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PROSPECTS

Defective Insulin Secretion in NIDDM: Integral Part of a Multiplier Hypothesis

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Abstract Non-insulin dependent diabetes mellitus (NIDDM) is characterized by a specific defect in glucose recognition by the pancreatic islet beta cell. This is in clear distinction to patients with insulin dependent diabetes mellitus (IDDM) who undergo pancreatic islet beta cell death and no longer have the ability to synthesize, store, and release insulin. Defective glucose-induced first phase insulin responses in patients with NIDDM can be partially restored by exogenous insulin treatment and by other pharmacologic therapy. These observations provide strength for the theory of glucose desensitization of the pancreatic beta cell as an important secondary defect in the pathogenesis of abnormal insulin secretion in NIDDM. However, even though defective insulin secretion is an essential part of the pathogenesis of NIDDM, in itself it is not sufficient. A multiplicative effect is required involving interaction between tissue resistance to insulin action and defective insulin secretion whose product is the syndrome of NIDDM.

Key words: multiplier hypothesis, NIDDM, insulin secretion

Non-insulin dependent diabetes mellitus (NIDDM) is readily distinguishable from insulin dependent diabetes mellitus (IDDM). NIDDM—which used to be called adult-onset diabetes mellitus and is also referred to as Type II diabetes mellitus—characteristically occurs in middle-aged to elderly adults. Clinically, the onset of this disease is insidious and approximately 80% of affected individuals are obese. A family history of NIDDM is common and the estimated concordance rate in identical twins approaches 100%. In contrast, IDDM characteristically affects younger individuals with a peak incidence in young adolescence. This disease has an explosive onset and the affected individuals are usually lean. In further contrast to NIDDM, patients with IDDM have circulating islet cell antibodies which strongly supports the theory that the pathogenesis of IDDM involves a strong autoimmune component. The concordance rate in identical twins is approximately 50% for IDDM. As this nomenclature implies, patients with IDDM will develop diabetic ketoacidosis if they are not treated daily with exogenous insulin. Patients with NIDDM have more therapeutic options such as dietary management, weight loss, and oral hypoglycemic agents. Although they may be better controlled by using exogenous insulin treatment, NIDDM patients generally will not develop ketoacidosis in the absence of insulin treatment no matter how poorly controlled the level of glycemia becomes. The explanation for these two markedly different disease states can be readily seen through the microscope. The pancreatic islet in patients with IDDM is devoid of beta cells whereas islets in patients with NIDDM have normal-appearing beta cells. Moreover, patients with NIDDM retain the ability to synthesize, store, and secrete insulin as will be discussed below. This is in obvious contrast to patients with IDDM who no longer have the ability to synthesize and release insulin.

This consideration of insulin secretory defects in NIDDM will begin with the description of normal insulin secretion in humans and be followed by a consideration of abnormal insulin secretion in patients with NIDDM. However, it is important to emphasize that contemporary theory of the pathogenesis of NIDDM involves two primary and equal factors—defective insulin secretion and tissue insulin resistance. Both factors play a role in the pathogenesis of NIDDM and neither alone is sufficient to induce the

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disease. Although an integration of both these factors will be presented at the conclusion of this manuscript, a full consideration of the determinants of insulin sensitivity and the pathogenesis of insulin resistance is beyond the scope of this manuscript which focuses on insulin secretory defects in NIDDM.

NORMAL REGULATION OF HUMAN INSULIN SECRETION

Stimulation of Insulin Secretion

The concept that dietary carbohydrate stimulates insulin release from pancreatic islet beta cells is a very familiar one. This occurs both by direct stimulation of the beta cell by glucose as well as augmentation of glucose-stimulated insulin secretion by gut factors that are released during meals. The oral glucose tolerance test is a common clinical tool that has been used for many years. Well-established criteria exist to decide whether results from the oral glucose tolerance test are consistent with the diagnosis of normality, impaired glucose tolerance, or overt diabetes mellitus. The intravenous glucose tolerance test is a more clinically cumbersome but much more informative test. Intravenous glucose causes biphasic insulin release [1,2]. Characteristically, first phase insulin release is maximal within 3 to 5 minutes of intravenous injection of glucose. This is followed by a second phase of insulin release which is normally evident by 15 minutes and persists as long as glucose elevations remain. A measure of glucose tolerance during injection with intravenous glucose can be obtained by measuring the glucose disappearance rate or K_G which is calculated as the slope of the line reflecting the natural log of glucose concentration as a function of elapsed time from 10 to 30 minutes following intravenous glucose injection.

