Gluco-lipotoxicity of the pancreatic beta cell

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INTRODUCTION

Type 2 diabetes is a heterogeneous syndrome of polygenic origin (DeFronzo 1999). Peripheral insulin resistance and absolute or relative insulin deficiency are the two cardinal metabolic alterations underlying type 2 diabetes. Although the respective roles of insulin resistance and insulin secretory defects in the etiology of type 2 diabetes are still debated, it is now generally accepted that both abnormalities must be present for the disease to occur. In addition, longitudinal studies in type 2 diabetic patients have clearly established that glucose homeostasis inexorably deteriorates after the onset of the disease, a phenomenon mostly due to an aggravation of the beta-cell defect (UKPDS 1995). This observation illustrates the role of beta-cell dysfunction in the pathogenesis of type 2 diabetes, both as an initial lesion and as an aggravating factor. Regarding the latter aspect, it has been postulated that the metabolic abnormalities associated with diabetes, particularly chronic hyperglycemia and hyperlipidemia, are harmful to the beta cell and thereby contribute to defective insulin secretion. These chronic, deleterious effects of hyperglycemia and hyperlipidemia on the beta cell are respectively referred to as glucotoxicity and lipotoxicity. Historically, these have often been considered distinct entities which both contribute to the beta-cell defect in an additive manner. The hypothesis proposed in this review is that glucotoxicity and lipotoxicity are in fact synergistic, and that lipotoxicity only exists in the context of chronic hyperglycemia (Poitout and Robertson 2002). The biochemical rationale underlying this hypothesis will be presented first, followed by a review of the experimental evidence supporting it.

THE METABOLISMS OF GLUCOSE AND LIPIDS IN THE BETA CELL ARE CLOSELY INTERRELATED

The biochemical basis underlying our hypothesis that lipotoxicity requires hyperglycemia derives from a model initially proposed by Prentki and Corkey in the context of stimulus-secretion coupling in the beta-cell (Prentki 1992), and illustrated in fig. 1. The main elements of this model, as it pertains to chronic situations, are as follows: When glucose concentrations are in the normal range, any excessive amount of extracellular fatty acids is readily oxidized in the mitochondria after transport of the activated form, long-chain fatty-acyl Coenzyme A (LC-CoA), across the mitochondrial membrane by the enzyme carnitine-palmitoyltransferase 1 (CPT1) (Poitout 2002). Physiologically, this phenomenon is important for the beta-cell to be able to switch to the utilization of fatty acids as a fuel when glucose levels are low, such as during fasting or starvation (Dobbins 1998). In contrast, when glucose and fatty acid concentrations are simultaneously elevated, glucose metabolism in the TCA cycle generates intermediates such as citrate, which can be exported out and produce acetyl CoA and malonyl CoA. Since in the beta cell the activity of the fatty acid synthase is low, malonyl CoA serves as a signaling molecule rather than a substrate for fatty acid synthesis. Malonyl CoA potently inhibits CPT-1, resulting in cytosolic accumulation of LC-CoA, which are then partitioned into alternate pathways, such as the esterification pathway or, when the fatty acid is a palmitate, ceramide synthesis (fig. 1). Activation of these cytosolic pathways then leads to the generation of lipid-derived signals which in turn impair beta-cell function in the presence of high glucose. This model, although not validated in all its aspects, is supported by a number of experimental observations. For example, glucose inhibits fatty acid oxidation in beta-cells (Segall 1999;
Further, high glucose increases the levels of malonyl CoA (Corkey 1989; Farfari 2000) and LC-CoA (Liang 1991; Prentki 1992). Finally, prolonged exposure of isolated islets to fatty acids leads, in the presence of high glucose only, to increased incorporation of fatty acids into neutral lipids (Briaud 2001). In addition, these metabolic effects are accompanied by coordinate changes in gene expression that contribute to increasing cytosolic levels of LC-CoA when both glucose and fatty acids are present. Thus, high glucose stimulates the expression of the genes for acetyl CoA synthase (Roche 1998), fatty acid synthase (Roche 1998), and hormone sensitive lipase (Winzell 2001), whereas it inhibits the expression of peroxisome proliferator-activated receptor α; (Roduit 2000) and the activity of adenosine monophosphate-activated kinase (Salt 1998; da Silva Xavier 2000), all of which result in decreased fatty acid oxidation and increased lipid synthesis.

Therefore, upon prolonged exposure of beta-cell to both glucose and fatty acids, LC-CoA accumulate in the cytosol and generate lipid-derived signals which, in our view, ultimately affect insulin secretion and gene expression. Because these signals are only generated when fatty acids and glucose are present simultaneously, one might argue that they represent mechanisms of glucotoxicity as much as mechanisms of lipotoxicity. Considering the tight relationship between glucose and lipid metabolisms in the beta-cell, it seems reasonable to group these phenomena under the single term glucolipotoxicity, or glucolipoxia, as originally coined by Prentki and Corkey (Prentki 1996).

