β-Cell Dysfunction Induced by Chronic Hyperglycemia

Current Ideas on Mechanism of Impaired Glucose-Induced Insulin Secretion

JOHN L. LEAHY, MD
SUSAN BONNER-WEIR, PHD
GORDON C. WEIR, MD

Non-insulin-dependent diabetes mellitus is characterized by abnormal β-cell function. The characteristic secretory defect is a selective loss of glucose-induced insulin secretion. Substantial data have been generated in animal models to support the concept that chronic hyperglycemia causes the loss of glucorecognition (the so-called glucose toxicity hypothesis). This review summarizes the data supporting the concept of hyperglycemia-induced β-cell dysfunction and then focuses on the ideas for the mechanism of the glucose unresponsiveness. The lack of access to islet tissue in humans means that these studies have all been conducted in animal models. Another major stumbling block continues to be the lack of in vitro systems that faithfully reproduce the secretory abnormalities that occur in vivo. Despite these limitations, many hypotheses are being investigated that span most of the major intracellular steps for glucose-induced insulin secretion, including abnormalities in glucose transport, storage, metabolism/oxidation, and the second messengers. No single hypothesis stands out as being able to explain all of the characteristics of the secretory abnormalities. In the last few years major advances have occurred in our knowledge about the events that normally cause glucose-induced insulin secretion. Similarly, biochemical and molecular tools have become available to probe the different steps. As better in vitro models of the selective glucose unresponsiveness become available, rapid progress can be expected in unraveling the biochemical basis for the loss of glucose responsiveness in diabetic rat models. The long-term hope is that this information will lead to innovative new strategies for the therapy of non-insulin-dependent diabetes mellitus.

The concept that has emerged over the last decade for the pathogenesis of non-insulin-dependent diabetes mellitus (NIDDM) is that genetic factors are combined with acquired defects in insulin secretion and insulin action to give the full-blown phenotypic picture (1; Fig. 1). The genetic factors are poorly understood. Our understanding is that they somehow confer susceptibility to glucose intolerance. When genetic susceptibility is present, the risk that glucose intolerance will develop depends on the myriad of nongenetic (environmental) factors that influence the balance between insulin secretory capacity and the tissue insulin effectiveness. This balance explains the increased risk for NIDDM seen with factors that reduce insulin sensitivity, such as obesity, age, and physical inactivity. Once mild hyperglycemia occurs, the acquired defects in insulin secretion and insulin action appear. A key finding with respect to the acquired defects is that they disappear, at least in part, after a period of tight metabolic control; this has led to the concept that they result from a metabolic change induced by the diabetes. An hypothesis that has received considerable attention is that chronic hyperglycemia is the factor causing the tissue dysfunction, the so-called glucose toxicity hypothesis.

β-Cell function in NIDDM has been the subject of intense investigation for several decades, and we have recently published an extensive review of this topic (2). Therefore, it is not the purpose of this article to review the natural history of β-cell dysfunction in NIDDM. Rather, we focus on one aspect: the concepts concerning the deleterious effects of chronic hyperglycemia on the β-cell. Although they are most relevant to NIDDM, the principles discussed also pertain to the early stages of insulin-dependent diabetes mellitus (IDDM) and to islet transplantation.

β-CELL SECRETORY ABNORMALITIES IN NIDDM

— The characteristic insulin secretory defect linked to chronic hyperglycemia is the loss of glucose-induced insulin secretion (2). The plasma glucose concentration is usually the main regulator of insulin secretion. A rapid rise in glucose level elicits a biphasic insulin response, with the first phase lasting 5–10 min and the second phase persisting for the duration of the hyperglycemia (3). It was first demonstrated in the late

FROM THE RESEARCH DIVISION, JOSLIN DIABETES CENTER; THE DEPARTMENTS OF MEDICINE, BRIGHAM AND WOMEN'S HOSPITAL AND NEW ENGLAND DEACONESS HOSPITAL; AND HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO JACK LEAHY, MD, JOSLIN DIABETES CENTER, 1 JOSLIN PLACE, BOSTON, MA 02215.

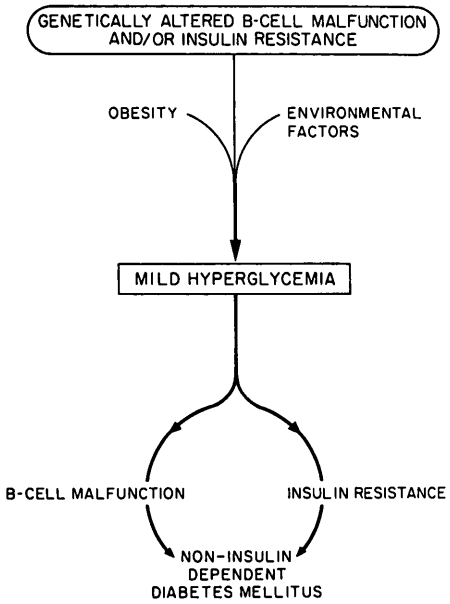

Figure 1—*Schematic representation of the events that culminate in non-insulin-dependent diabetes mellitus. Sequence is hypothetical and is based on data obtained from animal models, studies conducted in humans, and our own best guesses. Depicted events start with a genetic predisposition for glucose intolerance that could represent several different abnormalities in the β-cell or the insulin-sensitive tissues. Risk of decompensating to glucose intolerance then depends on balance between insulin secretory capacity and tissue insulin needs. Factors that act on either of these will also influence the risk for diabetes. Onset of mild hyperglycemia results in further deterioration of insulin secretion and insulin sensitivity. Net result is a vicious cycle that may culminate in the complete clinical picture of non-insulin-dependent diabetes mellitus.*

1960s that patients with NIDDM had a delayed insulin response to an intravenous glucose challenge (4,5). This delay represented a disappearance of the first phase (Fig. 2). The first phase is believed to serve an important physiologic function to maximize glucose clearance (6). As such, its loss accounts for part of the exaggerated postprandial rise in plasma glucose that occurs in NIDDM (7). The characteristics of this secretory defect have been extensively studied over the last two decades. These characteristics include the following: *1*) the first phase is relatively intact in nondiabetic people destined to develop NIDDM (8,9); *2*) in contrast, a first phase is no longer present when the fasting glucose level reaches 6.4 mM (10); *3*) tight metabolic control partially restores the first phase (11,12); and *4*) insulin responses to secretagogues other than glucose are relatively unimpaired in people with NIDDM (2,13; Fig. 2). Therefore, the missing early release of insulin to glucose in NIDDM is an acquired defect, it is specific for glucose, and the timing of its development coincides with the onset of mild hyperglycemia. An area of active research during the last decade has been the attempt to identify why the β-cell becomes refractory to glucose. Our research has focused on chronic hyperglycemia as being the toxic factor, and this review summarizes the substantial body of experimental data that support this idea. However, it should not be inferred by the lack of discussion of other possibilities that all other hypotheses have been excluded. For example, a long-standing idea is that overactivity of endogenous substances such as opioids and/or prostaglandins is important (14,15).

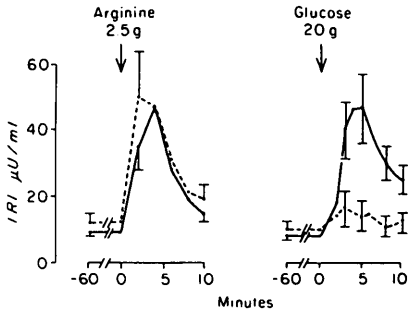

Figure 2—*Plasma insulin responses (IRI) to intravenous boluses of arginine (2.5 g) and glucose (20 g) in non-insulin-dependent diabetic (NIDDM; dashed line) and nondiabetic control (solid line) subjects. Note that glucose is without effect in NIDDM compared with arginine, where the insulin responses are brisk in both groups. From Palmer et al. (13). © by the Journal of Clinical Investigation.*

Several other insulin secretion abnormalities occur in NIDDM, including a reduced slope of glucose potentiation, disruption of the pulsatile release, and a raised plasma ratio of proinsulin to insulin, which may reflect a relative hypersecretion of proinsulin. The clinical characteristics of these defects have been described in detail (2,16). A fundamental question concerns the relationship of these β-cell abnormalities to the loss of glucose-induced insulin secretion. Is there a single intracellular defect in the β-cell that has multiple manifestations? Alternatively, are there multiple independent defects? The question most germane to this discussion is whether any of these other defects are acquired on the basis of chronic hyperglycemia. Relatively little information on this point is available with respect to the reduced slope of glucose potentiation and the disrupted pulsatile insulin release. Both defects appear to occur early in the course of diabetes, perhaps even predating the onset of hyperglycemia (16,17). Moreover, it is unclear whether they are reversed during improved metabolic control. The data for the raised plasma ratio of proinsulin to insulin more strongly suggest a link to chronic hyperglycemia. Multiple studies have shown a raised proinsulin/insulin ratio in patients with NIDDM (18). Cross-sectional data are consistent with the rise occurring after the onset of mild hyperglycemia (19–21). In addition, patients who become hyperglycemic from cystic fibrosis have a raised proinsulin-insulin ratio compared with cystic fibrosis patients who are not diabetic (22). Finally, the limited data available have shown normalization of the proinsulin-insulin ratio in NIDDM when euglycemia is restored by diet or sulfonylureas (23,24). These findings suggest a link between hyperglycemia and a raised proinsulin-insulin ratio. However, acceptance of this hypothesis first requires explanation of another observation: why the proinsulin-insulin ra-

tio is raised in normoglycemic identical twins of patients with IDDM (25,26).