Even though the pancreatic beta cell is exquisitely sensitive to glucose and glucose is normally considered the primary secretagogue for insulin secretion, it is important to realize that non-glucose agonists of beta cell function also exist. Biphasic insulin responses can be elicited by intravenous injection of other substances such as arginine [3], β-adrenergic agonists [4], glucagon [5], and secretin [6].

A somewhat complicated but experimentally useful assessment of beta cell function is referred to as glucose potentiation of non-glucose stimulated insulin secretion [7,8]. Glucose potentiation is quantified by comparing the insulin response to agents such as arginine given intravenously before and then during purposeful elevation of the circulating glucose level by a concurrent infusion of intravenous glucose. Higher levels of circulating glucose induced by the glucose infusion cause greater degrees of augmentation of the insulin response to the non-glucose secretagogue. Eventually, a plateau in potentiation is reached which allows the construction of a glucose concentration-potentiation response curve. From such curves are calculated the plasma glucose level at which 50% potentiation occurs (PG_{50}), the maximal level of potentiation obtainable, and the slope of potentiation which is calculated as the difference between the maximally potentiated insulin response minus the insulin response obtained at basal glucose levels divided by the glucose level at which the maximal response was obtained minus the basal glucose level. The physiologic importance of glucose potentiation studies lies in the theory that they provide an assessment of the functional reserve of pancreatic beta cells [7,8].

Inhibition of Insulin Secretion

Both basal and stimulated insulin secretion is normally regulated by hormones and autacoids. Perhaps the earliest demonstration of negative regulation of insulin secretion by a hormone came from studies of epinephrine which inhibits glucose-induced insulin secretion [9]. Epinephrine and norepinephrine stimulate α_2-adrenergic receptors in the pancreatic islet, presumably on the beta cell. This permits fine regulation of insulin secretion by norepinephrine released from adrenergic nerve terminals within the islet and stress-related regulation of islet function by epinephrine secreted from the adrenal medulla. Interestingly, α_2-adrenergic activity profoundly blocks glucose-stimulated insulin secretion but does not affect secretin-induced insulin secretion [10]. Similarly, the β-adrenergic antagonist, propranolol, completely blocks isoproterenol-induced insulin secretion but does not affect glucose-induced insulin secretion [4].

Other endogenous inhibitors of insulin secretion include somatostatin [11], prostaglandin E_2 (PGE_2) [12,13], and galanin [14]. All these substances can be viewed as local regulators of insulin secretion since there is synthesis and release of norepinephrine and galanin from pancreatic islet nerves, somatostatin from pancreatic islet delta cells, and PGE_2 from pancreatic islet beta cells. Guanine nucleotide binding pro-

teins (G-proteins) serve as a common mechanism of action for these four inhibitors [15]. In our laboratory three forms of G_i alpha and three forms of G_o alpha have been identified in beta cells in experiments by Seaquist et al. utilizing pertussis toxin to identify substrates sensitive to ADP-ribosylation and immunoblots with antisera specific for different species of G_i alpha and G_o alpha. Recently, we have observed that epinephrine, somatostatin, and PGE_2 have effects on insulin mRNA levels in HIT cells [16]. Within 24–48 hours of incubation with these substances, insulin mRNA levels decrease by approximately 50%. It has not yet been ascertained whether these effects are expressed at the level of insulin gene transcription or insulin message degradation. However, one likely mechanism of action may involve the cyclic AMP response element (CRE) of the insulin gene since epinephrine, somatostatin, and PGE_2 have in common the ability to lower cyclic AMP in the pancreatic islet. Thus, these regulators can have readily reversible, short-term effects on insulin exocytosis and secretion as well as more long-term effects on insulin mRNA levels and synthesis [16].

