Differentiating Glucose Toxicity From Glucose Desensitization: A New Message From the Insulin Gene

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Our perspective is that the concepts of glucose toxicity and glucose desensitization should be differentiated because they carry very different connotations. The term glucose desensitization most properly refers to a pharmacological event involving a temporary, readily induced, physiological and reversible state of cellular refractoriness because of repeated or prolonged exposure to high concentrations of glucose. The term glucose toxicity should be reserved for nonphysiological, irreversible alterations in cellular function caused by chronic exposure to high glucose concentrations. With regard to the pancreatic islet β-cell, the mechanism of action for glucose desensitization seems most likely to be expressed at the level of the insulin exocytotic apparatus or insulin stores within the β-cell, whereas the mechanism of action for glucose toxicity may be at the level of insulin gene transcription. This differentiation raises the possibility that exposure of patients to chronic hyperglycemia may cause glucose toxic effects on the process of insulin gene transcription and/or expression that are irreversible. If so, this may contribute to so-called secondary drug failure and, in any event, reemphasizes the need to intensify therapeutic efforts to better regulate glycemia in type II diabetes. Diabetes 43:1085-1089, 1994

Whether and how glucose directly causes damage to cellular function are questions that have fascinated diabetes researchers for decades. Because hyperglycemia is inevitably present in patients with diabetes, it seems reasonable to believe that it causes the complications of the disease. However, only rarely, if ever, have normal glucose levels been therapeutically achieved over the lifetime of a diabetic patient. Thus, there are no data in humans to test whether total avoidance of hyperglycemia totally prevents complications. Nonetheless, the results from the Diabetes Control and Complications Trial (DCCT) reinforce the position that glucose itself damages cell function. Certainly, the DCCT established irrefutably that control of hyperglycemia and other metabolic derangements of diabetes decreases progression of eye, nerve, kidney, and perhaps macrovascular complications (1).

If hyperglycemia is intrinsically harmful to some tissues, can it adversely affect the pancreatic islet? Specifically, can high circulating glucose levels cause deleterious effects on β-cell function? The consensus opinion is affirmative, which has given rise to terms such as glucose toxicity and glucose desensitization. Although these terms are used interchangeably, our perspective is that glucose toxicity and glucose desensitization should not be used synonymously because time of exposure to hyperglycemia is a critically differentiating variable. This differentiation is more than simply a semantic distinction. The adverse effects of glucose toxicity on β-cell function are inherently irreversible, whereas the effects of glucose desensitization are reversible upon restoration of normoglycemia. This distinction has clear clinical implications for the management of type II diabetes.

GLUCOSE TOXICITY VERSUS GLUCOSE DESENSITIZATION: THE DIMENSION OF TIME

The terms toxicity and desensitization connote two different notions. The concept of desensitization evolved from the world of pharmacology, whereas toxicity resides in the domain of toxicology. The former implies a temporary, readily induced, physiological, and reversible state of cellular refractoriness because of repeated exposure to an agonist. A common example is receptor downregulation caused by repeated exposure of a cell to a hormone; such downregulation is reversible when the cell is no longer exposed to high hormonal concentrations. The concept of toxicity conjures up a more sinister scenario. Toxicity implies cellular damage that often is irreversible, is in no sense of the word physiological, and may have a more insidious onset.

In recent reviews (2-6), glucose has been described as having both desensitizing and toxic effects. Unfortunately, these terms are often used interchangeably. In this context, several interesting articles have been published since the turn of the decade that deserve comment. Sako and Grill (7) sought to discover whether glucose desensitization is caused by chronic hyperglycemia itself or if it is a nonspecific consequence of continuous stimulation of insulin secretion. They made nondiabetic rats hyperglycemic by infusing glucose intravenously for 48 h and then removed the pancreas for studies of insulin secretion using a perfusion method. Some animals received diazoxide to inhibit insulin secretion during the 48-h intravenous glucose infusion. They observed that pancreas taken from animals receiving the 48-h glucose infusion had markedly impaired insulin secretion during

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DCCT, Diabetes Control and Complications Trial; IBMX, 3-isobutyl-1-methylxanthine; IGF, insulin upstream factor; CAT, chloramphenicol acetyltransferase; GSTF, glucose-sensitive transcription factor.
Differeniatng Glucose Toxicity

subsequent perfusion. However, inhibition of insulin secretion by diazoxide treatment during the 48-h glucose infusion not only prevented the deleterious effects but actually augmented insulin secretion seen during perfusion. The authors concluded that glucose desensitization of the β-cell is not due to exposure of the islet to high concentrations of glucose molecules.

