Perspectives in Diabetes

Hyperproinsulinemia and Amyloid in NIDDM

Clues to Etiology of Islet β-Cell Dysfunction?

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Impaired islet function is a feature of non-insulin-dependent diabetes mellitus (NIDDM), which is manifested in part by disproportionate proinsulin release. A disproportionate increase in proinsulin also occurs in insulinomas, suggesting that enhanced proinsulin release results from an increase in synthesis and premature release of proinsulin-rich immature granules in both conditions. However, recent human and animal studies suggest that normal β-cells respond to an increase in synthetic demand by enhancing their ability to process proinsulin. Thus, impaired processing of proinsulin is likely in NIDDM. A new point of similarity with insulinoma has been the demonstration of a novel pancreatic peptide isolated from insulinomas and the pancreas of patients with NIDDM. This peptide, named islet amyloid polypeptide or amylin, is also present in normal islets. Because of its association with two apparently dissimilar disease states, we propose a hypothesis that encompasses the observations related to proinsulin and islet amyloid polypeptide and suggest they are manifestations of the same abnormality. In this hypothesis, we suggest that this new pancreatic peptide is a normal participant in the process of proinsulin processing and storage. We also suggest that in the presence of defective proinsulin processing and insulin release, as occurs in NIDDM, hyperglycemia stimulates amylin biosynthesis so that this peptide is deposited in increased quantities in the islet as amyloid. This then further exacerbates the diabetic process, resulting in progressive hyperglycemia and deterioration in islet function. *Diabetes* **38:1333–36, 1989**

F or many years, it has been reported that patients with non-insulin-dependent diabetes mellitus (NIDDM) have a relative excess of proinsulin to insulin circulating in blood plasma compared with appropriately matched nondiabetic control subjects (1–7). In general, this excess has been found in the most hyper-glycemic patients and therefore has been considered to be an end stage of the disease in which progressive hyperglycemia causes an increased stimulation to the β-cell, which responds by secreting incompletely processed granules containing proinsulin and partial cleavage products of proinsulin with reduced biological potency. Support for this idea was given by our observation several years ago that experimentally increasing insulin demand further exacerbated the proinsulin abnormality (7); that is, when hyperglycemic NIDDM patients who had a relative proinsulin excess were treated with steroids for 3 days to increase insulin resistance, there was a further doubling in the proportions of circulating proinsulin-to-insulin levels. However, the same steroid treatment given to weight-matched healthy control subjects also produced a similar proportionate increase in proinsulin levels. We considered both increases to be due to increased release of immature granules, rather than to a change in the clearance of insulin or proinsulin, because a similar acute increment in proinsulin was found after the administration of an arginine bolus. Thus, control and diabetic islets seemed to respond with disproportionate proinsulin release to the corticosteroid-induced increased demand for insulin secretion. This is surprising if hyperproinsulinemia is believed to be an end stage of NIDDM. Therefore, we have explored alternative explanations.

In this article, we review some recent observations and literature that have caused us to reevaluate the conclusion that increased proinsulin release in NIDDM is just due to increased demand. Instead, we suggest that hyperproinsulinemia is related to an intrinsic β-cell defect in NIDDM and that such a finding is a clue to the etiology of the loss of β-cell secretory capacity we have observed (8–10).

HYPERPROINSULINEMIA WITHOUT HYPERGLYCEMIA

This alternate conclusion was first suggested by the observation that human leukocyte antigen–haploidentical or–identical relatives of individuals with insulin-dependent diabetes mellitus (IDDM) also have a circulating excess of proinsulin, but this is in the absence of fasting hyperglycemia and often without the presence of any abnormality of carbohydrate tolerance (11,12). This finding has been attributed to the possibility that early islet injury in IDDM causes a defect in islet β-cell function that causes abnormal granule processing and excessive proinsulin secretion. Thus, impaired islet function and defective proinsulin processing can occur before hyperglycemia, and this impairment would in turn contribute to the hyperglycemia of IDDM. Thus, neither hyperglycemia or increased demand is necessary for the disproportionate release of proinsulin.

INCREASED DEMAND WITHOUT HYPERPROINSULINEMIA

Further reasons to question the idea that insulin resistance or increased demand per se causes incomplete granule processing have been found during three studies in which we found β-cell insulin secretion to be increased without an increase in the proinsulin-to-insulin ratio.

The first observation was made in a study of partial pancreatectomy in dogs. Despite a 60% resection of pancreas mass, we found no change in the basal immunoreactive insulin level, fasting plasma glucose, or the acute response to glucose and a nonsignificant decrease in the acute insulin response to arginine at basal glucose (13). However, the maximum capacity of glucose to potentiate an insulin response to arginine was decreased by 80%. Thus, it would appear that the residual β-cells must have markedly increased their basal and glucose-stimulated output of insulin per cell to compensate for the loss of β-cell mass. Yet, when we measured the ratio of circulating proinsulin to insulin in these animals, it was equivalent to that found before pancreatectomy.