DEFECTIVE INSULIN SECRETION IN NIDDM

Defective Glucose Recognition

Patients with NIDDM retain the ability to synthesize, store, and normally release insulin in response to intravenous stimulation with all known agonists except glucose (Fig. 1). Thus, when presented with an intravenous challenge of arginine, isoproterenol, glucagon, or secretin, NIDDM patients release both first and second phases of insulin secretion [3–6]. However, when glucose is injected intravenously, NIDDM patients fail to have first phase insulin secretion and second phase secretion, although usually present, is markedly diminished [17]. This selective defect in beta cell responsiveness to intravenous glucose provides strong evidence for the theory of defective glucose recognition as a primary defect in NIDDM patients [18]. Experiments assessing glucose potentiation of non-glucose induced insulin secretion in NIDDM lends further support of this theory. Patients with NIDDM characteristically have similar PG_{50} values as non-diabetic patients, but the maximal degree of potentiation and the slope of potentiation are decreased [7,8]. This phenomenon can be observed if one compares results after arginine stimulation of NIDDM patients when hyperglycemic to the responses observed in normoglycemic individuals. Usually, the magnitude of insulin responses will be indistinguishable. However, after lowering the hyperglycemic blood levels in NIDDM patients to the normal range, the responses to arginine are clearly lower then those observed in non-diabetic controls. Conversely, after infusing glucose in normal controls to reach the hyperglycemic levels found spontaneously in NIDDM patients, the insulin secretory response to intravenous arginine is of greater magnitude. Thus, there is defective recognition of glucose as a potentiating factor for insulin responses to arginine and other non-glucose secretagogues.

Glucose Toxicity or Glucose Desensitization

The hypothesis of glucose desensitization envisions glucose *per se* as having deleterious effects on pancreatic islet beta cell function. While this may seem paradoxical at first glance, an important feature of this argument is the idea that chronic, prolonged hyperglycemia rather than normal, periodic elevations of circulating glucose can be harmful. While no mechanism for glucose desensitization has yet been identified, support for this hypothesis can be found from experiments in patients with NIDDM. For example, the magnitude of first phase insulin secretion in non-diabetics negatively correlates with the magnitude of the fasting plasma glucose level [17]. Importantly, first phase insulin secretion to intravenous glucose disappears if the plasma glucose level exceeds 115 mg/dl as found in NIDDM [17]. A more direct line of evidence that chronic hyperglycemia can adversely affect islet beta cell function can be found from experiments in which first phase insulin responses to intravenous glucose were assessed in patients before and after normalizing hyperglycemic blood levels by insulin treatment [19,20]. In one such study (Fig. 2), glucose-induced first phase insulin secretion was restored after fasting glucose levels had been returned to the normal range for approximately 20 hours [23]. More recently, it has been observed that feeding of sucrose in sufficient amounts to cause hyperglycemia for two weeks in partially pancreatectomized animals is sufficient to cause chronic hyperglycemia even when sucrose feeding is discontinued [21–23].

Importantly, absent glucose-induced first phase insulin responses in NIDDM are reversible even when these patients are hyperglycemic. At least three separate pharmacologic