Kaiser et al. (8) used cultures of adult rat islets to examine this issue over a longer period of time and came to the same conclusion. They cultured rat islets in the presence of 11.1, 16.7, or 33.3 mM glucose over several weeks and then studied glucose-induced insulin secretion in static incubations for 1 h. They observed that insulin secretion gradually declined as a function of the duration of chronic glucose treatment and that this decline appeared earlier when higher glucose concentrations were used in the culture media. Subsequently, this β-cell defect was reversible when the glucose concentration in the culture media was reduced. They also tested whether the cause of decreased β-cell responsiveness was specific to glucose or more generally related to the high rate of insulin secretion. They did this by comparing results from cultures exposed to either 33 mM glucose or 11.1 mM glucose plus 0.1 mM isobutylmethylxanthine, 3-isobutyl-1-methylxanthine (IBMX), which stimulated insulin secretion more than 11.1 mM glucose alone. Insulin secretion was markedly reduced after 1-week exposure to either the high glucose concentration or the lower glucose concentration plus IBMX. Therefore, they concluded that the defect was not a glucose-specific phenomenon and argued that the adverse effect of a high glucose concentration was a result of prolonged secretion and generalized exhaustion of the islet.

However, Davalli et al. (9) came to a different conclusion. They examined this issue in human islets cultured in 5.5 or 16.7 mM glucose for 48 h. During subsequent perfusion experiments, they observed that islets cultured in the medium containing the higher glucose concentration developed defective glucose-induced insulin responses, but no abnormalities in arginine-induced responses. They used these data to argue against β-cell exhaustion as an explanation for glucose desensitization. Interestingly, these experiments are reminiscent of earlier ones in subjects with type II diabetes published by Vague and Moulin (10), who reported that normalization of hyperglycemia by a 20-h insulin infusion restored glucose-induced, but did not augment tolbutamide-induced, first-phase insulin secretion. In other human islet experiments, Eizirik et al. (11) used culture medium containing 5.6, 11, or 28 mM glucose for 7 days to evaluate the adverse effects of exposure to high concentrations of glucose over a longer period of time. They observed less insulin content and defective glucose-induced insulin secretion when the high glucose concentrations were used. The authors also found these changes to be partially reversible upon subsequent culturing in a lower glucose concentration, but they did not assess whether these findings were specifically related to glucose or to general β-cell exhaustion.

These recent publications provide important amplifications of previous reports that exposure of the islet to abnormally high concentrations of glucose can lead to defective glucose-induced insulin secretion. Because all of these experiments were conducted over periods not exceeding 2 weeks, and because some of the studies demonstrated reversibility of defective insulin secretion upon subsequent exposure of islets to a lower glucose concentration, the phenomenon focused on by these studies is properly referred to as glucose desensitization of the β-cell. To a certain extent, some of these observations may have reflected decreased insulin stores or β-cell exhaustion. Nonetheless, the term glucose toxicity is not appropriate because the defects occurred over a short period of time and were reversible. Thus, the central question about the mechanism of glucose toxicity on the β-cell is not answered by these experiments, and, at a clinical level, we are left to wonder what might be the mechanism for toxic effects of glucose in humans with type II diabetes whose pancreatic β-cells have been exposed to abnormally high concentrations of glucose for a matter of years.

PARADOXICAL DYSREGULATION OF THE INSULIN GENE BY GLUCOSE

An essential ingredient of successful laboratory models for examining glucose toxicity is that they must involve exposure of pancreatic islets to varying concentrations of glucose for lengthy periods of time. Laboratory animals and primary islet cultures are valuable to examine the adverse effects of high concentrations of glucose for short periods of time only. It is neither practical to intravenously infuse animals with glucose over many months nor possible to reproducibly sustain primary islet cultures for this period of time.

However, several lines of pancreatic islet β-cells are now available that can be passaged for periods in excess of 1 year. We have used one of these cell lines, the HIT-T15 cell, which was derived by simian virus 40 transformation of Syrian hamster pancreatic islets (12), to examine the adverse effects of prolonged culturing in media containing high glucose concentrations (13,14). In agreement with others (12,15), we observed that HIT cells gradually lose their ability to secrete insulin over 6 months of culture in media containing 11.1 mM glucose during which they were split and passed weekly. This loss of insulin secretion was associated with marked decreases in insulin content and insulin mRNA levels. This led us to consider whether the evolution of this defect in insulin secretion in HIT cells was because of alterations in insulin gene transcription. To this end, we designed studies of insulin gene transcription using a reporter gene driven by the human insulin enhancer-promoter.

According to Docherty and colleagues (16,17), the enhancer-promoter region of the human insulin gene is comprised of positive and negative regulatory elements (Fig. 1). Several of the nuclear proteins that bind and activate these regulatory elements have been characterized. These transcriptional factors include IUF-1 (insulin upstream factor-1), a β-cell-specific nuclear protein that binds to a region that includes the critical sequence TAAT, which is essential for its regulatory activity on the insulin gene. IUF-1 binds to three separate but identical elements of the enhancer-promoter region of the human insulin gene that are termed CT1, CT2, and CT3.

