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Type 2 Diabetes: When Insulin Secretion Fails to Compensate for Insulin Resistance

Minireview

Barbara B. Kahn
Diabetes Unit
Department of Medicine
Beth Israel Deaconess Medical Center
and Harvard Medical School
Boston, Massachusetts 02215

Diabetes mellitus is the most common metabolic disease worldwide. Every day, 1700 new cases of diabetes are diagnosed in the United States, and at least one-third of the 16 million Americans with diabetes are unaware of it. Diabetes is the leading cause of blindness, renal failure, and lower limb amputations in adults and is a major risk factor for cardiovascular disease and stroke. Normal glucose homeostasis requires the finely tuned orchestration of insulin secretion by pancreatic β cells in response to subtle changes in blood glucose levels, delicately balanced with secretion of counter-regulatory hormones such as glucagon. Type 1 diabetes results from autoimmune destruction of pancreatic β cells causing insulin deficiency. Type 2 or noninsulin-dependent diabetes mellitus (NIDDM) accounts for >90% of cases and is characterized by a triad of (1) resistance to insulin action on glucose uptake in peripheral tissues, especially skeletal muscle and adipocytes, (2) impaired insulin action to inhibit hepatic glucose production, and (3) dysregulated insulin secretion (DeFronzo, 1997). In most cases, Type 2 diabetes is a polygenic disease with complex inheritance patterns (reviewed in Kahn et al., 1996; DeFronzo, 1997). Environmental factors, especially diet, physical activity, and age, interact with genetic predisposition to affect disease prevalence. Susceptibility to both insulin resistance and insulin secretory defects appears to be genetically determined (Kahn et al., 1996; DeFronzo, 1997). Defects in insulin action precede the overt disease and are seen in nondiabetic relatives of diabetic subjects. Initially, increased insulin secretion compensates for insulin resistance but overt disease occurs over time as β cell compensation fails. The possibility that Type 2 diabetes could result from primary defects in the β cell in some cases is demonstrated by rare subtypes with mutations in genes encoding glucokinase, or transcription factors such as hepatic nuclear factors (HNF)-1α, -1β, and -4α, or IPF1, causing maturity onset diabetes in the young (MODY). In spite of intense investigation, the genes responsible for the common forms of Type 2 diabetes remain unknown.

Insulin Signaling: The Basics

Insulin action on glucose uptake in muscle and fat results from a cascade of signaling events emanating from the insulin receptor and culminating in translocation of the major insulin responsive glucose transporter, GLUT4, from intracellular vesicles to the plasma membrane (Myers and White, 1996; Holman and Kasuga, 1997) (Figure 1). Insulin binds to its cell surface transmembrane receptor stimulating receptor autophosphorylation and activation of the intrinsic tyrosine kinase activity, which results in tyrosine phosphorylation of several cytosolic docking proteins called insulin receptor substrates (IRSs). IRSs bind to various effector molecules including the regulatory subunit of phosphoinositol 3-kinase via Src homology 2 (SH2) domains. Recruitment of the catalytic subunit results in activation of phosphoinositol 3-kinase, which is necessary for insulin action on glucose transport, glycogen synthase, protein synthesis, antilipolysis, and suppression of hepatic gluconeogenesis by regulation of phosphoenolpyruvate carboxykinase (PEPCK) gene expression. Evidence suggests that the serine/threonine kinase, Akt/PKB, is one downstream mediator of phosphoinositol 3-kinase and could play a role in insulin stimulation of glucose transport. Insulin receptor phosphorylation also activates the ras/MAP kinase cascade, which is not necessary for insulin action on glucose transport but plays an important role in mitogenic effects of insulin (Myers and White, 1996). Attempts to identify mutations or polymorphisms in genes encoding insulin signaling molecules that account for the genetic predisposition to the common forms of Type 2 diabetes has been generally unrevealing. However, recent studies in transgenic mice (reviewed in Patti and Kahn, 1996) provide important insights.