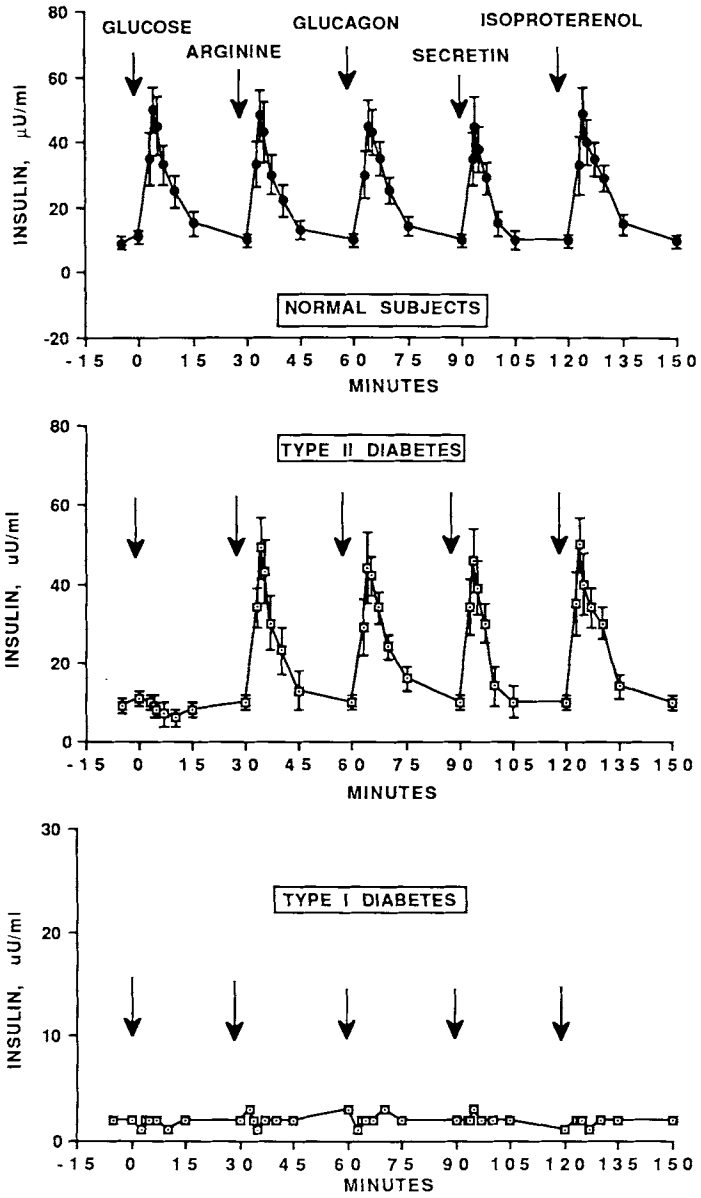

Fig. 1. Comparison of first phase insulin responses to glucose and non-glucose intravenous stimulation in normal subjects and patients with either Type II (NIDDM) or Type I (IDDM) diabetes mellitus. Note that no first phase insulin response is present in IDDM patients whereas first phase insulin responses to glucose only are absent in patients with NIDDM.

agents—phentolamine [24], sodium salicylate [25], and naloxone [26]—have been shown to partially restore defective first phase responses in NIDDM patients. When given intravenously for 1 hour, phentolamine, an α-adrenergic antagonist, partially restores absent glucose-induced first phase insulin responses in NIDDM patients. Similar observations were made with sodium salicylate (Fig. 3), a cyclooxygenase inhibitor that prevents PGE_2 synthesis by the pancreatic islet, and naloxone, an antagonist of endogenous opioid action. That the drugs are effective even when the patients are hyperglycemic raises the intriguing possibility that there may be mechanisms of action for glucose desensitization that depend on hypersensitivity to catecholamines, PGE_2, or opioids. Other potential mechanisms of action for glucose desensitization that should be considered include effects on the pancreatic islet glucose transporter