In another study, nicotinic acid was given to normal humans to produce experimental insulin resistance (14). This treatment was associated with a marked decrease in measured insulin sensitivity and an increase in insulin secretion similar to that we had found after steroid treatment. Yet, despite the similar increment in basal immunoreactive insulin (and presumably insulin resistance) found during steroid treatment, there was no change in the ratio of circulating proinsulin to insulin in the nicotinic acid study. Thus, the increased proinsulin-to-insulin ratio during steroid treatment seems to have been independent of increased β-cell secretory demand and suggests a direct steroid effect on islet β-cells.

The third observation has recently been made in nondiabetic Pima Indians (15). Although diabetic Pima Indians show an increase in proinsulin to insulin that is similar to that of Whites, in Pima Indians with normal glucose tolerance, obesity is associated with a significant decrease in the proinsulin-to-insulin ratio compared with leaner control subjects. Thus, a chronic increase in secretory demand was not associated with an increase in the proinsulin-to-insulin ratio in individuals with normal carbohydrate tolerance and intact islet β-cells.

Although there may be other explanations for these individual findings, in aggregate we have concluded that steroid treatment is associated with an independent change in islet β-cell function in addition to its effect on insulin sensitivity. We suggest that the increase in the proinsulin-to-insulin ratio in healthy subjects after steroids is more likely due to a change in proinsulin processing as a consequence of a direct effect of steroids on islet β-cells. This suggestion is consistent with literature demonstrating direct inhibitory effects of steroids on β-cell insulin secretion in vitro (16,17). Although hyperinsulinemia is often observed in vivo after steroid treatment, impaired insulin secretion is always observed in vitro and in fact has been demonstrated at matched glucose levels in vivo (18). Although there is no in vitro study to show the ability of the endocrine pancreas to change its proportion of proinsulin-to-insulin secretion, we suggest that steroid treatment directly impairs proinsulin processing and insulin secretion, but that in healthy subjects, the stimulation to the islet from insulin resistance may override the inhibition of secretion and result in hyperinsulinemia (19). That this compensation is incomplete is demonstrated by the almost universal elevation of fasting plasma glucose levels found in healthy subjects given steroids (9,20,21) compared with the preservation of basal euglycemia we observed during nicotinic acid treatment (14). It seems reasonable to conclude from this absence of effect on basal glucose level and the lack of effect on insulin secretion in vitro (22) that nicotinic acid does not alter islet β-cell function directly and, in the absence of abnormal processing and release, that β-cell compensation is better. Regardless of the exact explanation for the steroid effect on the proinsulin-to-insulin ratio, we suggest that the normal islet response to increased islet demand in the short term (partial pancreatectomy and nicotinic acid treatment) or in the long term (obese Pima Indians) is an increased release of mature granules and higher rates of processing to produce normal or even reduced circulating proinsulin-to-insulin ratios.

Based on these considerations, we argue that the increased proinsulin-to-insulin ratio in NIDDM is an indication of the presence of a primary islet lesion and is just not simply due to increased demand, although increased demand would be an important contributor. If there is in fact a problem in the processing of proinsulin, then the greater the demand, the greater the problem would become. Under such conditions, impaired processing would lead to impaired release of insulin, which would lead to hyperglycemia, which in turn would further increase demand. This in turn would increase the synthesis and provision of insulin. Combined with a delay or defect in processing, this increase would lead to the accumulation of proinsulin-related insulin precursors and the paradox of increased insulin synthesis with defective release. Of course, a primary defect in release might result in the same phenomenon, i.e., ineffective secretion would lead to hyperglycemia and increased proinsulin synthesis, which due to impaired release mechanisms, might impair proinsulin processing and granule maturity.

In either event, we suggest that such a defect in islet function may be fundamental to the pathogenesis of NIDDM. We predict that impaired or ineffective release would be associated with hyperglycemia and a compensatory increase in insulin synthesis rates. If processing was primarily impaired, there would be the build up of partially processed

proinsulin. If release was impaired, this might lead to a backup and the eventual release of immature granules via a constitutive pathway not normally used for exocytosis. In either case, there would be high insulin synthesis and a high proinsulin-to-insulin ratio. The situation is analogous in certain respects to that of an insulinoma, where high rates of insulin synthesis are associated with even higher relative rates of proinsulin secretion (23) in a situation where glucose is a relatively ineffective stimulus to exocytosis via the regulated pathway and glucose intolerance is a common finding (24). Again, constitutive release of proinsulin-rich granules with an abnormal regulated pathway is a possible explanation.