The primary strategy we used was to establish whether insulin gene transcription became defective in HIT cells cultured in a high glucose concentration and, if so, to ascertain whether decrements in IUF-1 binding occurred. We passed HIT cells weekly over 25 weeks under identical conditions except that one set of cells was grown in media containing 11.1 mM glucose, while the other was grown in 0.8 mM glucose. HIT cells grown in the high glucose concentration lost glucose-induced insulin secretion and had no mea-
was present in older HIT cells grown under conditions of high glucose as well as older HIT cells from passage 130 grown in high glucose. Furthermore, we speculated that GSTF and IUF-1 might be closely related, if not identical. IUF-1 might be closely related, if not identical. A: the IUF-1 gene produces IUF-1 that activates the CT regions of the insulin gene. B: prolonged exposure to abnormally high concentrations of glucose results in glucosylation of the IUF-1 gene and/or IUF-1 itself, thereby preventing IUF-1 interaction with the regulatory elements. Alternatively, glucosylation could also inactivate the three CT regulatory elements themselves.

FIG. 2. Proposed mechanism of glucose toxicity expressed at the level of insulin gene transcription. A: absence of GSTF will lead to compromised insulin gene transcription and is thus a likely explanation for the decline of insulin mRNA levels, insulin content, and glucose-induced insulin secretion in HIT cells cultured for a long period of time under conditions of high glucose. We have termed this phenomenon "paradoxical dysregulation" of insulin gene transcription (14) because under physiological circumstances glucose serves to increase insulin gene transcription.

To assess binding of nuclear proteins to the enhancer-promoter region, we performed electrophoretic mobility shift analysis. An oligonucleotide corresponding to the 30-base pair region containing the CT element was synthesized and radiolabeled. Gel shift analysis was performed using nuclear extracts from HIT cells at passage 70 grown in high glucose. No differences were found in population doubling times, insulin gene restriction fragment length polymorphism, or GLUT2 levels (13), which argued against changes in the rate of cellular aging, insulin gene mutations, and disappearance of glucose transporters as alternate explanations for losses in insulin gene transcription in cells grown under conditions of high glucose.

The mechanism through which chronic exposure of the insulin gene to high glucose concentrations might lead to glucose toxicity has yet to be defined. One candidate is glucosylation of the insulin gene, IUF-1, or the IUF-1 gene (Fig. 2). This possibility also extends to other genes and their transcriptional products that are critical for normal insulin gene transcription. Hence, the new message transmitted by the insulin gene during our experiments is that long-term exposure of the β-cell to high concentrations of glucose may result in toxic effects at the level of insulin gene transcription. This would lead, in turn, to decreased insulin synthesis, content, and secretion that would be irreversible. In support of this concept, it is important to point out that the adverse effects on insulin gene transcription we observed in HIT cells chronically cultured in a high glucose concentration were not reversed by returning these cells to low glucose concentrations for an additional 25 passages (unpublished data, R.P.R., L.K.O., and H.-J.Z.).

**THERAPEUTIC IMPLICATIONS FOR TYPE II DIABETES**

Most patients with type II diabetes are exposed to mild hyperglycemia for many years before they are clinically
type II diabetes is due to glucose toxicity, glucose desensitization, or a combination of both, these considerations in combination with the recent experience reported by the DCCT argue strongly for reexamination of our therapeutic attitude toward control of glycemia in type II diabetes. In a sense, we are at the same point with type II diabetes that we were with type I diabetes before the DCCT. Skepticism that the results of the DCCT apply across the board to type II diabetes is justified and points out the need to demonstrate that control of glycemia in type II diabetes will have the same beneficial consequences. Nonetheless, until such trials are completed, it appears prudent to intensify our efforts to better control glycemia in patients with type II diabetes so that they do not become glucose toxic.

**DISCUSSION**

This perspective examined the thesis that glucose toxicity is not the same as glucose desensitization and that this is more than a mere semantic differentiation. Glucose desensitization properly refers to a reversible consequence of short-term exposure of the islet to high glucose levels that results in defective β-cell function. The mechanism of action of glucose desensitization is unknown, but seems likely to be expressed at the level of the insulin exocytotic apparatus or insulin stores within the β-cell (Fig. 3). Glucose toxicity properly refers to long-term exposure of the β-cell to high concentrations of glucose that results in irreversible changes in β-cell function. We suggest that one prominent site of action for glucose toxic effects may be at the level of insulin gene transcription (Fig. 3). These deleterious effects probably involve interference with the production or the action of GLUTF or IUF-1 and perhaps other nuclear factors necessary for promoting insulin gene transcription.

**ACKNOWLEDGMENTS**

This work was supported by National Institutes of Health Grants R01-DK-38325 and 5F32-DK-08742.

We thank Elizabeth Oseid and Laurie Pohlman for superb technical assistance, Dr. Howard Towle for invaluable advice, and Lucy Mittag for excellent manuscript preparation.

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