INSULIN SECRETION IN HYPERGLYCEMIC ANIMAL MODELS

— Current studies that have searched for the metabolic factor responsible for the β-cell dysfunction have focused primarily on chronic hyperglycemia. The idea is not a new one. Classic studies from the 1940s showed that glucose injections caused permanent diabetes in partially pancreatectomized cats (27). Another study reproduced the same findings with glucose infusions in partially pancreatectomized dogs (28). The hypothesis is that chronically exposing a normal β-cell to an elevated plasma glucose level will make that cell become nonresponsive to glucose. Direct testing of this hypothesis in nondiabetic humans has been hindered by the lack of access to islet tissue and the difficulty of producing experimental hyperglycemia. Instead, like most investigators, we have used animal models to determine whether sustained hyperglycemia has deleterious effects on β-cells.

Impaired glucose-induced insulin secretion

Our initial studies caused hyperglycemia in normal rats by reducing the β-cell mass. Rats were given streptozocin in the neonatal period (29), or they underwent a 90% pancreatectomy at 5 wk of age (30). Both models developed modest nonfasting hyperglycemia. Insulin secretion studies performed on the remaining β-cells showed the typical pattern of selective glucose unresponsiveness: Glucose failed to induce a rise in insulin output, compared with other secretagogues where insulin responses were present, and at times were abnormally large (2,31; Fig. 3). To show that hyperglycemia was responsible for the glucose unresponsiveness, normal rats were made hyperglycemic with intravenous glucose infusions. Selective β-cell glucose unresponsiveness was again found (32). In this model hyperglycemia was

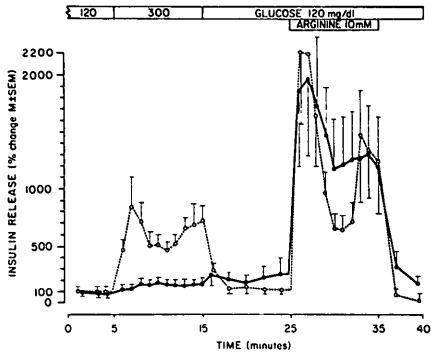

Figure 3—*Insulin secretion in response to glucose and to arginine in rats 8–11 wk after a 90% pancreatectomy (solid line; n = 9). Data were obtained with the in vitro perfused pancreas technique. Note that there is a brisk response to arginine in these rats, whereas glucose is without effect—the classic picture of selective glucose unresponsiveness. As expected, sham-operated control rats (dashed line; n = 7) show clear responses to both secretagogues. From Bonner-Weir et al. (30). © by the Journal of Clinical Investigation.*

accompanied by a raised plasma insulin level compared with most other diabetic models, including the two previously described, which are relatively or absolutely hypoinsulinemic. Finding the same secretory defect in the infused rats therefore indicated that hyperglycemia, not insulin deprivation, was the key pathogenic factor.

In addition to the studies cited above, several other genetic and experimental diabetic models have been studied by investigators around the world. All of these models share the same secretory defect: selective glucose unresponsiveness. Moreover, many have specific controls that underscore the key role that hyperglycemia plays. For instance, male Zucker *fa/fa* rats are hyperglycemic and develop glucose unresponsiveness. In contrast, genetically identical female Zucker *fa/fa* rats are neither hyperglycemic nor glucose unresponsive (33). Hyperglycemic corpulent rats (SHR/N-cp) are glucose unresponsive; normoglycemic corpulent rats (LA/N-cp) are not (34). A 60% pancreatectomy in rats does not cause hyperglycemia, and these rats retain normal glucose-induced insulin secretion. In contrast, the addition of sucrose to their water supply causes mild hyperglycemia and the emergence of β-cell glucose unresponsiveness (35). Viewed together, these studies clearly show that the presence of hyperglycemia is always accompanied by selective glucose unresponsiveness and that hyperglycemia is a prerequisite for that secretory defect.

Recovery of glucose-induced insulin secretion

Additional support for the concept of hyperglycemia-induced β-cell glucose unresponsiveness has come from studies that used phlorizin in these rat models. Phlorizin renders diabetic rats euglycemic by inhibiting kidney tubule glucose reabsorption (36). A unique property of phlorizin is its ability to normalize plasma glucose levels in the absence of a change in the plasma insulin level. Restoration of normal tissue function then directly implicates hyperglycemia as the cause of the abnormality; this explains why phlorizin has been viewed as a specific glucose-lowering agent. Phlorizin restored glucose-induced insulin secretion in 90% pancreatectomized rats (37) and also in glucose-infused normal rats (38).

Reversal studies have not been limited to phlorizin. We and others have observed a return of glucose-induced insulin secretion with insulin therapy (39,40). The surprising aspect of these results has been just how fast the reversal can occur. We studied rats 4–6 wk after a 90% pancreatectomy. This model develops mild hyperglycemia within 1 wk of the surgery (30); thus, these rats had been diabetic for at least 3 wk. Despite the prolonged duration of hyperglycemia, a 6-h insulin infusion was sufficient to cause a fourfold increase in glucose-induced insulin secretion (40). In vitro studies have provided even more startling evidence for the rapid reversibility of the glucose unresponsiveness. Grill et

al. (41) studied rats treated with streptozocin as neonates. These modestly hyperglycemic rats had the classic picture of selective β-cell glucose unresponsiveness: loss of glucose-induced insulin secretion but preservation of insulin responses to nonglucose secretagogues (29). These investigators were able to restore glucose-induced insulin secretion in these rats by perfusing their pancreases in vitro for just 40 min with perfusate containing no glucose (41). The same result has been reproduced in several other diabetic rodent models (40,42,43); this has lead to the conclusion that rapid reversibility is a fundamental feature of hyperglycemia-induced β-cell glucose unresponsiveness. An important understanding from these results is that any attempt to define the mechanism of the suppressed glucose-induced insulin secretion must be able to explain the rapid reversibility. For instance, a 40-min reversibility period is compelling evidence against any process for recovery that requires protein synthesis. Such a conclusion effectively eliminates defects in gene expression which presuppose that the impaired β-cell response to glucose is secondary to the lack of a key protein. On the other hand, defective processing, activation, or regulation of a key protein is perfectly compatible with the observed rapid reversibility.

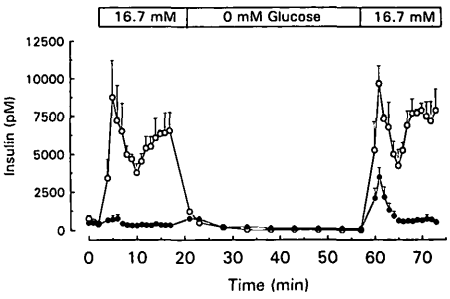

Figure 4—*Effect of 40 min of 0 mM glucose on glucose-induced insulin secretion in 8- to 12-wk-old rats. Insulin secretion was assessed in vitro by use of perfused pancreas technique.* ●, *Rats administered 90 mg/kg i.p. streptozocin at 2 days of age* ○, *Littermate controls treated with the diluent. Insulin response to 16.7 mM glucose in the controls was unaffected by the 40 min of 0 mM glucose. In diabetic rats there was no insulin response to the first glucose challenge, but there was a definite first-phase rise in insulin output after the 40 min of 0 mM glucose. β-Cell mass in these rats was 30–40% of normal (31); thus, first-phase insulin response after 0 mM glucose was nearly normal when adjusted for the reduced mass of β-cells.*

Absent glucose-induced insulin secretion represents more than one defect

A new concept is beginning to emerge that the deficient glucose-induced insulin secretion represents not a single defect, but multiple defects. The most persuasive data for this idea have come from the reversal studies in which the pancreas was perfused in vitro for 40 min with perfusate devoid of glucose. All of these studies have observed a clear return of the first-phase insulin response but virtually no change in the second phase (40–43; Fig. 4). This variability in reversal is strong evidence for the existence of separate mechanisms for the suppressed early and late phases of insulin secretion. A second intriguing observation has come from islets isolated from glucose-infused normal rats: a leftward shift in the dose-response curve for glucose-induced insulin secretion (44). A similar observation has been made in normal dogs after a two-thirds pancreatectomy (45). These results have raised the question of whether β-cells, when faced with increasing secretory demands, might compensate by becoming hyperresponsive to glucose. The reason this question is so interesting is that there is recent evidence in glucose-infused normal rats that implicates excessive stimulation of β-cells as a cause of the hyperglycemia-induced loss of glucose-induced insulin secretion (46). As described in this review, the glucose toxicity hypothesis states that sustained hyperglycemia causes the glucose unresponsiveness. However, a difficult part of this hypothesis has been that glucose-induced insulin secretion is lost in diabetic rat models when the plasma glucose level is only minimally elevated (35,38,47). It has remained unclear how such a trivial rise in glucose level could induce β-cell dysfunction. A tantalizing extension to this hypothesis is that there is a cascade of events. Mild hyperglycemia first induces a leftward shift in the dose-response curve. The β-cells are now hyperresponsive to glucose; thus, more insulin is secreted than normally would occur. It is this overactivity that initiates the intracellular events that culminate in the next step: the suppressed glucose-induced insulin secretion.