HYPERPROINSULINEMIA AND PANCREATIC AMYLOID

This similarity between the increased relative secretion of proinsulin in NIDDM and in insulinoma brought to our attention another striking parallel between these two disease states. Within the past 5 yr, two groups of investigators have called attention to an old observation made at the autopsy table at the turn of the century—the presence of amyloid in the pancreatic islet in NIDDM (25) and in insulinoma tissue (26). Both groups isolated and characterized an amyloid protein from these two sources and have published the structure of its major peptide (27,28). Of considerable surprise was the finding that the diabetes-associated peptide from NIDDM pancreases and the insulinoma/islet amyloid polypeptide from an insulinoma were the same molecule. The molecule is unique but has a 50% homology with calcitonin gene–related peptide. Because the molecule has been identified in two sources, a new name, amylin, has been proposed (29). Although this molecule was originally thought to be unique to one or another of these two pathologic states, antibodies to this peptide have been raised that appear to identify cross-reacting material in normal β-cells (30,31). If this cross-reacting material is in fact amylin, then the possibility exists that amylin is a normal peptide that is accumulating in excess extracellularly in patients with NIDDM and in some (pro)insulin-secreting tumors. If this peptide were found only in insulinoma, it might be thought to participate in the neoplastic process, but the increased frequency of its presence in NIDDM suggests some common pathologic process might be involved. Because both insulinoma and NIDDM are associated with high circulating levels of proin-

sulin, it is tempting to speculate that the proinsulin and amylin excesses are somehow related.

One parsimonious hypothesis is that insulin synthesis is in fact increased in both conditions and that amylin is normally used in the process of storing or processing proinsulin. We further suggest that excessive internal uptake and processing of granules by lysosomes (crinophagy) leads over time to the accumulation of peptides associated with the normal granule. In this sense, amylin accumulation would be a consequence of accelerated synthesis with impaired release and processing of β-cell granules. Other peptides that, like amylin, contain a high degree of antiparallel β-sheet chemical conformation are known to be more likely to precipitate as amyloid (32). Therefore, high local concentrations of this peptide may precipitate in the islet. Such extracellular accumulation might eventually lead to further impairment of islet β-cell function and perhaps even to loss of β-cell mass. This process would be slow and would depend on the relative need for insulin. A slow process is certainly consistent with the late clinical onset of NIDDM and its exacerbation and well-documented association with insulin resistance (33,34). Recently, it has even been shown that amylin and calcitonin gene–related peptide produce insulin resistance in vitro (29), and therefore, it is possible that both islet dysfunction and insulin resistance could be directly related via amylin.

The recent suggestion that proinsulin processing may be a two-stage event occurring in the Golgi body and the granule with at least one and possibly two unique enzymes involved (35) raises hope that further study of β-cell signaling, processing, and release may lead to new insights into the underlying heritable abnormalities in the β-cell of this heterogeneous syndrome we call NIDDM. Possible sites of defective processing are illustrated and discussed in Fig. 1.

CONCLUSION

The relative excess of circulating proinsulin and related proinsulin intermediates in hyperglycemic NIDDM patients was originally thought to be due to increased demand from insulin resistance and hyperglycemia, leading to the release of granules with insufficient time for complete granular processing of proinsulin to insulin. However, a closer look at other forms of increased demand in nonhyperglycemic states and the finding of a similarity of proinsulin hyperse-

FIG. 1. Normal sequence of synthesis and processing of preproinsulin to mature granule containing 5 products for release. Scheme is based on data and hypothesis from Davidson et al. (35) suggesting sequential processing of proinsulin by 2 separate enzymes in Golgi body and granule. Relative circulating excess of 32,33-desproinsulin compared with proinsulin, which is itself higher than 64,65-desproinsulin, has been reported in normal control subjects and non-insulin-dependent diabetic patients (6). Findings suggest incomplete processing in Golgi body rather than delayed processing and release of immature granules in diabetes.
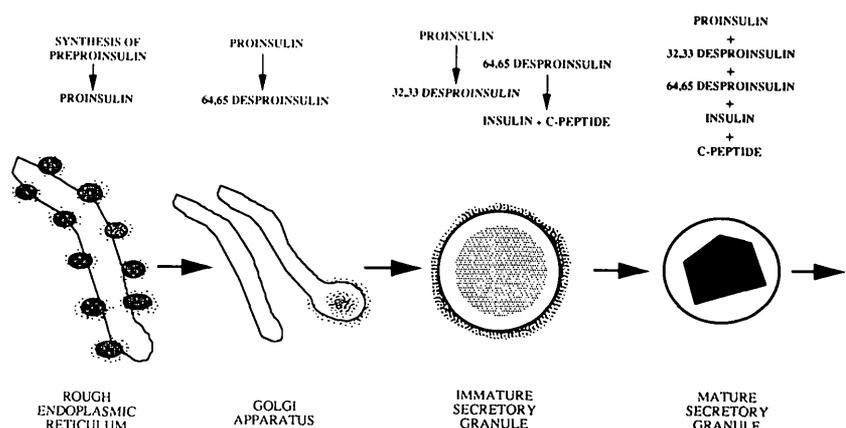
cretion and the accumulation of amyloid in insulinoma and NIDDM suggests that the elevated proinsulin secretion is an important clue to primary β-cell dysfunction, which we have emphasized as a key factor in the hyperglycemia of NIDDM. Increased demand from obesity or other forms of insulin resistance would be important contributing factors to the expression of a defect(s) in the processing or secretion of insulin.

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