Diabetes in IRS-2 *Knockout Mice*

Last week, Withers et al., reported that disruption of *IRS-2* causes diabetes in mice. The most compelling aspect of this report is that inactivation of this single gene causes defects in both insulin action and insulin secretion. *IRS-2* knockout mice have hyperglycemia as early as 3 days postbirth, and insulin levels are initially increased, the hallmark of insulin resistance. These mice show progressive deterioration of glucose homeostasis with severe diabetes at 10–16 weeks of age due to insulin resistance in liver and skeletal muscle and β cell failure. This is distinctly different from the consequences of

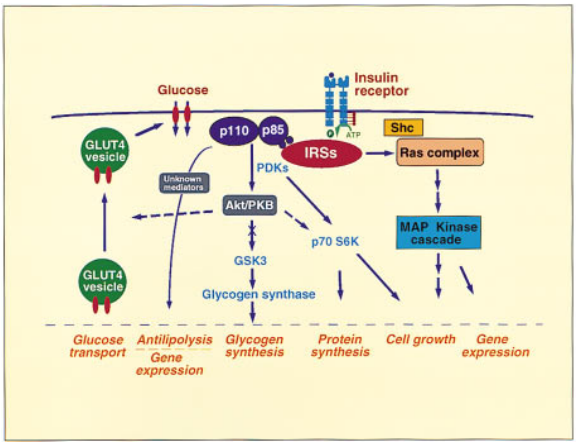

Figure 1. Insulin Signaling Pathways

IRSs, insulin responsive substrates; PDKs, phosphoinositide-dependent protein kinases; GSK3, glycogen synthase kinase 3; Akt/PKB, protein kinase B; p110, catalytic subunit; p85, regulatory subunit of phosphoinositide 3-kinase. Dotted lines indicate effects that are still under investigation.

disruption of *IRS-1* (Araki et al., 1994; Tamemoto et al., 1994), which causes growth retardation and mild insulin resistance but not frank diabetes because insulin secretion increases to compensate for the resistance. Indeed, the relatively mild insulin resistance and residual ability of insulin to partially stimulate glucose uptake in tissues of *IRS-1* knockout mice (Araki et al., 1994; Tamemoto et al., 1994) were important clues to the existence of other *IRS*s including *IRS-2*. In retrospect, differences in the consequences of *IRS-1* and *IRS-2* disruption might be expected since, in spite of high homology of these proteins, recent data demonstrate unique interactions with activated insulin receptors and different tyrosine phosphorylation motifs in the COOH termini suggest unique signaling patterns (see references in Bernal et al., 1998). Tissue distribution indicates that *IRS-2* mRNA is more abundant than *IRS-1* in liver and pancreas (Bernal et al., 1998), although both are widely expressed and are abundant in muscle. In insulin-resistant states in rodents and humans, IRS-1 and IRS-2 are differentially regulated in different tissues and the relative importance of each IRS (including also IRS-3, IRS-4, Gab1, etc.) for specific actions of insulin in a given tissue is currently under investigation.

Pancreatic β Cell Compensation Distinguishes IRS-1 *from* IRS-2 *Knockouts*

The reduction in pancreatic β cell mass even in neonatal *IRS-2* knockout mice and the inability to increase β cell mass in the face of insulin resistance are critical to the development of diabetes. In contrast, the capacity for β cell hyperplasia in *IRS-1* knockout mice most likely protects them against frank diabetes. In the early stages of the development of diabetes in the *IRS-2* knockout mice, glucose-stimulated insulin release in vivo is normal. Subsequently, the impact of the failure of normal β cell expansion postweaning becomes manifest, hyperglycemia progresses, and insulin release is attenuated. This sequence is reminiscent of Type 2 diabetes in humans and may result, at least in part, from "glucose toxicity" or glucose-induced desensitization, the phenomenon by which increased metabolites of the hexosamine biosynthetic pathway abrogate glucose sensing/insulin secretion in β cells as well as insulin action in muscle and fat (McClain and Crook, 1996; DeFronzo, 1997). This propels a vicious cycle, further aggravating hyperglycemia. The presence of IRS-2 in pancreatic ductal epithelium, the site of neogenesis of islets from ductal precursor cells, in normal mice and the reduced islet mass and failure to undergo hyperplasia in *IRS-2* null mice (Withers et al., 1998) suggest potentially a more direct role for IRS-2-dependent signaling in the regulation of islet cell replication, neogenesis and/or apoptosis.