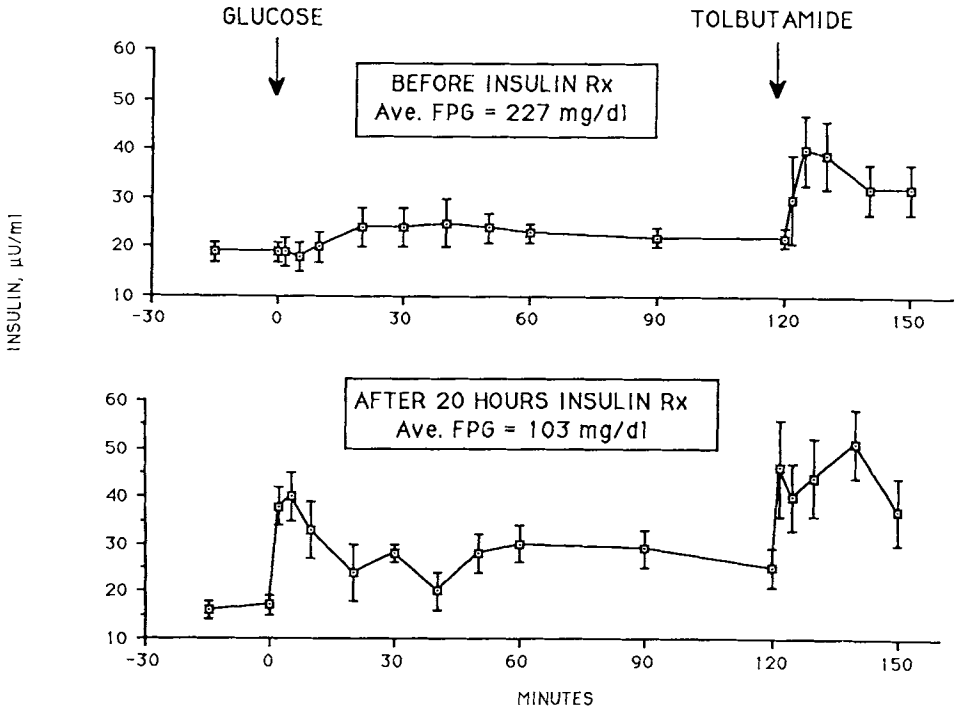

Fig. 2. Comparison of first phase insulin responses to intravenous glucose and intravenous tolbutamide before and after a 20-hour overnight insulin infusion in NIDDM patients. Restoration of normal fasting plasma glucose levels by the insulin infusion allowed partial restoration of glucose-induced first phase insulin responses and no change in tolbutamide-induced first phase insulin responses. Reproduced from [20] with permission of W.B. Saunders Company.

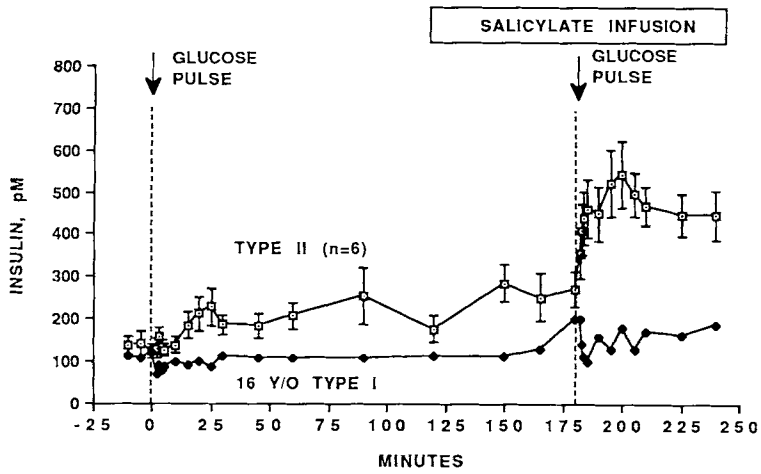

Fig. 3. Comparison of glucose-induced first phase insulin responses before and during an intravenous infusion of sodium salicylate in six patients with NIDDM and one patient with recent onset IDDM who had not yet undergone insulin therapy. The absent first phase insulin responses in the NIDDM patients were partially restored after 60 minutes infusion of sodium salicylate whereas treatment with this agent had no beneficial effects on the patient with IDDM. These data are consistent with reversibly defective glucose recognition by the beta cell in patients with NIDDM and, in contrast, beta cell death in patients with IDDM which explains absence of glucose-induced first phase insulin secretion. Reproduced from [18] with permission.

(GLUT-2) and glucokinase, the glucose sensor of the islet [27]. However, no solid evidence has been reported suggesting defects in GLUT-2 or glucokinase in NIDDM patients.

Finally, chronic exposure to hyperglycemia may have deleterious effects at various sites along the exocytotic pathway that are important for insulin release such as adenylate cyclase, potassium and calcium channels, and the exocytotic apparatus. We have recently found that prolonged high concentrations of glucose may adversely affect the availability of insulin mRNA. These observations were made in HIT cells which had undergone extensive passaging in media containing high glucose concentrations. Such cells eventually lose insulin mRNA whereas cells passaged for the same amount of time in media containing low glucose concentrations retain insulin mRNA. Moreover, after loss of insulin mRNA in HIT cells passaged in media containing high glucose, mRNA can be regained if cells are subsequently cultured in media containing low glucose concentrations. The mechanism of this effect is not yet known but may involve paradoxically adverse effects of high glucose concentrations on insulin gene transcription and/or mRNA stability.

