MOLECULAR ASPECTS OF NON-INSULIN-DEPENDENT DIABETES

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The last five years have been an exciting time for those interested in understanding the fundamental aetio-pathogenesis of non-insulin-dependent (type 2) diabetes mellitus (NIDDM). Studies have begun to move from examination of gross alterations in whole-body metabolism to the investigation of specific molecules central to both insulin secretion and action (Fig. 1). NIDDM has always been suspected to be a highly heterogeneous grouping of syndromes and the recent discovery of individuals whose hyperglycaemia is due to specific mutations in the insulin gene, the insulin receptor gene and glucokinase gene, has confirmed that this is the case. The extent to which inherited defects in these genes contribute to NIDDM as a whole is still open to question. It has long been assumed that two major factors contribute to the development of NIDDM: an ill-defined inherited component which is reflected in the high concordance in identical twins¹ and the environmentally induced obesity and inactivity occurring in 'Western-style' cultures.² Recent data concerning the relationship between birth weight and later development of diabetes have led to the suggestion that the intra-uterine environment may also have a major influence on susceptibility to NIDDM many years later.³

Whether the basic problem is inherited or environmental (and in the latter case whether pre- or post-natal) the disorder of NIDDM ultimately expresses itself by a combination of defects: the beta cell does not secrete insulin normally and insulin-sensitive tissues (liver, fat and muscle) fail to respond to insulin adequately.⁴ Enormous strides have been made in the understanding of the basic mechanisms of insulin secretion and action. I shall briefly describe the function of some of the key molecules in both these processes and in each case summarise the evidence that inherited or acquired abnormalities of these molecules may contribute to NIDDM.

THE BETA CELL

Normal Physiology

The question of how the normal beta cell senses and

responds to plasma glucose with unerring accuracy has been a much-investigated question (Fig 2). A major advance in understanding was the discovery that the beta cell has a specific glucose transporter (GLUT2: which it shares with the liver) in its cell membrane, the transport kinetics of which are such that intracellular glucose concentrations parallel extracellular concentrations.⁵ Coupled with this was the finding that the beta cell phosphorylates its glucose to glucose-6-phosphate using glucokinase, an enzyme which has a high Michaelis constant (K_m) and is not subject to product inhibition.⁶ This combination of GLUT2 and glucokinase ensures that the amount of glucose-6-phosphate available for glycolysis and generation of ATP is directly proportional to plasma glucose. The ATP generated during glycolysis (in the cytosol) and oxidative phosphorylation (in the mitochondria) is responsible for the closing of an ATP-sensitive potassium channel in the plasma membrane. This causes depolarisation of the cell and opening of a voltage-sensitive calcium channel.⁷ The influx of calcium leads to exocytosis of secretory granules containing insulin. Investigation of the constituents of the secretory granule has revealed the presence of several other peptides and neurotransmitters, in particular islet amyloid polypeptide (IAPP) or amylin,⁸ which will be discussed further below.

Fig. 1. An overview of the genetic and environmental influences on the development of NIDDM.

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Fig. 2. Some critical steps in the beta cell secretory response to glucose. See text for detailed description.

Clinical investigators have exploited these fundamental discoveries and examined the role of inherited or acquired defects in many of these molecules in the aetiology of NIDDM.

GLUT2 Gene

The GLUT2 gene has been studied in families with early-onset forms of NIDDM and no linkage has been found between the gene and the disease.⁹ Likewise in 'common' NIDDM there is little convincing evidence of any genetic association. Studies of GLUT2 expression in islets in animal models of diabetes have, however, suggested an important role for this transporter in the mediation of the phenomenon of 'glucose toxicity'. It has long been recognised clinically that diabetes tends to be self-perpetuating and that if severe hyperglycaemia is treated there is often a striking recovery of insulin secretion. In various models of NIDDM Thorens and colleagues have clearly demonstrated that GLUT2 is down-regulated by hyperglycaemia and that this can be reversed by any treatment which restores the blood glucose to normal.¹⁰

Glucokinase

One of the most exciting recent findings in NIDDM has been the demonstration that a substantial number of families with an early-onset, relatively mild, autosomal dominant form of diabetes (sometimes termed maturity onset diabetes of the young: MODY) show close linkage of their disorder with the glucokinase gene.^{11,12} Several point mutations have now been found in this gene which lead directly to diabetes.¹³ Phenotypic studies of these patients clearly show that their diabetes is due to a defective insulin secretory response and this presumably reflects a pure 'glucose-sensing' defect in the beta cell. The definition of such a defect, for the first time, allows rationally based therapy for a subset of patients with NIDDM. As the defect in these patients is early in the metabolism of glucose and the generation of ATP, sulphonylureas, which act on the ATP-sensitive potassium channel, should be able to bypass this defect and allow normal insulin secretion from

normal secretory granules. The extent to which mutant glucokinase genes contribute to the broader spectrum of patients with NIDDM remains open to question. It should be noted, however, that many of the patients in the MODY pedigrees were not diagnosed until middle age, and had been clinically classified as conventional NIDDM patients.

Mitochondrial Genome

As the mitochondria are the major site of ATP generation, and ATP is a critical signal for insulin secretion, it might perhaps be expected that defects in the mitochondrial oxidative phosphorylation complex would be associated with defective insulin secretion. Recently, families have been described in which deafness and NIDDM are due to defects of the mitochondrial genome.¹⁴ As the mitochondrial genome is maternally inherited it is interesting to note that several studies of NIDDM have recognised a predominance of maternal inheritance. It is premature to assume that the diabetes in these families is due to a defect of mitochondrial metabolism in the beta cell alone as the relevant pathophysiological studies have not been performed and it is possible that insulin action is also affected.