Implications for Human Diabetes

Diabetes in the *IRS-2* knockout mouse provides a monogenic mechanism for the long-standing observation that overt Type 2 diabetes occurs when insulin secretory capacity can not adequately compensate for impairments in insulin action (DeFronzo, 1997). In humans, this phenomenon is illustrated by the fact that obesity is a major risk factor for Type 2 diabetes. Whereas obesity is invariably associated with insulin resistance, most obese people are not diabetic due to their capacity to hypersecrete insulin. Only individuals in whom β cell compensation is inadequate develop diabetes. The critical partnership between genetic determinants of insulin action and insulin secretion has previously been demonstrated in transgenic mice with simultaneous disruption of two separate genes, for example, IRS-1 impairing insulin action and glucokinase impairing insulin secretion (Terauchi et al., 1997). However, the current report gives substance to the speculation that defects in a single gene product could impair both insulin action and secretion, thereby causing diabetes.

Humans with Type 2 diabetes have reduced β cell mass compared to weight-matched nondiabetic subjects (Kloppel et al., 1985), consistent with the animal data showing that failure of β cell compensation is associated with diabetes. Intense research has focused on promoting islet cell growth and/or survival in models of Type 2 diabetes as well as in islet transplantation studies aimed at both Type 1 and 2 diabetes. Understanding the mechanisms by which IRS-2 can foster islet hyperplasia could have implications for therapeutic approaches to augment islet mass either in vivo or ex vivo in combination with islet transplantation. The parallels between the *IRS-2* knockout mice and Type 2 diabetes in humans raises the tantalizing question as to whether human diabetes is caused by mutations in the *IRS-2* gene. Disappointingly, studies in press in several populations including Danish Caucasians (Bernal et al., 1998) reveal no association between polymorphisms in the *IRS-2* gene and Type 2 diabetes.

Do All Paths Lead to the Same Syndrome?

The Insulin Receptor. While complete absence of the insulin receptor results in severe metabolic abnormalities and neonatal death in humans and rodents, mice with 50% reduction of insulin receptors due to one null allele compensate by hypersecreting insulin, resulting in normal glucose tolerance (Accili, 1997). Overexpression of normal insulin receptors in skeletal muscle, the major site of insulin-mediated glucose uptake, has a relatively mild effect to increase insulin sensitivity and expression of a kinase-deficient receptor mutant results in only mild insulin resistance without diabetes (reviewed in Patti and Kahn, 1996). These data are consistent with the "spare receptor" concept reflecting the fact that activation of the full cohort of insulin receptors is not required for normal insulin action. In humans, a number of mutations in the insulin receptor have been described and the phenotypes range from mild glucose intolerance to severe diabetes (Kahn et al., 1996). Such mutations constitute a very rare cause of Type 2 diabetes.

Glucose Transporters. Genetic manipulation of GLUT4, a more distal (Figure 1) but major mediator of insulin action on glucose transport in muscle and fat, results in relatively dramatic phenotypes. Overexpression of GLUT4 in muscle and/or adipose tissue in transgenic mice results in fasting hypoglycemia, enhanced glucose tolerance, and increased insulin sensitivity (reviewed in Katz et al., 1996). GLUT4 overexpression has a therapeutic effect to improve glucose homeostasis and insulin sensitivity in genetically obese, diabetic *db/db* mice or mice rendered insulin resistant by high fat feeding or frankly diabetic by injection of the β cell toxin streptozotocin. In the *db/db* and high fat fed models, increased

GLUT4 overcomes multiple defects in insulin signaling. Hence, if gene therapy were more feasible for humans, GLUT4 would be a promising target. Interestingly, the same phenotype is not seen with overexpression of GLUT1, which results in fasting hypoglycemia but resistance to insulin stimulated glucose uptake. This most likely results from constitutively high glucose flux through the hexosamine biosynthetic pathway (glucose toxicity hypothesis). Thus, increasing glucose transport by any mechanism does not necessarily enhance insulin sensitivity.