Summary

The studies cited above have provided substantial evidence for a cause and effect relationship between chronic hyperglycemia and β-cell glucose unresponsiveness. Proof of this connection must await delineation of the intracellular events in the β-cell that link chronic hyperglycemia and the loss of glucose-induced insulin secretion. Our working concept is that the same series of events causes the loss of glucose-induced insulin secretion in NIDDM. The animal models have provided a way to characterize this secretory defect and to address questions that are not easily tested in humans. Results from investigators around the world have shown remarkable consistency. These results can be summarized in the following: sustained hyperglycemia makes β-cells glucose unresponsive. The extent of the abnormal function depends on the glucose level and the duration of exposure. Even trivial hyperglycemia of prolonged duration causes β-cell dysfunction. Once glucose unresponsiveness develops, continuous but very mild hyperglycemia is required for the defect to persist. Without hyperglycemia, glucose responsiveness returns quickly.

MECHANISM OF β-CELL GLUCOSE UNRESPONSIVENESS —

During the last few years many of the major intracellular steps that lead to glu-

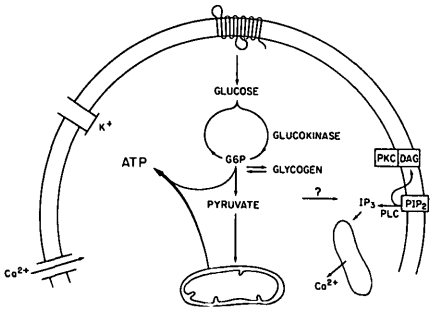

Figure 5—*Schematic representation of the major intracellular steps that lead to glucose-induced insulin secretion. Glucose enters the β-cell through a high K_m glucose transporter (GLUT2). It is phosphorylated by glucokinase, then undergoes metabolism and oxidation to CO_2 and H_2O. An offshoot of this process, probably a rising ATP-ADP ratio, closes the ATP-sensitive K^+ channel, leading to cellular depolarization, opening of voltage-dependent Ca^{2+} channel, and an influx of Ca^{2+}. This rise in intracellular Ca^{2+}, along with a variety of other second messengers such as the breakdown of membrane inositol phospholipids, leads to granule recruitment and extrusion.*

cose-induced insulin secretion have been delineated (Fig. 5). Glucose enters the β-cell through a high K_m glucose transporter. It is phosphorylated by glucokinase, then undergoes metabolism and oxidation to CO_2 and H_2O. An offshoot of this process, probably a rising ATP-ADP ratio, closes the ATP-sensitive K^+ channel, leading to cellular depolarization, opening of the voltage-dependent Ca^{2+} channel, and an influx of Ca^{2+}. This rise in intracellular Ca^{2+}, along with various other second messengers such as the breakdown of membrane inositol phospholipids, leads to granule recruitment and extrusion. Glucose-induced insulin secretion is therefore a complex cascade of events. A defect in any part of that cascade could theoretically cause the glucose unresponsiveness linked to chronic hyperglycemia.

Attempts to identify the specific defects have been complicated by the lack of a suitable in vitro model. Biochemical studies in β-cells are conducted on isolated islets so that islet tissue can be studied independently from acinar tissue. Isolated islets from hyperglycemic rodent models have been a major disappointment because they fail to show the same profound lack of glucose-induced insulin secretion that is found in vivo (48–50): The secretory defect largely disappears during the several-hour isolation procedure. A recent innovation that may prove helpful is to use buffers during islet isolation that contain higher than normal glucose concentrations (44,51). A second limitation of islet studies is that 20% of the cells in a normal islet are non–β-cells (A, D, and PP cells), which complicates localizing the site of a defect. This problem is further complicated when islets from normal and diabetic rats are compared; most diabetic rodent models have a reduced β-cell mass, and thus the proportion of non–β-cells is even higher (31,52). An important consequence of these problems is that extreme caution must be taken not to overinterpret studies that characterize the biochemical properties of isolated islets from hyperglycemic animal models. An alternate approach, prolonged culture of normal islets at high glucose concentrations, also has not resulted in β-cells with profound glucose unresponsiveness. Therefore, an ongoing obstacle in this field is that we have no in vitro system that exhibits the full-blown secretory defect. However, several hypotheses are being investigated. When these data are reviewed, it is important to remember that most of these results were generated in systems that do not express the β-cell functional abnormality of interest: selective glucose unresponsiveness. The previous comments need to be clarified in that the lack of a suitable in vitro model pertains to systems that are amenable to study using standard biochemical techniques such as isolated islets and cell lines. The insulin secretion data previously reviewed from hyperglycemic animal models showing the profound loss of glucose-induced insulin secretion were obtained in vitro with the perfused pancreas technique; i.e., the secretory defect is fully expressed in the intact pancreas (31). Therefore, one strategy to attempt to overcome the lack of an in vitro model is to develop biochemical techniques that can be used in the whole pancreas.

Impaired glucose transport into β-cells

Glucose enters the β-cell through a membrane-bound facilitative transporter that is designated as GLUT2 or the liver/β-cell transporter (53,54). Some characteristics of this transporter make it unique among the superfamily of facilitative transporters: it has a high K_m of 15–20 mM glucose (55), it is located on unique tissues (β-cells, liver, intestinal epithelium, and kidney tubules) (56–58), and it is relatively insensitive to inhibition by cytochalasin B (55). It has been proposed that impaired glucose entry into the β-cell causes the loss of glucose-induced insulin secretion in NIDDM (59). This hypothesis is based on the observation that every hyperglycemic rodent model that has been studied has a marked reduction in the amount of GLUT2 protein in their β-cells as determined by indirect immunofluorescence on pancreas sections and/or from Western blots of membrane preparations (33,60–63; Fig. 6). High K_m glucose transport into islet cells is also reduced in the few studies where transport kinetics have been measured (33). One mechanism for the fall in GLUT2 protein may be reduced synthesis, because GLUT2 mRNA levels also decline (33,59). Unlike the β-cell, the level of GLUT2 protein in hepatocytes from these same diabetic rats is not reduced (60); this raises fascinating questions about the mechanism for the differential tissue regulation of this protein.

Finding a reduced expression of GLUT2 in β-cells and a loss of glucose-induced insulin secretion in the same animals, although provocative, does not prove a cause and effect relationship.

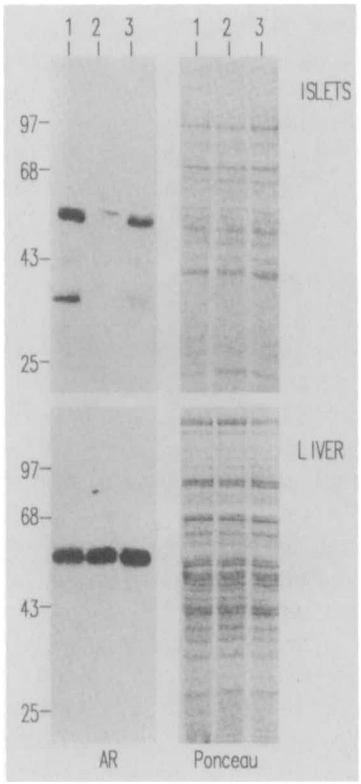

Figure 6—*Immunoblot analysis of the liver/β-cell glucose transporter (GLUT2) in islets (top) and liver (bottom) from 8 wk-old-rats. AR, autoradiogram; Ponceau, Ponceau S staining of the filters. Lane 1 represents control rats. Lanes 2 and 3 represents rats that had been given streptozocin at 2 days of age; lane 2 represents markedly diabetic rats (plasma glucose 513 ± 129 mg/dl), and lane 3 represents less severely diabetic rats (204 ± 25 mg/dl). Left-hand panels show immunodetection of the transporter using an antibody directed against amino acids 510–522 of rat GLUT2 and I^{125}-labeled protein A. Numbers on left are molecular masses in kilodaltons. Note reduced amount of transporter in islets from the diabetic rats, with the decrease being greater in markedly hyperglycemic group. In contrast, liver from the same rats had the same amount of transporter in all three groups. Band on the islet blots migrating between 25 and 43 kDa is probably a degradation product of the glucose transporter. Right-hand blots are Ponceau S staining of the same blots before immunodetection of the transporter to show that equal amounts of protein were loaded in all lanes. From Thorens et al. (60). © by the Proceedings of the National Academy of Science.*

Several other findings have obscured the role the reduced amount of GLUT2 plays in the secretory dysfunction. First, glucose phosphorylation, not glucose transport, is believed to be the rate-limiting step for glucose metabolism and glucose-induced insulin secretion. It has been known for years that the capacity for glucose transport into a normal β-cell exceeds that for phosphorylation by as much as 10-fold (64). Second, there is precedence in the brain for chronic hyperglycemia causing downregulation of another glucose transporter (GLUT1), presumably as a way to reregulate glucose entry to a "normal" level and prevent hyperglycemia-induced overaccumulation of glucose in the cell (65). The reduction in β-cell GLUT2 might represent a similar reregulation mechanism. Third, a long-standing observation is that β-cell glycogen stores are raised as a result of chronic hyperglycemia (66); this is not what would be expected in a fuel-deficient state. Fourth, insulin responses to many nonglucose secretagogues are supernormal in hyperglycemic rat models (31,67). If the problem were simply a reduced intracellular glucose level, then the expected result would be suppressed insulin responses because of the lack of a glucose potentiating effect. Finally, in an unpublished study, we used the rapid in vitro reversal approach of Grill et al. (41) to restore glucose-induced insulin secretion in the neonatal streptozocin rat model. Glucose responsiveness reappeared without any increase in β-cell GLUT2 level as determined by indirect immunofluorescence on pancreas sections (C. Chuan, B. Thorens, S. B.-W., G. C. W., J. L. L., unpublished observations).

