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Minireview: Secondary β-Cell Failure in Type 2 Diabetes—A Convergence of Glucotoxicity and Lipotoxicity

VINCENT POITOUT AND R. PAUL ROBERTSON

Pacific Northwest Research Institute (V.P., R.P.R.), Seattle, Washington 98122; and Departments of Medicine (V.P., R.P.R.) and Pharmacology (R.P.R.), University of Washington, Seattle, Washington 98195

Chronic hyperglycemia and hyperlipidemia can exert deleterious effects on β-cell function, respectively referred to as glucotoxicity and lipotoxicity. Over time, both contribute to the progressive deterioration of glucose homeostasis characteristic of type 2 diabetes. The mechanisms of glucotoxicity involve several transcription factors and are, at least in part, mediated by generation of chronic oxidative stress. Lipotoxicity is probably mediated by accumulation of a cytosolic signal derived from the fatty acid esterification pathway. Our view that hyperglycemia is a prerequisite for lipotoxicity is supported by several recent studies performed in our laboratories. First, prolonged *in vitro* exposure of isolated islets to fatty acids decreases insulin gene expression in the presence of high glucose concentrations only, and glucose is rate-limiting for the incorporation of fatty acids into neutral lipids. Second, normalization of blood glucose in Zucker diabetic fatty rats prevents accumulation of triglycerides and impairment of insulin gene expression in islets, whereas normalization of plasma lipid levels is without effect. Third, high-fat feeding in Goto-Kakizaki rats significantly impairs glucose-induced insulin secretion *in vitro*, whereas a similar diet has no effect in normoglycemic animals. We propose that chronic hyperglycemia, independent of hyperlipidemia, is toxic for β-cell function, whereas chronic hyperlipidemia is deleterious only in the context of concomitant hyperglycemia. (*Endocrinology* 143: 339–342, 2002)

TYPE 2 DIABETES mellitus is a heterogeneous syndrome of polygenic origin and involves both defective insulin secretion and peripheral insulin resistance. β-Cell dysfunction is a *sine qua non* for the development of the disease, but the nature of the primary β-cell defect is still elusive. Once diabetes is established, chronic hyperglycemia and hyperlipidemia can exert deleterious effects on β-cell function, respectively referred to as glucotoxicity and lipotoxicity. Over time, both of these phenomena contribute to the progressive deterioration of glucose homeostasis characteristic of this disease. The purpose of this minireview is to present recent advances in the understanding of the interrelationships between glucotoxicity and lipotoxicity, and to propose the hypothesis that lipotoxicity occurs only in the presence of hyperglycemia, whereas glucotoxicity occurs independently of hyperlipidemia.

Glucotoxicity

Glucotoxicity, β-cell exhaustion, and glucose desensitization

Considerable evidence has been reported suggesting that chronic hyperglycemia impairs glucose-induced insulin secretion and insulin gene expression (reviewed in Ref. 1). Adverse effects of chronic hyperglycemia on β-cell function encompass three distinct phenomena: glucose desensitization, β-cell exhaustion, and glucotoxicity. Glucose desensitization refers to the rapid and reversible refractoriness of the β-cell exocytotic machinery that occurs after a short exposure to elevated glucose[1] and is a physiological adaptive mechanism that occurs even when insulin secretion is inhibited, thus differentiating it from β-cell exhaustion (2). β-Cell exhaustion refers to depletion of the readily releasable pool of intracellular insulin following prolonged exposure to a secretagogue (3, 4). In contrast, the term glucotoxicity describes the slow and progressively irreversible effects of chronic hyperglycemia on pancreatic β-cell function, which occur after prolonged exposure to elevated glucose. The fact that these associated β-cell defects are reversible up until a certain point in time and become irreversible thereafter suggests a continuum between β-cell exhaustion and glucotoxicity, the latter becoming predominant after prolonged exposure (5, 6). In addition to inducing functional changes, chronic hyperglycemia can also decrease β-cell mass by inducing apoptosis (7, 8).

Mechanisms of glucotoxicity

Impairment of insulin gene expression after prolonged exposure to elevated glucose levels is associated with diminished activity of two major β-cell transcription factors, pancreatic-duodenum homeobox-1 (9, 10) and the activator of the rat insulin promoter element 3b1 (11, 12). Increased expression of the insulin gene transcriptional repressor CCAAT/enhancer-binding protein β (13, 14) and of the proto-oncogene c-*myc* (15) have also been reported. The latter has been postulated to reflect a loss of differentiation of β-cells exposed to elevated glucose, which could explain, in

Abbreviations: FA, Fatty acids; GK, Goto-Kakizaki; LC-CoA, long-chain fatty acyl CoAs; NAC, *N*-acetyl-cysteine; TG, triglycerides; UCP-2, uncoupling protein-2; ZDF, Zucker diabetic fatty.