Mice that are heterozygous for a null allelle for *GLUT4* are normal at birth but males develop progressive hyperinsulinemia and >50% become overtly diabetic by 4–5 months (Stenbit et al., 1997). Thus, a relatively mild defect can initiate a sequence of events that eventuates in Type 2 diabetes in midlife, similar to the progression in humans. Interestingly, homozygous disruption of *GLUT4* results in growth retardation, severe reduction in adipose tissue, cardiac hypertrophy and failure, but not diabetes (Katz et al., 1996). Multiple alterations in organ function and fuel availability and metabolism are evident, and delineation of the molecular physiology will be possible with tissue-specific reconstitution as well as tissue-specific *GLUT4* knockout using the Cre recombinase *loxP* system, ideally with inducible promoters in transgenic mice.

p21^{ras}. Overexpression of the low molecular weight GTP binding protein, p21^{ras} driven by an adipose-specific promoter/enhancer in transgenic mice results in reduced adipose mass and increased insulin sensitivity (Houseknecht et al., 1996). Studies in isolated adipocytes from transgenic mice indicate that this may be mediated by increased translocation of GLUT4 to the cell surface. However, other in vitro evidence indicates that blocking ras activation does not impair insulin action on glucose transport (Holman and Kasuga, 1997). Importantly, this transgenic model illustrates that even when manipulation of a signaling pathway mimics insulin's effects, one cannot conclude that this pathway is critical for the normal action of insulin on glucose homeostasis. Preliminary data indicate that such models will provide important insights into the cross-talk between signal transduction pathways.

PEPCK. Overexpression of PEPCK, the rate limiting enzyme in gluconeogenesis, in transgenic mice targets insulin action in liver (Valera et al., 1994). The consequences are seen in all insulin target tissues and resemble the constellation of defects in Type 2 diabetes. Mice overexpressing PEPCK have increased hepatic glucose production and hepatic insulin resistance resulting in hyperglycemia and hyperinsulinemia. Secondarily, expression of the GLUT4 glucose transporter is downregulated in skeletal muscle causing resistance to insulin action on glucose uptake. Such models indicate that whether insulin resistance originates in liver, muscle, or fat, eventually all insulin target tissues may be affected. This may be mediated in part by the compensatory hyperinsulinemia itself that down-regulates insulin receptors and desensitizes postreceptor pathways (Patti and Kahn, 1996), and by associated metabolic derangements such as elevated plasma free fatty acids (DeFronzo, 1997). This phenomenon has been confirmed in transgenic mice overexpressing normal insulin in the liver. These mice are hyperinsulinemic and show an age-related reduction in insulin receptor expression, glucose intolerance, and hyperlipidemia without any primary defects in insulin action or secretion (reviewed in Patti and Kahn, 1996). Interestingly, in insulin-resistant, normoglycemic mice with one disrupted *GLUT4* allele, insulin action is impaired in muscle but not in liver. It will be important to determine whether hepatic insulin resistance develops as these mice become more severely hyperinsulinemic and hyperglycemic.

Importance of Genetic Background

Mice with combined heterozygous deficiencies in the insulin receptor and *IRS-1* genes were created to recapitulate the polygenic nature of Type 2 diabetes (Brüning et al., 1997). While mice heterozygous for either defect alone have subclinical phenotypes, the double heterozygotes are highly insulin-resistant and 40% develop Type 2 diabetes at 4–6 months of age despite massive hyperinsulinemia and pancreatic β cell hyperplasia. The fact that the highest insulin levels and greatest β cell hyperplasia occur among the mice that develop overt diabetes indicates that in this model, even exuberant β cell compensation for insulin resistance is inadequate in some mice. But what about the other 60% of mice that do not develop diabetes despite the same genetic defects? Most likely the "clinical" impact of these defects is modified by other genetic factors, especially since these knockout experiments are performed in outbred populations.