A reasonable conclusion from the data that have emerged thus far on β-cell GLUT2 in diabetic rodent models is that there is a link between chronic hyperglycemia and a reduction of β-cell GLUT2. Whether hyperglycemia downregulates the transporter is not known. The role the lowered amount of transporter protein plays in the loss of glucose-induced insulin secretion is also unknown. Is the reduced expression of GLUT2 a direct effect of chronic hyperglycemia? One way to attempt to answer this question is to look for a rise in GLUT2 protein when diabetic rats are made euglycemic. The available data on this point are scanty. Orci et al. (61) observed only a small rise in GLUT2 protein level in the β-cells of Zucker *fa/fa* rats treated with the α-glucosidase inhibitor acarbose. We studied another genetic model, the *db/db* mouse. These obese hyperglycemic mice have a loss of glucose-induced insulin secretion and a marked reduction in β-cell GLUT2 (63). In an unpublished study, *db/db* islets were transplanted into a euglycemic host, and the result was to essentially normalize the GLUT2 levels in the grafted β-cells (B. Thorens, Y.-J. Wu, J. L. L., G. C. W., unpublished observations). Therefore, at least in this model, the reduced GLUT2 level is acquired on the basis of some metabolic defect linked to the hyperglycemia.

An alternate approach to study the relationship between chronic hyperglycemia and the GLUT2 level has been to determine whether experimental hyperglycemia has a regulatory effect on GLUT2 expression in normal β-cells. Unger studied normal rats made hyperglycemic (11 mM glucose) for up to 7 days by glucose infusions. They found an increase, not a decrease, in the β-cell GLUT2 mRNA level (68) and no change in GLUT2 immunostaining (62). Prolonged culture of islets at 33 mM glucose also failed to cause a fall in the amount of GLUT2 (62). Therefore, identifying the link between chronic hyperglycemia and the fall in β-cell GLUT2 remains a key question.

The preceding discussion focused on the transporter protein. Substantial data on this point have been published now that the tools to study the protein and gene expression are available. However, it should not be forgotten that the key issue is transport. The major question is whether sufficient glucose enters the β-cell during chronic hyperglycemia to support glucose-induced insulin se-

cretion. This question is still unanswered. Studies in isolated islets from diabetic rats have found no major reduction in glucose metabolism as determined by [^{3}H]glucose conversion to ^3H_2O (50,69). These studies are sometimes interpreted to exclude a major reduction in glucose entry into the β-cell. However, as previously discussed, this reasoning is invalidated because these isolated islets fail to show a complete lack of glucose-induced insulin secretion (48–50). In collaboration with F. Matschinsky, we attempted to assess transport in 90% pancreatectomized rats and in glucose-infused normal rats with a technique that avoids the problem of the secretory defects reversing. Pancreases were perfused in vitro. After a washout period, glucose was infused for 30 s; then the pancreases were rapidly removed and frozen. The glucose content of individual islets was measured with microsurgical and microanalytical techniques (70). Neither model had a decrease in glucose content, which seems to contradict the idea of a major reduction in transport (71). However, these results are not definitive, for the contribution that came from endogenous glycogen breakdown cycling back to glucose is unknown. An important area of study for the future is the development of tools that will allow β-cell glucose transport kinetics to be studied in the whole pancreas.

Accumulation of β-cell glycogen

Under normal conditions, β-cells contain minuscule amounts of glycogen. In contrast, β-cells exposed to an elevated glucose level accumulate large glycogen stores (66). For instance, islet glycogen content is raised 40-fold in normal rats infused with glucose for 48 h (72). It is also well known that, when exogenous fuel is removed, the glycogen is rapidly mobilized and provides substrates for insulin secretion (73). Malaisse (73) argued that glycogen accumulation explains a number of the insulin secretion defects that occur with chronic hyperglycemia, including the loss of anomeric specificity for α-D-glucose over β-D-glucose, the exaggerated basal rate of insulin release, and the paradoxical rise in insulin release seen during an acute reduction in glucose concentration. Normally, α-D-glucose is a more potent insulin secretagogue than the β-anomer, presumably because the α-anomer is more efficiently metabolized (74). This anomeric preference is lost in human and experimental diabetes (74,75). The reasoning behind how glycogen causes the loss of anomeric specificity is based on the idea that the stored glycogen is metabolically active, providing substrates that contribute to insulin secretion. A high glucose concentration inhibits glycogenolysis through inactivation of phosphorylase α, a process that also displays preference for the α-anomer (76). The loss of anomeric preference for the α-anomer in terms of insulin secretion could then represent competing effects that cancel each other out: more efficient utilization of the extrinsic α-D-glucose, but also a more efficient reduction in the intrinsic substrates from glycogen breakdown (74). Similar reasoning can explain the paradoxical increase in insulin secretion seen during an acute reduction in glucose concentration (77). Acutely lowering the glucose concentration prevents inactivation of the glycogen phosphorylase so that the endogenous substrates from glycogenolysis increase.

An important question that remains unanswered is what role, if any, stored glycogen plays in the suppressed glucose-induced insulin secretion. Could intermediates from glycogen breakdown, as they feed into the glycolytic pathway, compete with the extracellular glucose? It was demonstrated many years ago in islets from obese hyperglycemic mice that the excessive glycogen stores were fully mobilized within 45 min of being incubated at 0 mM glucose (66). It may be a coincidence, but the timing and experimental conditions of this observation are reminiscent of the rapid reversibility for glucose-induced insulin secretion that is found in vitro using the perfused pancreas and perfusate containing no glucose (41).

Mitochondrial defect

Glucose transport and phosphorylation are the initial steps of glucose usage in the β-cell. The more distal steps, glycolysis and oxidation, are also critical events in glucose-induced insulin secretion, as evidenced by the fact that blocking glucose metabolism effectively eliminates the insulin response (64,78). Glycolysis occurs in the cytosol, and oxidation occurs in the mitochondria. It was presumed for many years that the link between the two in terms of glucose-induced insulin secretion was pyruvate, the final metabolite in glycolysis. Pyruvate is transported into the mitochondria; then it is converted to acetyl-CoA by pyruvate dehydrogenase and enters the Krebs cycle. This assumption was challenged by the unexpected observation that exogenous pyruvate is well oxidized by β-cells, and it can potentiate glucose-induced insulin secretion, but it has no insulin-releasing activity of its own (79,80). Therefore, some other link between glycolysis and the mitochondria must exist to initiate insulin secretion, and there is increasing interest that the glycerol phosphate shuttle may be that link. During glycolysis, fructose-1,6-biphosphate is converted to two trioses: glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate (DHAP). Glyceraldehyde-3-phosphate proceeds to the next step in glycolysis: 1,3-bisphosphoglycerate. DHAP can be converted to glyceraldehyde-3-phosphate by an isomerase enzyme and also proceed through glycolysis. Alternatively, there is a second pathway called the *glycerol phosphate shuttle*, whereby DHAP is converted to glycerol-3-phosphate with NADH being oxidized to NAD in the process (Fig. 7). This reaction occurs in the cytoplasm. Glycerol-3-phosphate is then reoxidized to DHAP mitochondrial by the enzyme flavin adenine dinucleotide (FAD)-dependent glycerol-3-phosphate dehydro-

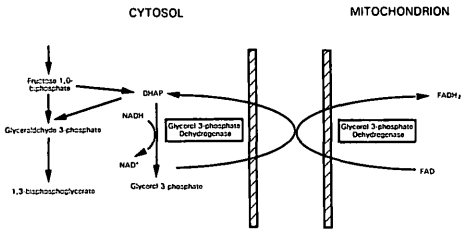

Figure 7—*Schematic representation of the glycerol phosphate shuttle. During glycolysis, fructose-1,6-biphosphate is converted to 2 trioses, glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate (DHAP). Glyceraldehyde-3-phosphate proceeds to the next step in glycolysis. DHAP can be converted to glyceraldehyde-3-phosphate by an isomerase enzyme and also proceed through glycolysis. Alternatively, there is another pathway called the glycerol phosphate shuttle whereby DHAP is converted to glycerol-3-phosphate with NADH being oxidized to NAD^+. This reaction occurs in the cytoplasm. Glycerol-3-phosphate is reoxidized to DHAP mitochondrial by the enzyme FAD-dependent glycerol-3-phosphate dehydrogenase with generation of $FADH_2$. The net effect of this pathway is to enhance mitochondrial Ca^{2+} uptake and to shuttle hydrogen ions from the cytosol to the mitochondria, where they enter the respiratory chain for generation of ATP. It has been proposed that this reaction may be an important step in glucose-induced insulin secretion.*

genase with generation of reduced flavin adenine dinucleotide ($FADH_2$). The net effect of this alternate pathway is to enhance mitochondrial Ca^{2+} uptake and to shuttle hydrogen ions from the cytosol to the mitochondria, where they enter the respiratory chain for generation of ATP (81). It has been hypothesized that this reaction may be a critical step in glucose-induced insulin secretion, partly because of the observation that the activity of the FAD-dependent glycerol-3-phosphate dehydrogenase is 40- to 70-fold higher in islets compared with other tissues (82). On the other hand, the portion of DHAP that enters the glycerol phosphate shuttle under normal circumstances versus being directly converted to glyceraldehyde-3-phosphate by the isomerase enzyme is still unknown.