CONCLUSION

The pancreatic islet beta cell is normally under complex regulation by a variety of substrates, fuels, and hormones. Patients with NIDDM, in contrast to patients with IDDM, retain the ability to normally synthesize, store, and release insulin to virtually all insulin secretagogues with the single exception of intravenous glucose. This defect is evidenced by lack of first phase insulin secretion to intravenous glucose although second phase responses to intravenous glucose and insulin responses to oral glucose remain intact. This and other observations strongly support the notion of abnormal glucose recognition by the pancreatic islet as a fundamental defect in NIDDM. Another important element of this defect is that it is at least partially reversible by restoring circulating glucose levels to normal or by treating hyperglycemic NIDDM patients with pharmacologic agents. Such observations lend support for the concept that exposure to chronic hyperglycemia itself may secondarily have deleterious effects on pancreatic islet function.

No consideration of the pathogenesis of hyperglycemia in NIDDM would be complete without emphasizing that this syndrome involves equally the processes of defective insulin secretion and tissue insulin resistance. Although a discussion of insulin resistance is beyond the scope of this manuscript, it is clearly a primary force in the pathogenesis of NIDDM. However, neither defective insulin secretion nor insulin resistance by itself is sufficient to cause NIDDM. Thus, al-

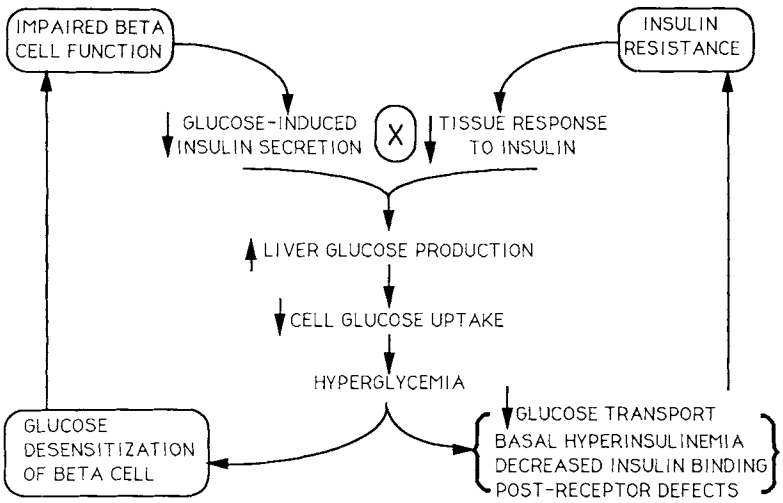

Fig. 4. A multiplier hypothesis. Two defects are required for NIDDM to be manifest—that is, defective glucose-induced insulin secretion and defective tissue response to circulating insulin. These two defects in concert lead to increased liver glucose production, decreased cellular glucose uptake, and hyperglycemia. Hyperglycemia then *secondarily* leads to glucose desensitization of the beta cell which in turn leads to further impairment of beta cell function. Hyperglycemia is also associated with decreased glucose transport, basal hyperinsulinemia, decreased insulin binding, and post-receptor defects—all of which promote insulin resistance. Relentlessly progressive impairments in insulin secretion and insulin sensitivity ultimately produce the syndrome of NIDDM.

though insulin resistance is commonly found in obese patients, most obese patients do not have NIDDM. It is generally assumed that nondiabetic obese patients have sufficient pancreatic beta cell reserve to secrete enough insulin to overcome insulin resistance in tissues. Similarly, there are individuals with insulin secretory defects who do not have NIDDM. For example, patients with cystic fibrosis [28] and healthy, hemi-pancreatectomized donors for pancreas transplantation [29] often fail to have normal glucose-induced first phase insulin secretion yet may have glucose disappearance rates that are within the normal range and do not have NIDDM. It can only be assumed that such patients have enhanced insulin- or glucose-mediated glucose disposal in tissues. Hence, it appears that a multiplicative effect exists between defects in insulin secretion and tissue insulin resistance such that NIDDM is the product of these two forces. A corollary of this multiplier hypothesis (Fig. 4) is that if either of these abnormal defects is absent, NIDDM fails to develop.

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