**Figure 1: Effects of glucose on lipid metabolism in the beta cell.** In the presence of elevated glucose levels, the increase in cytosolic malonyl–CoA levels resulting from glucose metabolism inhibits the enzyme carnitine-palmitoyl transferase-1. Transport of long-chain fatty acyl-CoA in the mitochondria is reduced, and the esterification pathway is activated, leading to cytosolic accumulation of lipid-derived signaling molecules. When the fatty acid is palmitate, condensation of palmitoyl-CoA with L-serine gives rise to ceramide. CPT-1, carnitine-palmitoyl transferase-1; DG, diglyceride; FA, fatty acid; LC-CoA, long-chain fatty acyl-CoA; PA, phosphatidic acid; PL, phospholipid; TG, triglyceride. Adapted from (Poitout 2002).

**CHRONICALLY ELEVATED FATTY ACIDS ONLY AFFECT THE BETA-CELL IN THE PRESENCE OF HYPERGLYCEMIA**

From the functional standpoint, the effects of prolonged exposure to fatty acids are highly dependent on the concomitant glucose concentrations. Initial evidence for this notion was obtained in our laboratory by measuring insulin mRNA levels after 72-h of culture of isolated rat islets in the absence or presence of palmitate at low or high glucose (Jacqueminet 2000). We observed that palmitate inhibited insulin mRNA levels only if high glucose was present during the culture. To test this hypothesis in vivo, we used a hyperglycemic, normolipemic rat model, the Goto-Kakizaki (GK) rat. We reasoned that manipulation of plasma lipid levels in this model by high-fat feeding would allow us to investigate the effects of hyperlipemia in the context of hyperglycemia. To that end, we administered a high fat diet for 6 weeks to hyperglycemic GK rats, normoglycemic Wistar rats, or GK rats treated with insulin for the duration of the diet. We observed that high-fat feeding does impair glucose-induced insulin release in islets isolated from GK rats, but not from age-matched normoglycemic Wistar rats or GK rats treated with insulin (Briaud 2002). Similarly, Harmon et al. (Harmon 2001) used an obese, hyperlipidemic and markedly insulin resistant rodent model of type 2 diabetes, the Zucker Diabetic Fatty (ZDF) rat, to investigate the effects of normalizing blood glucose levels on insulin gene expression. They showed that normalization of blood glucose levels by phlorizin, but not that of lipid levels by bezafibrate, reduced islet triglyceride (TG) content and normalized insulin mRNA levels (Harmon 2001). Recently, El-Assaad et al. (El-Assaad 2003) sowed that fatty acids and glucose synergized to cause beta-cell death in insulin-secreting INS-1 cells and human beta-cells.

It appears therefore that prolonged exposure of beta-cells to simultaneously elevated fatty acids and glucose leads, on the one hand, to accumulation of LC-CoA and generation of lipid-derived cytosolic signals and, on the other hand, to deleterious, functional changes that are only observed when both fatty acids and glucose are elevated. That the latter functional changes are due to the former metabolic signals is supported by experimental evidence. For instance, the non-metabolizable analogue palmitate-methyl ester, or to the short-chain fatty...
acid octanoate which does not require CPT-1 for mitochondrial transport, fail to inhibit insulin gene expression (Briaud 2001). In addition, pharmacological blockade of fatty acid activation into LC-CoA prevents fatty-acid induced cell death (El-Assaad 2003). Therefore, it is likely that the mechanisms of gluco-lipotoxicity are mediated by lipid-derived, intracytosolic signals generated when both fatty acids and glucose are elevated. The results of several studies, summarized in the next section, provide some clues on the identity of such signals.

MOLECULAR MECHANISMS OF GLUCO-LIPOTOXICITY

As illustrated in fig. 1, there are essentially two potential fates for LC-CoA in the cytosol. Quantitatively, the most predominant one is the esterification pathway, which is activated in islets upon the concomitant presence of fatty acids and glucose (Berne 1975; Briaud 2001). Less quantitatively important, but qualitatively relevant, is de novo synthesis of ceramide from palmitate, which was initially shown to mediate fatty acid-induced beta-cell apoptosis in ZDF rats (Shimabukuro, Higa 1998; Shimabukuro, Zhou 1998). Recent studies in our laboratory have attempted to determine the respective role of these two pathways in the mechanisms of gluco-lipo-toxicity.