[1] "Elevated glucose" refers to concentrations above the physiological plasma levels of 5.6 mM, such as those measured in type 2 diabetic patients.

part, defective β-cell function (15). The biochemical mechanisms of glucotoxicity have been proposed to involve generation of chronic oxidative stress (16–19). In the insulin-secreting HIT-T15 cell, generation of reactive oxygen species in the presence of a reducing sugar (17) or chronic exposure to elevated glucose (18) leads to decreased transcription of the insulin gene, an effect prevented by the antioxidants aminoguanidine and *N*-acetyl-cysteine (NAC). Chronic exposure of isolated islets to elevated glucose levels *in vitro* leads to accumulation of advanced glycation end-products, impaired β-cell function, and apoptosis, all of which can be prevented by aminoguanidine and NAC (16, 20). Finally, treatment of Zucker diabetic fatty (ZDF) rats with aminoguanidine or NAC normalizes plasma glucose levels and restores insulin secretion, insulin content, and insulin mRNA levels (18). These findings firmly support the hypothesis that glucotoxicity is mediated, at least in part, by chronic oxidative stress.

Lipotoxicity

Chronically elevated fatty acids affect β-cell function

Similar to the paradoxically deleterious effects of chronic hyperglycemia, fatty acids (FA), which are essential β-cell fuels in the normal state, become toxic when chronically present in excessive levels. Prolonged exposure of pancreatic β-cells to FA increases basal insulin release but inhibits glucose-induced insulin secretion (reviewed in Ref. 21). In addition, FA inhibit insulin gene expression in the presence of elevated glucose levels (22–24), in part via negative regulation of the transcription factor pancreatic-duodenum homeobox-1 (22). Finally, excessive FA induce β-cell death by apoptosis both *in vitro* (25, 26) and in ZDF rat islets (7, 27).

Mechanisms of lipotoxicity

One central question in understanding the mechanisms of FA effects is whether they are due to increased FA oxidation and a resulting decrease in glucose oxidation, or to generation of a cytosolic signal via esterification of FA. We favor the view that one or several intermediate metabolites generated in the FA esterification pathway mediate deleterious effects of chronically elevated FA, mostly because prolonged exposure to FA is associated with profound alterations in lipid metabolism and minimal changes in glucose metabolism (28). The biochemical basis for this hypothesis was first proposed by Prentki and Corkey (29) and has been recently reviewed in detail (30). According to this model, the simultaneous presence of elevated glucose and FA results in accumulation of cytosolic citrate, the precursor of malonyl-CoA, which in turn inhibits carnitine-palmitoyl-tranferase-1, the enzyme responsible for transport of FA into the mitochondrion. Sustained inhibition of carnitine-palmitoyl-tranferase-1 results in cytosolic accumulation long-chain fatty acyl CoAs (LC-CoA), which are proposed to mediate the deleterious effects of chronically elevated FA (29). This model proposes that the glucose concentration plays a critical role in the effects of FA. Whether LC-CoA accumulation directly affects β-cell function, or whether it serves as a precursor for other active molecules such as diacylglycerols or phospholipids is not known. Similarly, the nature of the effectors downstream of lipid metabolite accumulation is unknown, although several candidates have been proposed, including the ATP-sensitive potassium channel, PKC, uncoupling protein-2 (UCP-2), direct effects on the exocytotic machinery, or modulation of gene expression (reviewed in Ref. 30).

Glucolipotoxicity: Glucose and FA Synergistically Harm the β-Cell

The "malonyl-CoA/LC-CoA hypothesis" proposed as a biochemical basis for lipotoxicity implies that the effects of FA are greatly influenced by the concomitant glucose concentration. If this hypothesis is correct, in the presence of physiological glucose concentrations elevated FA should be readily oxidized in the mitochondrion and should not harm the β-cell. In contrast, under circumstances where both FA and glucose are elevated, accumulation of metabolites derived from fatty acid esterification would inhibit glucose-induced insulin secretion and insulin gene expression. Recent studies performed in our laboratories support this hypothesis.