The importance of genetic background has clearly been demonstrated by feeding different strains of mice high fat diets. The strains respond with widely varying degrees of hyperinsulinemia and only some develop overt diabetes (Surwit et al., 1991). Backcrossing indicates that insulin resistance in these mice is determined by a dominant gene whereas hyperglycemia (i.e., failure of β cell compensation) is determined by a recessive gene. Similarly, the severity of diabetes in mice with mutations in genes encoding leptin (*ob*) or the leptin receptor (*db*) is significantly altered by genetic background. When *ob* or *db* is expressed on the C57BL/Ks background, severe diabetes occurs in association with β cell atrophy (Coleman, 1978). The same mutations on the C57BL/6 background result in obesity and extreme insulin resistance but only mild hyperglycemia because β cell hyperplasia and hypertrophy occur. Positional cloning studies are aimed at determining the gene(s) responsible for differences in β cell neogenesis, hypertropy, and/or apoptosis in these strains.

In humans, it is possible that defined genetic variations, for example in insulin signaling molecules, may be modified by constellations of other genes, accounting for the complex inheritance patterns of Type 2 diabetes. For example, the *IRS-1* gene is highly polymorphic with coding sequence variations in ~5% of normal people and in 10%–20% of subjects with Type 2 diabetes (Kahn et al., 1996). Furthermore, decreased levels of both the insulin receptor and IRS-1 are seen in insulin target tissues of humans and rodents with Type 2 diabetes. The impact of these polymorphisms or reduced expression of signaling molecules could be modified by traits as genetically complicated as those that influence

the level of or response to physical activity, thus modifying the genetic predisposition to diabetes. The insulin receptor/IRS1 double null heterozygotes demonstrate that a multiplex of genetic mutations may not be required and alterations in only two genes could be sufficient to cause diabetes on certain genetic backgrounds. Backcrosses of these new transgenic models of Type 2 diabetes combined with quantitative trait linkage analysis could lead to identification of additional susceptibility loci, which may suggest syntenic regions for genome scans of Type 2 diabetes in human populations (Brüning et al., 1997).

Environmental Stress

In humans the ability of environmental stress to modify genetic predisposition is seen in states as physiologic as pregnancy. The latter half of pregnancy is an insulin-resistant state in normal women due largely to the actions of placental lactogen and other hormones. This "stress test" for the β cell results in gestational diabetes in 3%–5% of pregnancies in the Western world and resolves within 48 hr of delivery in ≥90% of cases. Thus, genetic predisposition for limited β cell reserve is unmasked by pregnancy. Although gestational diabetes is reversible in the short-term, ≥50% of these women will develop Type 2 diabetes with time and the risk is increased after multiple diabetic pregnancies (DeFronzo, 1997). Molecular scanning of candidates genes for insulin resistance or impaired insulin secretion among populations of these women has so far been largely unrevealing.

Conclusions and Future Directions

In most cases, the genetic susceptibility to Type 2 diabetes fails to follow simple Mendelian inheritance, consistent with the dogma that the common forms of Type 2 diabetes are polygenic with superimposed environmental influences. Now Withers et al. demonstrate that a single gene defect can compromise both insulin action and the ability of β cells to compensate with hyperplasia and hypersecretion of insulin, suggesting a possible single molecular cause for the two key components in the pathogenesis of Type 2 diabetes. The anticlimax is that the specific genes causing the vast majority of cases of human diabetes remain unknown. This could be due to the fact that (1) interactions between two or more genes are required and are difficult to uncover with current genetic approaches, (2) additional important molecules in insulin action are yet to be discovered, and/or (3) the focus for identification of candidate genes has been on the wrong site, i.e., the primary defect leading to insulin resistance may not be in classic insulin target tissues. Generation of signals or biochemical molecules that predispose to diabetes may originate in the brain; a likely example is insulin resistance in the *db/db* (leptin receptor mutant) mouse. Efforts to identify diabetogenes are now taking a broader focus extending to secretory products of adipocytes (leptin, TNF) and neuropeptides. Thorough testing for concurrent mutations in several candidate genes, albeit a major undertaking, could pay off, expecially when paired with specific biochemical or physiologic subphenotypes. Transgenic models in which one or more candidate genes are manipulated either singly or in combination will continue to be a valuable approach to identifying new susceptibility loci, uncovering synergy between two subclinical defects, testing the impact of superimposed metabolic and lifestyle variations (e.g., diet, inactivity, stress), and developing new strategies for the prevention and treatment of diabetes and its complications.

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