Our observation that TG levels were inversely correlated with the amount of insulin mRNA (Briaud 2001) prompted us to determine whether TG accumulation was a cause for beta-cell dysfunction. To address this question, we overexpressed the last enzyme of TG synthesis, diacylglycerol-acyltransferase-1 (DGAT), in isolated islets by means of an adenovirus (Kelpe 2002). We showed that DGAT overexpression effectively and selectively increased the flux of fatty acids towards TG synthesis, and that DGAT-overexpressing islets, when cultured in the presence of high glucose, showed impaired glucose-induced insulin secretion. These findings demonstrated that forcing TG synthesis impairs insulin release, although the mechanisms whereby this occurs remain unknown. One possibility is that excessive fuel supply is associated with mitochondrial uncoupling via increased expression of uncoupling protein-2 (UCP2). This in turn is expected to diminish insulin release in response to glucose, simply by virtue of a lower amount of ATP generated from glucose metabolism, and, thereby, decreased coupling between glucose utilization, energy production, and insulin secretion. Indeed, UCP2 expression is increased by high-fat feeding in various tissues (Fleury 1997; Surwit 1998), including islets (Briaud 2002). In vitro exposure of islets to fatty acids increases UCP2 expression (Lameloise 2001), and mice with a deletion of the UCP2 gene are hyperinsulinemic, hypoglycemic, and protected against diabetes (Zhang 2001). In addition, islets isolated from UCP2 knock-out mice are insensitive to the inhibitory effects of fatty acids on insulin secretion (Joseph 2002). Finally, UCP2 gene transcription is regulated by fatty acids in beta-cells (Medvedev 2002). Therefore, although this hypothesis is not unanimously accepted (Li 2002), evidence suggests that the simultaneous presence of fatty acids and glucose sends a “signal of plenty” to the beta-cell which, as a protective mechanisms against fuel oversupply, switches into “storage mode”, accumulates TG, and increases mitochondrial uncoupling via stimulated UCP2 expression. This protective mechanism has the undesirable effect of lowering the coupling efficiency between glucose metabolism and insulin secretion, resulting in impaired insulin release (fig. 2). Whether TG accumulation and mitochondrial uncoupling are mechanistically linked or are two independent phenomena remains to be determined, but the fact that forcing TG synthesis impairs insulin secretion (Kelpe 2002) and that exposure of islets to an analogue of diglyceride (our unpublished data) has a similar effect seems to argue in favor of UCP2 being downstream of TG.

Surprisingly, in islets overexpressing DGAT insulin secretion was impaired but insulin gene expression was unaffected (Kelpe 2002). This suggested that the mechanisms whereby fatty acids affect insulin secretion and gene expression might be distinct. To test this possibility, we examined whether ceramide generation, rather
than TG accumulation, could be responsible for decreased insulin gene expression. Indeed, we observed that prolonged exposure of isolated islets to elevated glucose and palmitate is associated with increased de novo synthesis of ceramide, and that blockade of ceramide generation prevents palmitate inhibition of insulin gene expression (Kelpe 2003). In addition, we demonstrated that palmitate inhibits insulin gene expression via a transcriptional mechanism rather than via modulation of insulin mRNA stability (Kelpe 2003). More recently, we observed that exposure of islets to exogenous ceramide impairs insulin gene expression but not insulin secretion and that conversely, only palmitate, but not oleate, decreases insulin gene expression, whereas both fatty acids impair insulin secretion (M. Ugas and V.P., unpublished observations). Since only palmitate can serve as a substrate for ceramide synthesis, these findings support the notion that ceramide synthesis impairs insulin gene expression but not insulin secretion (fig. 2). How ceramide inhibits insulin gene expression is currently unknown. An interesting hypothesis is that ceramide, as shown in other cells, decreases Akt/PI3K activity (Basu 1998; Summers 1998; Zhou 1998; Schmitz-Feiffer 1999), which may result in nuclear translocation of the transcription factor Foxo1 and, in turn, nuclear exclusion of PDX-1, resulting in decreased insulin gene transcription (Kitamura 2002). The fact that palmitate inhibits PDX-1 expression in islets (Gremlich 1997) supports this hypothesis, which is currently being tested in our laboratory.

CONCLUSION

It is clear that glucose toxicity can affect beta-cell function by mechanisms that are not related to lipid metabolism, namely the generation of oxidative stress (Tanaka 1999; Tanaka 2002). It is also clear from the data summarized above that excessive lipid levels only affect beta-cell function in the presence of concomitant hyperglycemia, and that generation of the lipid-derived, cytosolic signal which mediates lipotoxicity require the simultaneous presence of elevated glucose. In that sense, the term glucolipotoxicity is more appropriate to describe the phenomenon of fatty-acid impairment of beta-cell function. In the presence of elevated glucose, excessive fatty acids are partitioned into storage in the form of TG and, in the case of palmitate, generation of ceramide. We propose that TG accumulation in turn activates UCP2 and mitochondrial uncoupling, resulting in impaired insulin secretion in response to glucose. On the other hand, ceramide generation from palmitate inhibits insulin gene transcription.

These experimental observations have potential clinical implications. First, the notion that hyperlipidemia is only deleterious to the beta-cell in the presence of chronic hyperglycemia is consistent with the common clinical observation that many patients with dyslipidemia and/or obesity have normal beta-cell function. Second, they suggest that in the context of hyperglycemia, excessive lipid levels exert multiple effects at several key steps of the regulation of insulin production and secretion. Third, they emphasize the importance of achieving good metabolic control of glucose and lipid levels in patients with type 2 diabetes.

REFERENCES

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