The evidence is mounting in hyperglycemic animal models that a mitochondrial defect contributes to the loss of glucose-induced insulin secretion. Raising the glucose concentration normally causes glucose oxidation (assessed by conversion of [^{14}C]glucose to $^{14}CO_2$) to be stimulated more than overall glucose metabolism (combination of glycolysis and oxidation assessed by conversion of [^{3}H]glucose to ^3H_2O and CO_2) (83,84). In other words, glucose preferentially stimulates mitochondrial oxidation relative to the overall rate of glucose utilization. This preferential increase is reflected by a rise in the relative proportion of glucose usage that is made up by oxidation as determined by tracer methods that quantify the different usage pathways. Studies in isolated islets from neonatal streptozocin-induced rats have provided evidence for a defect in glucose oxidation (50). The data are stronger for a diminished preferential increase in oxidation by glucose than for an absolute reduction in glucose oxidation (85,86). This defect may seem minor compared with the profound loss of glucose-induced insulin release that occurs with chronic hyperglycemia (31). However, some important facts need to be considered: *1*) the data are from isolated islets where the secretory defect is incompletely expressed, and thus a more severe defect in mitochondrial oxidation cannot be ruled out; *2*) glucose oxidation in these islets was more severely impaired than oxidation of leucine, although the differences were subtle (85); and *3*) a recent report in a variant of this rat model used techniques based on the in vitro perfused pancreas (i.e., the secretory defect was fully expressed) and also obtained evidence for a defect in mitochondrial oxidation (87). This study observed potentiation of succinate-induced insulin secretion (4.9-fold) by pyruvate in control rats, but no potentiation (1.2-fold) in the diabetic rats. The authors concluded that these results were most consistent with a defect in pyruvate oxidation (87).

At what step(s) in the mitochondria is the defect located? The available data argue against a defect in the Kreb's cycle, since insulin responses to succinate, octanoate (converted to acetyl-CoA), and glutamine (converted to ketoglutarate) are relatively intact in neonatal streptozocin-induced rats as determined with the perfused pancreas (87). Instead, attention has focused on the glycerol phosphate shuttle. Mitochondria obtained from isolated islets from neonatal streptozocin-induced rats convert L-[2-^{3}H]glycerol-3-phosphate to ^3H_2O at a rate that is only 30 % of normal compared with L-[1-^{14}C]-2-ketoglutarate, which is converted to $^{14}CO_2$ at a rate that is 75 % of normal (86). Liver mitochondria from these same rats convert both of these compounds identically to the control rats (86). These results appear to be consistent with a unique defect in the islet at the level of the mitochondrial FAD-dependent glycerophosphate dehydrogenase. Given the growing appreciation for the critical role that the glycerol phosphate shuttle may play in glucose-induced insulin secretion (81), it is entirely possible that a defect at this step could cause the profound lack of glucose-induced insulin secretion that occurs with chronic hyperglycemia. On the other hand, these observations must be viewed very cautiously. It should not be forgotten that these results were obtained in a system (mitochondria obtained from isolated islets) that shows relatively minor changes in glucose-induced insulin (48–50). Also, islets from these rats have a marked reduction in β-cell mass (31,52). As stated previously, the activity of the FAD-dependent glycerol-3-phosphate dehydrogenase is 40- to 70-fold higher in normal islets than in other tissues (82). It is unclear whether this increased activity is present only in β-cells, or in all of the islet cells. If the increased activity is a unique feature of β-cells, the reduced conversion of L-[2-^{3}H]glycerol-3-phosphate to ^3H_2O

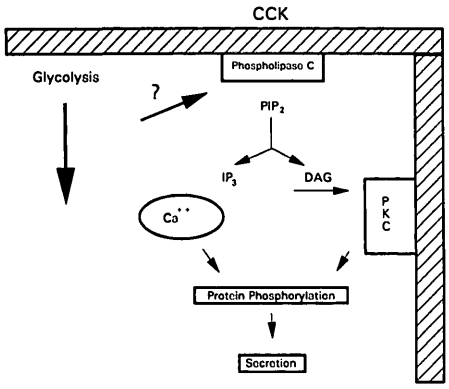

Figure 8—*Schematic representation of membrane phosphoinositide hydrolysis in the β-cell. Hydrolysis of membrane inositol phospholipids appears to be a key system for the insulin responses to a variety of secretagogues. Initial step is activation of the enzyme phospholipase C, which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 mobilizes calcium from intracellular pools, thereby activating calmodulin-dependent protein kinases. DAG causes translocation of α-protein kinase C (PKC) to the cell membrane, which initiates phosphorylation of other key proteins. The net result is to trigger the granule recruitment/extrusion process. Not shown are links between this system and other second messenger systems, such as IP_4, arachidonic acid, and cAMP. CCK directly activates phospholipase C. Also, it has been proposed that this system plays an important role in glucose-induced insulin secretion, with the link occurring between glycolysis and phospholipase C activation. The nature of the signal is unknown.*

might simply represent the reduced proportion of β-cells.

Defective hydrolysis of membrane inositol phospholipids

It has been proposed that hydrolysis of membrane inositol phospholipids is a key modulatory system for the insulin responses to a variety of secretagogues (88,89). The initial step is activation of the enzyme phospholipase C, which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (Fig. 8). IP_3 mobilizes calcium from intracellular pools, thereby activating calmodulin-dependent protein kinases. DAG causes translocation of α-protein kinase C (PKC) to the cell membrane (90), which initiates phosphorylation of other key proteins. The net result is to trigger the granule recruitment/extrusion process. This system also acts as a link to other second messenger systems such as IP_4, arachidonic acid, and cAMP (91, 92). Several nonglucose insulin secretagogues are known to act through this pathway, including CCK, which directly activates phospholipase C (93). Also, agents that stimulate adenylate cyclase and raise intracellular cAMP levels, such as glucagon, gastric inhibitory polypeptide, and glucagonlike peptide I increase phosphoinositide breakdown (88). Activation of this pathway by these gut hormones is thought to explain the marked rise in insulin level that occurs during a meal despite the rather modest increase in the plasma glucose level (88). In other words, this system probably plays a key role in modulating insulin release to a meal.

Zawalich et al. have provided evidence that this pathway is also important for glucose-induced insulin secretion. They have observed a close correlation between the timing and magnitude of glucose-induced insulin secretion and the activity of membrane phosphoinositide breakdown (94). Second, mannoheptulose blocks glucose-induced insulin secretion through its competitive inhibition of glucose phosphorylation by glucokinase (90,95). Mannoheptulose also blocks the glucose-induced translocation of PKC to the membrane (90) and the glucose-induced rise in phosphoinositide hydrolysis (94). Finally, the PKC inhibitor staurosporine blocks glucose-induced insulin secretion (96). Taken together, these data suggest that there is a link between glucose metabolism, glucose-induced activation of phosphoinositide breakdown, and glucose-induced insulin secretion, although the nature of the link is not known.

More controversial is the suggestion that defective inositol phospholipid hydrolysis is the cause of the impaired glucose-induced insulin secretion attributed to chronic hyperglycemia. This suggestion is based on the observation that short exposures of normal rat islets to agents that stimulate phosphoinositide hydrolysis increase insulin secretion but that longer exposures suppress insulin output. The effect has been seen with several agents, including glucose, and has been given the term *desensitization* (88). The experiments entail 2-h incubations with the agent of choice (e.g., 16.7 mM glucose), then finding a marked inhibition of both phosphoinositide hydrolysis and stimulated insulin output. Also, monooleoglycerol, an agent that inhibits DAG kinase, thereby blocking DAG breakdown, is able to restore the suppressed insulin responses. Similar results have been obtained with interleukin-1 (98), CCK (97), 16.7 mM glucose (97), tolbutamide (99), and forskolin and isobutylmethylxanthine (agents that raise cAMP) (100). The overall conclusion that has been made from these studies is that prolonged stimulation of phosphoinositide hydrolysis and/or chronic activation of PKC has a negative feedback on the β-cell, thereby reducing the ability of subsequent agents to stimulate inositol phospholipid hydrolysis. Similar results have been obtained in isolated islets from normal rats made hyperglycemic for 44 h with in vivo glucose infusions (101). This rat model is characterized by selective glucose unresponsiveness (32), which has lead to the suggestion that an impairment in phosphoinositide hydrolysis is the cause of the hyperglycemia-induced loss of glucose-induced insulin secretion.