In vitro studies

First, we asked whether prolonged exposure of isolated islets to palmitate differentially affects insulin gene expression in the presence of low *vs.* high glucose concentrations (23). We showed that a 72-h culture in the presence of palmitate does not affect insulin content or insulin mRNA levels at low glucose, but these both significantly decrease in the presence of high glucose. Second, we sought to determine whether prolonged culture of islets with palmitate is associated with glucose-dependent incorporation of FA into neutral lipids (31). We found that glucose and palmitate have additive effects on FA metabolism upon prolonged exposure: glucose increases overall cellular lipid synthesis, whereas palmitate specifically directs lipid partitioning toward neutral lipid synthesis. As a result, palmitate-induced accumulation of intracellular triglycerides (TG) only occurs in the presence of high glucose. The glucose-dependent accumulation of neutral lipids was inversely correlated to insulin mRNA levels.

In vivo studies

To differentiate between hyperlipidemia and hyperglycemia as secondary metabolic forces leading to TG accumulation and defective insulin gene expression in ZDF rat islets, we treated ZDF animals between 6 and 12 wk of age with either the lipid-lowering drug bezafibrate or the blood glucose-lowering agent phlorizin (32). Neither treatment had an effect on body weight. As expected, phlorizin treatment prevented the rise in blood glucose levels between 6 and 12 wk without affecting lipid levels, whereas bezafibrate prevented the rise in plasma TG without affecting glucose levels. Islet TG content was decreased by phlorizin treatment, but not by bezafibrate treatment, suggesting that islet TG accumulation in this model requires hyperglycemia. Phlorizin, but not bezafibrate, prevented the decrease in insulin mRNA levels between 6 and 12 wk of age. Thus, we concluded from these

studies that antecedent hyperglycemia, not hyperlipidemia, is associated with increased islet TG content and decreased insulin gene mRNA levels in ZDF rats. To determine whether hyperlipidemia induced by high-fat feeding differentially affects β-cell function in normoglycemic *vs.* hyperglycemic rats, we administered a high-fat diet for 6 wk to either Goto-Kakizaki (GK) or age-matched Wistar rats (33). Six weeks of high-fat feeding did not affect glucose-stimulated insulin release in islets from Wistar rats but decreased the maximal response to glucose in islets from GK rats by approximately 50%. Administration of insulin during the 6 wk of diet normalized both blood glucose and plasma FA levels and completely prevented the decrease in GSIS in islets from high-fat-fed GK animals. The mechanisms of high-fat diet-induced impairment of insulin secretion did not seem to involve intraislet TG accumulation, because we were not able to detect any differences in TG levels in islets between either Wistar or GK rats fed a regular or high-fat diet. We observed, however, an increase in the expression of UCP-2 in islets from high-fat-fed GK rats, which was prevented by insulin treatment.

These results clearly support the hypothesis that hyperglycemia is required for lipotoxicity to occur. They are consistent with the clinical observation that the majority of hyperlipidemic individuals are not diabetic. That β-cell function is usually normal in patients with disorders of lipid metabolism suggests that obesity or dyslipidemia are not sufficient to cause β-cell dysfunction.

Conclusion

β-Cell failure in type 2 diabetes is an evolving process, which, regardless of the nature of the initial defect, gradually worsens over time. Chronically elevated blood glucose levels adversely affect insulin secretion. The mechanisms of glucotoxicity involve defective insulin gene expression and are at least partly mediated by chronic oxidative stress. Presented here is the view that chronically elevated FA levels do not harm the β-cell as long as blood glucose levels are normal, but profoundly affect β-cell function in the presence of concomitant hyperglycemia. Thus, glucotoxicity and lipotoxicity are closely interrelated, in the sense that lipotoxicity does not exist without chronic hyperglycemia. Furthermore, the effects of glucose on lipid metabolism are so profound that lipotoxicity can be viewed as one mechanism of glucotoxicity. Generation of reactive oxygen species may be an alternative mechanism of both glucotoxicity and lipotoxicity. Exposure of islets to palmitate induces generation of reactive oxygen species (34), and treatment of islets with metformin, which has antioxidant properties, protects from deleterious effects of FA (35). It is therefore conceivable that glucotoxicity and lipotoxicity interdependently converge toward the generation of damaging effectors on β-cell function.

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Address all correspondence and requests for reprints to: Vincent Poitout, D.V.M., Ph.D., Pacific Northwest Research Institute, 720 Broadway, Seattle, Washington 98122. E-mail: vpoitout@pnri.org.

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