Insulin

The insulin molecule itself is normal in the vast majority of patients with NIDDM but a small number of subjects have been reported to have mutant insulins.¹⁵ Such patients may or may not be hyperglycaemic. Of more relevance to NIDDM is the realisation that the disordered beta cell in NIDDM releases an excess of imperfectly processed insulin precursors so that as much as 50% of the insulin measured by radio-immunoassay in the plasma of a patient with NIDDM may actually represent processing intermediates of much lower biological activity.¹⁶

Islet Amyloid Polypeptide (Amylin)

At post-mortem the majority of patients with NIDDM have been shown to have amorphous material in the islets which has the staining characteristics of amyloid.¹⁷ The long-standing and surprising neglect of this provocative observation is probably due to the fact that this amyloid material was also found in some normals and was dismissed as a non-specific feature of ageing. Careful histopathological studies by Clark and others re-established the close relationship of amyloid and diabetes.¹⁸ It is notable that in the species in which NIDDM is associated with pancreatic amyloid (humans, cats, racoons) the normal IAPP molecule is capable of forming amyloid fibrils, unlike that of species such as rats, mice and dogs whose NIDDM is not associated with amyloid deposits.¹⁹ The sequence of IAPP has been normal in all NIDDM subjects studied to date.²⁰ It is possible, however, that a defect in its processing and/or clearance is of fundamental relevance to the development and progression of NIDDM.

INSULIN-SENSITIVE TISSUE

The attempt to reach a comprehensive understanding of

Fig. 3. A simplified overview of insulin action. See text for further details.

the actions of insulin at the molecular level has proved to be a difficult and demanding task (Fig. 3). This relates to the multiplicity of actions of insulin on carbohydrate, lipid and protein metabolism and the tissue-specific nature of some of its effects. Considerable advances have been made in the understanding of the proximal and distal ends of several of insulin's signalling pathways, but gaps remain in our understanding of the signalling intermediates. Insulin mediates essentially all its effects by binding to the insulin receptor, a dimeric cell surface molecule the intracellular beta subunits of which become rapidly phosphorylated on specific tyrosine residues upon binding of insulin. This phosphorylation activates the ability of the insulin receptor itself to phosphorylate other intracellular proteins.²¹ One of these proteins (IRS-1) has recently been cloned and, although it does not appear to have any kinase activity itself, its phosphorylation may allow docking of one or more kinases which initiate signalling cascades involving serine and threonine kinases.²² Taking glycogen synthesis as the distal step in one of insulin's main effects on glucose metabolism, major advances have been made in the understanding of the elegant kinase/phosphatase interactions which allow the stimulation of glycogen synthase and the inhibition of phosphorylase by insulin to occur.²³ The precise links between the proximal and distal elements of this signalling pathway are the subject of intensive investigation.

The stimulation of glucose transport by insulin in muscle and fat involves the translocation of a specific glucose transporter (GLUT4) from intracellular vesicles to the plasma membrane.²⁴ It is unclear whether the kinase cascade initiated by insulin is of direct relevance to this pathway or whether alternative insulin-receptor-generated signals are involved. I will restrict my discussion of the clinically relevant molecules to the insulin receptor and GLUT4.

Insulin Receptor

In 1988 Kadowaki *et al.* described the first patient with a mutant insulin receptor. This was a baby with extreme insulin resistance and the 'leprechaunism' phenotype.²⁵ It

is now clear that leprechaunism represents one end of a spectrum of 'inherited insulin receptoropathies.' Mutations with less severe consequences for function are found in children with the Rabson-Mendenhall syndrome.²⁶ Several adolescent females whose insulin resistance has led to ovarian hyperandrogenism (type A insulin resistance) have been found to be heterozygous for point mutations in the tyrosine kinase domain.²⁷ Such mutations in a single allele have surprisingly severe consequences which can be explained by the inhibitory effect of the mutations on receptors where they are only present in one of the two beta subunits. This results in 75% of cellular receptors being defective (25% mutant dimers and 50% mutant/wild-type dimers) – the so-called dominant-negative effect. The contribution of insulin receptor mutations to common NIDDM has been investigated by ourselves and others and mutations of the insulin receptor have not been frequently found.^{28,29} However, we and others have found heterozygous relatives of patients with severe homozygous insulin resistance who themselves have no clinical phenotype other than glucose intolerance/NIDDM and hyperinsulinaemia (unpublished observations).³⁰ Thus it is likely that among the heterogeneous population of NIDDM patients there will be subjects in whom insulin receptor mutations are contributing to their disease. On the other hand, acquired defects of the insulin receptor kinase activity have been widely reported in subjects with NIDDM.³¹ The mechanism whereby the diabetic state affects the function of the insulin receptor is unknown.

Insulin-Responsive Glucose Transporter

Unlike the insulin receptor, no pathogenic human mutations of GLUT4 have, to date, been found, although one variant sequence has been reported.³² In animal models of NIDDM, GLUT4 expression is reduced, particularly in adipose tissue and to a lesser extent in muscle.³³ Studies of muscle and fat tissue in patients with NIDDM have failed to detect any consistent reduction in expression at either the protein or mRNA level.

CONCLUSION

There can be no doubt that the discoveries mentioned in this brief review represent only the beginning of understanding of the precise molecular alterations which can lead to the diabetic state. It is to be hoped that further advances in these fields will lead to more precise classification with ultimate implications for prognosis, treatment and prevention of NIDDM.

Key words: Diabetes, Genetics, Insulin action, Insulin secretion.

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