The major difficulty with these results is that it is uncertain whether the in vitro culture system is a model of the same process that causes the loss of glucose-induced insulin secretion ascribed to chronic hyperglycemia. It is striking that islets incubated for just 2 h at 16.7 mM glucose have a marked reduction in the second phase of glucose-induced in-

sulin secretion (97), whereas >24 h of in vivo hyperglycemia is required for the full-blown loss of glucose-induced insulin secretion to occur (102). Also, a fundamental feature of hyperglycemia-induced glucose unresponsiveness is that insulin responses to nonglucose secretagogues are relatively spared (31). This characteristic has not been validated in the culture system. One way to verify this system is to test predictions made by the desensitization theory. For instance, a desensitization effect of CCK should lead to blunted insulin responses to glucose; CCK enters the pathway by activating phospholipase C, whereas glucose enters more proximally by an as yet unknown signal that links glycolysis and the activation of phospholipase C (Fig. 8). Therefore, the block would be distal to the point at which glucose enters the pathway. This prediction has already been validated in that glucose-induced insulin secretion is reduced as a result of CCK-induced desensitization (97). A second prediction that insulin responses to non–glucose-insulin secretagogues such as CCK should be unaffected by glucose-induced desensitization has not been tested. This prediction is based on the assumption that the location of the defect induced by glucose-induced desensitization must be proximal to phospholipase C—the site where CCK acts (88). This idea has been extrapolated from a study which used adult rats that had been injected with enough streptozocin to give them only modest hyperglycemia. These rats had the expected near total loss of glucose-induced insulin secretion as determined with the in vitro perfused pancreas and also raised insulin responses to ceruletide (a CCK analogue) and to the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (directly stimulates PKC) (103). The only site that fits with this result would be a block in the signal that links glycolysis and the activation of phospholipase C. In that case all secretagogues that enter distal to this site (i.e., CCK, TPA, agents that raise cAMP) should be unaffected. If that proves to be the case, such a defect could in theory explain why glucose-induced insulin secretion is uniquely altered by chronic hyperglycemia. The theory concerning agents that enter the phosphoinositide hydrolysis cascade distal to the signal from glycolysis has already been explained. Similarly, the myriad of insulin secretagogues that act through mechanisms other than phosphoinositide hydrolysis would also be unaffected.

Alternate mechanisms for hyperglycemia-induced insulin secretory defects

Several hypotheses in addition to those already discussed have been suggested to explain the insulin secretory defects in hyperglycemic rodent models. These include an increased rate of glucose-6-phosphate cycling back to glucose rather than undergoing oxidative phosphorylation (104–106) and an impairment of K^+ channel function (107). Finally, a long-standing idea that has been difficult to test experimentally is that glucose phosphorylation by glucokinase is impaired (108,109).

SUMMARY— During the last decade remarkable progress has been made in identifying and characterizing the defects in insulin secretion that develop during chronic hyperglycemia. Substantial data have been generated in animal models to support the concept that chronic hyperglycemia causes β-cells to become selectively unresponsive to glucose. Efforts are now under way to determine the molecular basis for this defect, although a major stumbling block continues to be the lack of in vitro systems that faithfully reproduce the secretory abnormality. Despite this limitation, several hypotheses are being investigated that span almost all of the major intracellular steps for glucose-induced insulin secretion: defects in glucose transport, storage, metabolism, oxidation, and the second messengers. Thus far no hypothesis is able to explain all of the characteristics of the secretory abnormalities. As better in vitro systems become available, we can expect an explosion of research activity directed at identifying the site(s) of the intracellular abnormalities, as well as the beginning of attempts at designing treatments targeted at specific cellular defects. The long-term goal of this line of investigation is to uncover innovative new strategies for the therapy of NIDDM.

Acknowledgments—Studies cited in this article were supported by National Institutes of Health Grants DK-38543, DK-35449, and DK-36836 (Diabetes Endocrinology Research Center Animal Research Core).

References

1. Leahy JL, Weir GC: Non-insulin-dependent diabetes mellitus: current concepts of pathogenesis. In *Insulin Secretion (Molecular and Cellular Biology of Diabetes Mellitus)*. Draznin B, Melmed S, LeRoith D, Eds. New York, Alan R. Liss, 1989, p. 149–58
2. Leahy JL: Natural history of β-cell dysfunction in NIDDM. *Diabetes Care* 13: 992–1010, 1990
3. Ward WK, Beard JC, Porte D Jr: Clinical aspects of islet β-cell function in non-insulin-dependent diabetes mellitus. *Diabetes Metab Rev* 2:297–313, 1986
4. Perley MJ, Kipnis DM: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest* 46:1954–62, 1967
5. Cerasi E, Luft R: The plasma insulin response to glucose infusion in healthy subjects and in diabetes mellitus. *Acta Endocrinol* 55:278–304, 1967
6. Luzi L, DeFronzo RA: Effect of loss of first-phase insulin secretion on hepatic glucose production and tissue glucose disposal in humans. *Am J Physiol* 257: E241–46, 1989
7. Bruce DG, Chisholm DJ, Storlien LH, Kraegen EW: Physiological importance of deficiency in early prandial insulin secretion in non-insulin-dependent diabetes. *Diabetes* 37:736–44, 1988
8. Lillioja S, Mott DM, Howard BV, Ben-

nett PH, Yki-Järvinen H, Freymond D, Nyomba BL, Zurlo F, Swinburn B, Bogardus C: Impaired glucose tolerance as a disorder of insulin action: longitudinal and cross-sectional studies in Pima Indians. *N Engl J Med* 318:1217–25, 1988
9. Warram JH, Martin BC, Krowleski AS, Soeldner JS, Kahn CR: Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann Intern Med* 113:909–15, 1990
10. Brunzell JD, Robertson RP, Lerner RL, Hazzard WR, Ensinck JW, Bierman EL, Porte D Jr: Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance tests. *J Clin Endocrinol Metab* 42:222–29, 1976
11. Kosaka K, Kuzuya T, Akanuma Y, Hagura R: Increase in insulin response after treatment of overt maturity-onset diabetes is independent of the mode of treatment. *Diabetologia* 18:23–28, 1980
12. Vague P, Moulin J-P: The defective glucose sensitivity of the β-cell in noninsulin dependent diabetes: improvement after twenty hours of normoglycemia. *Metabolism* 31:139–42, 1982
13. Palmer JP, Benson JW, Walter RM, Ensinck JW: Arginine-stimulated acute phase of insulin and glucagon secretion in diabetic subjects. *J Clin Invest* 58: 565–70, 1976
14. Giugliano D: Morphine, opioid peptides, and pancreatic islet function. *Diabetes Care* 7:92–98, 1984
15. Robertson RP: Type II diabetes, glucose "non-sense," and islet desensitization. *Diabetes* 38:1501–5, 1989
16. Porte D Jr: β-Cells in type II diabetes mellitus. *Diabetes* 40:166–80, 1991
17. O'Rahilly S, Turner RC, Matthews DR: Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 318:1225–30, 1988
18. Porte D Jr, Kahn SE: Hyperproinsulinemia and amyloid in NIDDM: clues to etiology of islet β-cell dysfunction? *Diabetes* 38:1333–36, 1989
19. Yoshioka N, Kuzuya T, Matsuda A, Taniguchi M, Iwamota Y: Serum proinsulin levels at fasting and after oral glucose load in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 31:355–60, 1988
20. Saad MF, Kahn SE, Nelson RG, Pettitt DG, Knowler WC, Schwartz MW, Kowalyk S, Bennett PH, Porte D Jr: Disproportionately elevated proinsulin in Pima Indians with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 70:1247–53, 1990
21. Osei K, Cohen RM, Wray DM, Cottrell DA, O'Dorisio TM: Proinsulin component of circulating immunoreactive insulin in glucose-tolerant offspring of patients with NIDDM (Abstract). *Clin Res* 39:246A, 1991
22. Hartling SG, Garne S, Binder C, Heilmann C, Petersen W, Petersen KE, Koch C: Proinsulin, insulin, and C-peptide in cystic fibrosis after an oral glucose tolerance test. *Diabetes Res* 7:165–69, 1988
23. Mako ME, Starr JI, Rubenstein AH: Circulating proinsulin in patients with maturity onset diabetes. *Am J Med* 63: 865–69, 1977
24. Yoshioka N, Kuzuya T, Matsuda A, Iwamoto Y: Effects of dietary treatment on serum insulin and proinsulin response in newly diagnosed NIDDM. *Diabetes* 38:262–66, 1989
25. Heaton DA, Millward BA, Gray P, Tun Y, Hales CN, Pyke DA, Leslie RDG: Evidence of β cell dysfunction which does not lead on to diabetes: a study of identical twins of insulin dependent diabetics. *Br Med J* 294:145–46, 1987
26. Heaton DA, Millward BA, Gray IP, Tun Y, Hales CN, Pyke DA, Leslie RDG: Increased proinsulin levels as an early indicator of B-cell dysfunction in non-diabetic twins of type 1 (insulin-dependent) diabetic patients. *Diabetologia* 31: 182–84, 1988
27. Dohan FC, Lukens FDW: Experimental diabetes produced by the administration of glucose. *Endocrinology* 42:244–62, 1948
28. Imamura T, Koffler M, Helderman JH, Prince D, Thirlby R, Inman L, Unger RH: Severe diabetes induced in subtotally depancreatized dogs by sustained hyperglycemia. *Diabetes* 37:600–9, 1988
29. Weir GC, Clore ET, Zmachinski CJ, Bonner-Weir S: Islet secretion in a new experimental model for non-insulin-dependent diabetes. *Diabetes* 30:590–95, 1981
30. Bonner-Weir S, Trent DF, Weir GC: Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest* 71:1544–53, 1983
31. Weir GC, Leahy JL, Bonner-Weir S. Experimental reduction of β-cell mass: implications for the pathogenesis of diabetes. *Diabetes Metab Rev* 2:125–61, 1986
32. Leahy JL, Cooper HE, Deal DA, Weir GC: Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion: a study in normal rats using chronic in vivo glucose infusions. *J Clin Invest* 77:908–15, 1986
33. Johnson JH, Ogawa A, Chen L, Orci L, Newgard CB, Alam T, Unger RH: Underexpression of β cell high K_m glucose transporters in noninsulin-dependent diabetes. *Science* 250:546–49, 1990
34. Recant L, Voyles NR, Timmers KI, Bhathena SJ, Solomon D, Wilkins S, Michaelis OE IV: Comparison of insulin secretory patterns in obese nondiabetic LA/N-cp and obese diabetic SHR/N-cp rats. Role of hyperglycemia. *Diabetes* 38:691–97, 1989
35. Leahy JL, Bonner-Weir S, Weir GC: Minimal chronic hyperglycemia is a critical determinant of impaired insulin secretion after an incomplete pancreatectomy. *J Clin Invest* 81:1407–14, 1988
36. Starke A, Grundy S, McGarry JD, Unger RH: Correction of hyperglycemia with phloridzin restores the glucagon response to glucose in insulin-deficient dogs: implications for human diabetes. *Proc Natl Acad Sci USA* 82:1544–46, 1985
37. Rossetti L, Shulman GI, Zawalich W, DeFronzo RA: Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomized rats. *J Clin Invest* 80:1037–44, 1987
38. Leahy JL, Copper HE, Weir GC: Impaired insulin secretion associated with near normoglycemia: study in normal

