprise signaling via glucose-derived malonyl-CoA, TG/FFA cycling including lipolysis, and FFAR1 (Fig. 2). In this view, signaling via the AMPK/malonyl-CoA/CPT-1 network and TG/FFA cycling are tightly linked processes. Glucose promotes activity in the TG/FFA cycle by elevating malonyl-CoA, which inhibits partitioning of LC-CoA to FA oxidation (via CPT-1 inhibition), such that LC-CoAs are more available for esterification processes. Glucose also provides the glycerol-3-phosphate necessary for FA esterification into complex lipids. Lastly, glucose activates lipase enzyme(s), possibly through a direct effect of LC-CoA on the activity of lipases (51), although confirmation of this and assessment of other potential mechanisms are needed. A reduction in AMPK activity promoted by fuel stimulation that would enhance β-cell lipolysis is an alternative possibility in view that AMPK activation has been associated with reduced lipolysis in adipocytes (95,96). While glucose, by activating lipolysis, promotes TG/FFA cycling from endogenous lipid stores, the provision of exogenous FFA supply to the β-cell will amplify the pathway, increasing the intracellular concentrations of the lipid signals LC-CoA, DAG, phospholipids, and unesterified FFAs.

This refined model (Figs. 1 and 2) reconciles a potential

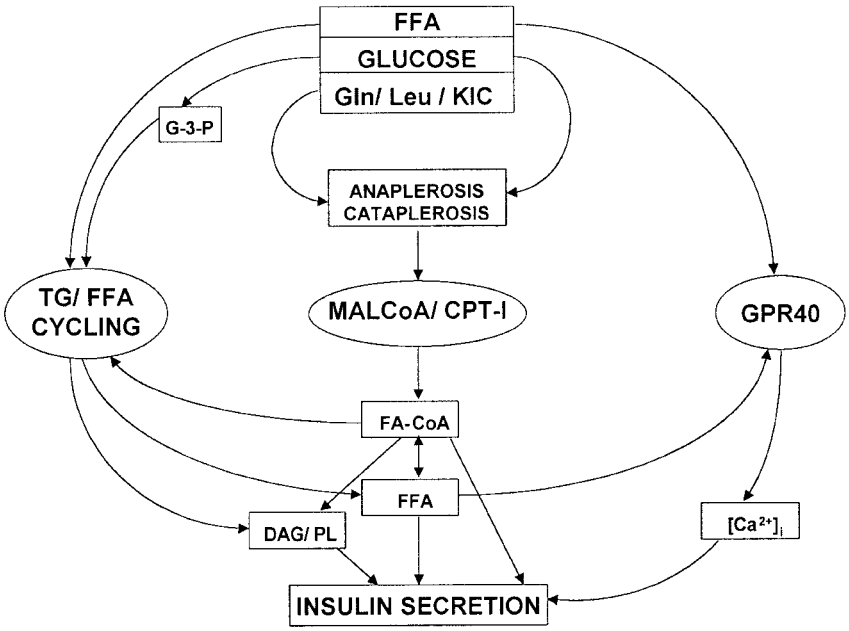

FIG. 2. The trident model of β-cell lipid signaling. Three interdependent arms of lipid signaling are proposed by which FFAs augment insulin secretion. First, glucose and other nutrient secretagogues such as glutamine (Gln), Leucine (Leu), and α-ketoisocaproate (KIC) contribute to anaplerosis, which allows cataplerotic efflux of citrate from mitochondria. This results in malonyl-CoA (MALCoA) formation, inhibition of CPT-1 activity, and FA oxidation, and accumulation of LC-CoAs that stimulate insulin secretion directly or by the formation of complex lipids such as DAG and various phospholipids (PL). The second arm involves glucose-responsive TG/FFA cycling, due to the effects of glucose to concomitantly promote FA esterification (provision of glycerol-3-phosphate [G-3-P] and Mal-CoA inhibition of FA oxidation) and lipolysis processes. This allows, particularly in the presence of exogenous FFAs, for the accumulation of cycle intermediates (LC-CoA, DAG, PL, and FFA) that have signaling roles. Third, exogenous FFAs activate the cell surface FA receptor, FFAR1/GPR40, which causes an increase in intracellular Ca^{2+}. FFAs formed from the hydrolysis of TG can cross the cell membrane and, together with exogenous FFAs, activate FFAR1. Thus, the "trident pathways" of lipid amplification intercommunicate and synergize to promote insulin secretion.

caveat of the malonyl-CoA model alone, namely that in some (97,98) (J. Lamontagne, M.P., unpublished data), but not all (38,44), studies, the FA oxidation inhibitors etomoxir and bromopalmitate did not promote insulin secretion. The explanation can be found in the interaction between the TG/FFA cycling and malonyl-CoA/CPT-1 pathways. Thus, lipid signaling molecules (FFA and DAG) released from endogenous TG stores by glucose-induced lipolysis can be sustained because FA oxidation is simultaneously inhibited by the rise in malonyl-CoA. In other words, if malonyl-CoA would not rise simultaneously with enhanced lipolysis, the released FFA and derived signals would be degraded via the CPT-1/FA oxidation pathway. An additional way to formulate this novel view that reconciles these contradictions in the literature (97,98) is to propose that the primary "on" signal of glucose, as far as lipid signaling is concerned, is enhanced lipolysis, whereas the rise in malonyl-CoA and CPT-1 inhibition mediate the suppression of an "off" signal (FA oxidation/degradation). Thus, inhibition of FA oxidation, per se, by agents such as etomoxir may not promote insulin secretion because they do not also activate lipolysis.

It should be underscored that TG/FFA and malonyl-CoA signaling are likely linked to FFAR1/GPR40 signaling, because FFAs released from lipolysis can be "secreted" from the β-cell and stimulate the cell surface receptor in an autocrine/paracrine manner (Fig. 2). In support of this, FFA release from islets in response to elevated glucose has been observed (99). While it seems likely that FFA signaling via FFAR1 involves increases in intracellular Ca^{2+}, the mechanisms by which TG/FFA cycling provides signals for insulin secretion are poorly understood and are likely to be multiple. As discussed above, they may involve protein acylation (3,100), the DAG receptor Munc-13 (56), and atypical C-kinase enzymes activated by LC-CoA (101). The lipase enzymes involved remain to be determined. It is essential that all aspects of lipid signaling in normal insulin secretion are solved such that the pathophysiological mechanisms of β-cell failure in obesity-associated type 2 diabetes can be more fully understood.

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