rats with 96-h in vivo glucose infusions. *Diabetes* 36:459–64, 1987
39. Kergoat M, Bailbe D, Portha B: Insulin treatment improves glucose-induced insulin release in rats with NIDDM induced by streptozocin. *Diabetes* 36: 971–77, 1987
40. Leahy JL, Weir GC: Beta-cell dysfunction in hyperglycaemic rat models: recovery of glucose-induced insulin secretion with lowering of the ambient glucose level. *Diabetologia* 34:640–47, 1991
41. Grill V, Westberg M, Östenson C-G: B-cell insensitivity in a rat model of non-insulin-dependent diabetes: evidence for a rapidly reversible effect of previous hyperglycemia. *J Clin Invest* 80:664–69, 1987
42. Portha B, Serradas P, Bailbé D, Suzuki K-I, Goto Y, Giroix M-H: β-Cell insensitivity to glucose in the GK rat, a spontaneous nonobese model for type II diabetes. *Diabetes* 40:486–91, 1991
43. Voyles NR, Powell AM, Timmers KI, Wilkins SD, Bhathena SJ, Hansen C, Michaelis OE IV, Recant L: Reversible impairment of glucose-induced insulin secretion in SHR/N-cp rats: genetic model of type II diabetes. *Diabetes* 37: 398–404, 1988
44. Timmers KI, Powell AM, Voyles NR, Solomon D, Wilkins SD, Bhathena S, Recant L: Multiple alterations in insulin responses to glucose in islets from 48-h glucose-infused nondiabetic rats. *Diabetes* 39:1436–44, 1990
45. Ward WK, Wallum BJ, Beard JC, Taborsky GJ Jr, Porte D Jr: Reduction of glycemic potentiation: sensitive indicator of β-cell loss in partially pancreatectomized dogs. *Diabetes* 37:723–29, 1988
46. Sako Y, Grill VE: Coupling of β-cell desensitization by hyperglycemia to excessive stimulation and circulating insulin in glucose-infused rats. *Diabetes* 39:1580–83, 1990
47. Trent DF, Fletcher DJ, May JM, Bonner-Weir S, Weir GC: Abnormal islet and adipocyte function in young β-cell deficient rats with near-normoglycemia. *Diabetes* 33:170–75, 1984
48. Halban PA, Bonner-Weir S, Weir GC: Elevated proinsulin biosynthesis in vitro from a rat model of non-insulin-dependent diabetes mellitus. *Diabetes* 32:277–83, 1983
49. Portha B: Decreased glucose-induced insulin release and biosynthesis by islets of rats with non-insulin-dependent diabetes: effects of tissue culture. *Endocrinology* 117:1735–41, 1985
50. Portha B, Giroix M-H, Serradas P, Welsh N, Hellerström C, Sener A, Malaisse WJ: Insulin production and glucose metabolism in isolated pancreatic islets of rats with NIDDM. *Diabetes* 37: 1226–33, 1988
51. Bedoya FJ, Jeanrenaud B: Evolution of insulin secretory response to glucose by perifused islets from lean *(fa/fa)* rats chronically infused with glucose. *Diabetes* 40:7–14, 1991
52. Bonner-Weir S, Trent DF, Honey RN, Weir GC: Responses of neonatal rat islets to streptozotocin: limited β-cell regeneration and hyperglycemia. *Diabetes* 30:64–69, 1981
53. Thorens B, Charron MJ, Lodish HF: Molecular physiology of glucose transporters. *Diabetes Care* 13:209–18, 1990
54. Bell GI, Kayano T, Buse JB, Burant CF, Takeda J, Lin D, Fukumoto H, Seino S: Molecular biology of mammalian glucose transporters. *Diabetes Care* 13: 198–208, 1990
55. Johnson JH, Newgard CB, Milburn JL, Lodish HF, Thorens B: The high K_m glucose transporter of islets of Langerhans is functionally similar to the low affinity transporter of liver and has an identical primary sequence. *J Biol Chem* 265:6548–51, 1990
56. Thorens B, Sarkar HK, Kaback HR, Lodish HF: Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and β-pancreatic islet cells. *Cell* 55:281–90, 1988
57. Orci L, Thorens B, Ravazzola M, Lodish HF: Localization of the pancreatic beta cell glucose transporter to specific plasma membrane domains. *Science* 245:295–97, 1989
58. Thorens B, Cheng Z-Q, Brown D, Lodish HF: Liver glucose transporter: a basolateral protein in hepatocytes and intestine and kidney cells. *Am J Physiol* 259:C279–85, 1990
59. Unger RH: Diabetic hyperglycemia: link to impaired glucose transport in pancreatic β cells. *Science* 251:1200–1205, 1991
60. Thorens B, Weir GC, Leahy JL, Lodish HF, Bonner-Weir S: Reduced expression of the liver/beta-cell glucose transporter isoform in glucose-insensitive pancreatic beta cells of diabetic rats. *Proc Natl Acad Sci USA* 87:6492–96, 1990
61. Orci L, Ravazzola M, Baetens D, Inman L, Amherdt M, Peterson RG, Newgard CB, Johnson JH, Unger RH: Evidence that down-regulation of β-cell glucose transporters in non-insulin-dependent diabetes may be the cause of diabetic hyperglycemia. *Proc Natl Acad Sci USA* 87:9953–57, 1990
62. Orci L, Unger RH, Ravazzola M, Ogawa A, Komiya I, Baetens D, Lodish HF, Thorens B: Reduced β-cell glucose transporter in new onset diabetic BB rats. *J Clin Invest* 86:1615–22, 1990
63. Wu Y-J, Thorens B, Leahy JL, Lodish HF, Weir GC: Increased basal and normal glucose-potentiated arginine-induced insulin secretion in the presence of reduced GLUT-2 expression in the *db/db* mouse (Abstract). *Clin Res* 39: 307A, 1991
64. Meglasson MD, Matschinsky FM: Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab Rev* 2:163–214, 1986
65. Pardridge WM, Triguero D, Farrell CR: Downregulation of blood-brain barrier glucose transporter in experimental diabetes. *Diabetes* 39:1040–44, 1990
66. Hellman B, Idahl L-Å: Presence and mobilization of glycogen in mammalian pancreatic β cells. *Endocrinology* 84: 1–8, 1969
67. Giroix M-H, Portha B, Kergoat M, Bailbe D, Picon L: Glucose insensitivity and amino-acid hypersensitivity of insulin release in rats with non-insulin-dependent diabetes: a study with the perfused pancreas. *Diabetes* 32:445–51, 1983
68. Chen L, Alam T, Johnson JH, Hughes S, Newgard CB, Unger RH: Regulation of

β-cell glucose transporter gene expression. *Proc Natl Acad Sci USA* 87:4088–92, 1990
69. Colella RM, May JM, Bonner-Weir S, Leahy JL, Weir GC: Glucose utilization in islets of hyperglycemic rat models with impaired glucose-induced insulin secretion. *Metabolism* 36:335–37, 1987
70. Matschinsky FM, Ghosh AK, Meglasson MD, Prentki M, June V, von Allman D: Metabolic concomitants in pure, pancreatic beta cells during glucose-stimulated insulin secretion. *J Biol Chem* 261:14057–61, 1986
71. Weir GC, Leahy JL, Wilson J, Matschinsky FM: Defective insulin secretion induced by hyperglycemia is not due to altered glucose transport in the β-cell (Abstract). *Diabetes* 37 (Suppl. 1):100A, 1988
72. Marynissen G, Leclercq-Meyer V, Sener A, Malaisse WJ: Perturbation of pancreatic islet function in glucose-infused rats. *Metabolism* 39:87–95, 1990
73. Malaisse WJ, Sener A, Koser M, Ravazzola M, Malaisse-Lagae F: The stimulus-secretion coupling of glucose-induced insulin secretion: XXV. Insulin release due to glycogenolysis in glucose-deprived islets. *Biochem J* 164:447–54, 1977
74. Malaisse WJ: Physiology and pathology of the pancreatic β-cell glucose-sensor device. In *Diabetes, Obesity and Hyperlipidemias-IV*. Crepaldi G, Tiengo A, Enzi G, Eds. Amsterdam, Elsevier, 1990, p. 3–22
75. Niki A, Niki H, Niki I, Kinoh Y: Insulin release by glucose anomers in a rat model of non-insulin-dependent diabetes. *Diabetologia* 31:65–67, 1988
76. Bollen MF, Malaisse-Lagae F, Malaisse WJ, Stalmans W: The interaction of phosphorylase α with D-glucose displays α-stereospecificity. *Biochim Biophys Acta* 1038:141–45, 1990
77. Leahy JL, Weir GC: Unresponsiveness to glucose in a streptozocin model of diabetes: inappropriate insulin and glucagon responses to a reduction of glucose concentration. *Diabetes* 34:653–59, 1985
78. Ashcroft SJH, Weerasinghe CC, Randle PJ: Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. *Biochem J* 132:223–31, 1973
79. Sener A, Kawazu S, Hutton JC, Boschero AC, Devis G, Somers G, Herchuelz A, Malaisse WJ: The stimulus-secretion coupling of glucose-induced insulin release. Effect of exogenous pyruvate on islet function. *Biochem J* 176:217–32, 1978
80. MacDonald MJ, Fahien LA, Mertz RJ, Rana RS: Effect of esters of succinic acid and other citric acid cycle intermediates on insulin release and inositol phosphate formation by pancreatic islets. *Arch Biochem Biophys* 269:400–406, 1989
81. MacDonald MJ: Elusive proximal signals of β-cells for insulin secretion. *Diabetes* 39:1461–66, 1990
82. MacDonald MJ: High content of mitochondrial glycerol 3-phosphate dehydrogenase in pancreatic islets and its inhibition by diazoxide. *J Biol Chem* 256:8287–90, 1981
83. Malaisse WJ: Insulin release: physiology and pathophysiology of nutrient metabolism in pancreatic islet cells. In *Pathogenesis of Non-Insulin Dependent Diabetes Mellitus*. Grill V, Efendić S, Eds., New York, Raven, 1988, p. 27–38
84. Sener A, Malaisse WJ: Stimulation by D-glucose of mitochondrial oxidative events in islet cells. *Biochem J* 246:89–95, 1987
85. Giroix M-H, Sener A, Bailbe D, Portha B, Malaisse WJ: Impairment of the mitochondrial oxidative response to D-glucose in pancreatic islets from adult rats injected with streptozotocin during the neonatal period. *Diabetologia* 33: 654–60, 1990
86. Giroix M-H, Rasschaert J, Bailbe D, Leclercq-Meyer V, Sener A, Portha B, Malaisse WJ: Impairment of glycerol phosphate shuttle in islets from rats with diabetes induced by neonatal streptozocin. *Diabetes* 40:227–32, 1991
87. Grill V, Sako Y, Östenson C-G, Jalkanen P: Multiple abnormalities in insulin responses to nonglucose nutrients in neonatally streptozocin diabetic rats. *Endocrinology* 128:2195–2203, 1991
88. Rasmussen H, Zawalich KC, Ganesan S, Calle R, Zawalich WS: Physiology and pathophysiology of insulin secretion. *Diabetes Care* 13:655–66, 1990
89. Zawalich WS: Phosphoinositide turnover in the stimulus-response coupling of pancreatic beta cells. In *Insulin Secretion (Molecular and Cellular Biology of Diabetes Mellitus)*. Draznin B, Melmed S, LeRoith D, Eds. New York, Liss, 1989, p. 69–80
90. Ganesan S, Calle R, Zawalich K, Smallwood JI, Zawalich WS, Rasmussen H: Glucose-induced translocation of protein kinase C in rat pancreatic islets. *Proc Natl Acad Sci USA* 87:9893–97, 1990
91. Metz SA: Role of phospholipase activation in signal transduction in the pancreatic beta cell. In *Insulin Secretion (Molecular and Cellular Biology of Diabetes Mellitus)*. Draznin B, Melmed S, LeRoith D, Eds. New York, Liss, 1989, p. 81–92
92. Fleischer N, Erlichman J: Intracellular signals and protein phosphorylation: regulatory mechanisms in the control of insulin secretion from the pancreatic beta cell. In *Insulin Secretion (Molecular and Cellular Biology of Diabetes Mellitus)*. Draznin B, Melmed S, LeRoith D, Eds. New York, Liss, 1989, p. 107–16
93. Zawalich WS, Takuwa N, Tahuwa Y, Diaz VA, Rasmussen H: Interactions of cholecystokinin and glucose in rat pancreatic islets. *Diabetes* 36:426–33, 1987
94. Zawalich WS, Zawalich KC: Phosphoinositide hydrolysis and insulin release from isolated perifused rat islets. Studies with glucose. *Diabetes* 37:1294–1300, 1988
95. Malaisse WJ, Sener A, Levy J: The stimulus-secretion coupling of glucose-induced insulin release: fasting-induced adaption of key glycolytic enzymes. *J Biol Chem* 251:1731–37, 1976
96. Zawalich WS, Zawalich KC: Influence of staurosporine on insulin secretion and phosphoinositide hydrolysis (Abstract). *Diabetes* 40 (Suppl. 1):179A, 1991
97. Zawalich WS, Zawalich KC, Rasmussen H: The conditions under which rat islets are labelled with [^{3}H]inositol alter

the subsequent responses of these islets to a high glucose concentration. *Biochem J* 259:743–49, 1989
98. Zawalich WS, Zawalich KC, Rasmussen H: Interleukin-1 alpha exerts glucose-dependent stimulatory and inhibitory effects on islet cell phosphoinositide hydrolysis and insulin secretion. *Endocrinology* 124:2350–57, 1989
99. Zawalich WS: Phosphoinositide hydrolysis and insulin secretion in response to glucose stimulation are impaired in isolated rat islets by prolonged exposure to the sulfonylurea tolbutamide. *Endocrinology* 125:281–86, 1989
100. Zawalich WS, Zawalich KC: Forskolin-induced desensitization of pancreatic β-cell insulin secretory responsiveness: possible involvement of impaired information flow in the inositol-lipid cycle. *Endocrinology* 2307–12, 1990
101. Zawalich WS, Zawalich KC, Shulman GI, Rossetti L: Chronic in vivo hyperglycemia impairs phosphoinositide hydrolysis and insulin release in isolated perifused rat islets. *Endocrinology* 126: 253–60, 1990
102. Leahy JL, Weir GC: Evolution of abnormal insulin secretory responses during 48-h in vivo hyperglycemia. *Diabetes* 37:217–22, 1988
103. Okabayashi Y, Otsuki M, Ohki A, Tani S, Baba S: Increased β-cell secretory responsiveness to ceruletide and TPA in streptozocin-induced mildly diabetic rats. *Diabetes* 38:1042–47, 1989
104. Kahn A, Chandramouli V, Östenson C-G, Ahren B, Schumann WC, Löw H, Landau BR, Efendić S: Evidence for the presence of glucose cycling in pancreatic islets of the *ob/ob* mouse. *J Biol Chem* 264:9732–33, 1989
105. Kahn A, Chandramouli V, Östenson C-G, Löw H, Landau BR, Efendić S: Glucose cycling in islets from healthy and diabetic rats. *Diabetes* 39:456–59, 1990
106. Östenson C-G, Kahn A, Efendić S, Landau BR, Goto Y, Suzuki K: Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK-rat (Abstract). *Diabetes* 40 (Suppl. 1):293A, 1991
107. Purrello F, Vetri M, Vinci C, Gatta C, Buscema M, Vigneri R: Chronic exposure to high glucose and impairment of K^+-channel function in perifused rat pancreatic islets. *Diabetes* 39:397–99, 1990
108. Meglasson MD, Matschinsky FM: New perspectives on pancreatic islet glucokinase. *Am J Physiol* 246:E1–13, 1984
109. Matschinsky FM: Glucokinase as glucose sensor and metabolic signal generator in pancreatic β-cells and hepatocytes. *Diabetes* 39:647–52, 1990