Mouse models of insulin resistance

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E977–E981, 2002; 10.1152/ajpendo.00561.2001.—The hallmarks of type 2 diabetes are impaired insulin action in peripheral tissues and decreased pancreatic β-cell function. Classically, the two defects have been viewed as separate entities, with insulin resistance arising primarily from impaired insulin-dependent glucose uptake in skeletal muscle, and β-cell dysfunction arising from impaired coupling of glucose sensing to insulin secretion. Targeted mutagenesis and transgenesis involving components of the insulin action pathway have changed our understanding of these phenomena. It appears that the role of insulin signaling in the pathogenesis of type 2 diabetes has been overestimated in classic insulin target tissues, such as skeletal muscle, whereas it has been overlooked in liver, pancreatic β-cells, and brain, which had been thought not to be primary insulin targets. We review recent progress and try to reconcile areas of apparent controversy surrounding insulin signaling in skeletal muscle and pancreatic β-cells.

knockout mice; genetics; β-cells; hormone receptors

THE PATHOGENESIS OF TYPE 2 diabetes involves defects in peripheral insulin signaling and β-cell function (1). Isolated genetic defects of one branch of the fuel-sensing mechanism can cause diabetes in rare patients with extreme insulin resistance due to insulin receptor (Ir) gene mutations or with “Maturity-Onset Diabetes of the Young” (15). However, most type 2 diabetics have combined defects in insulin production and insulin action. Thus the question is not whether insulin resistance precedes β-cell dysfunction or whether β-cell dysfunction exacerbates insulin resistance, but rather what signaling mechanisms regulate these complex events.

Type 2 diabetes is genetically heterogeneous and multigenic (33). Experimental crosses in mice and genome-wide scans in humans are consistent with an oligogenic model in which few susceptibility alleles account for the entire genetic load of the disease. For example, mice with double heterozygous mutations of Ir and Ir substrate 1 (Irs1) develop diabetes with greater frequency than mice with single heterozygous mutations (6, 20). Likewise, the susceptibility to diabetes in Mexican-Americans who have inherited a predisposing allele on chromosome 2 is increased by having a second susceptibility allele on chromosome 15 (12).

The definition of insulin resistance has undergone significant changes in recent years (18). In subjects with overt diabetes, insulin resistance is found in all “classic” insulin target tissues such as muscle, adipose cells, and liver. However, this should be considered a secondary result of chronic hyperinsulinemia and glucotoxicity (14). The site of the primary defect is unclear, as is the relationship between insulin resistance and impaired β-cell function.

WHAT IS THE ROLE OF INSULIN SIGNALING IN SKELETAL MUSCLE?

In humans, skeletal muscle accounts for the largest fraction of insulin-dependent glucose disposal (14). Epidemiological data indicate that resistance of skeletal muscle to insulin-dependent glucose uptake and phosphorylation is an early step in the development of type 2 diabetes (11). Several studies have analyzed the role of insulin receptor (IR) signaling in skeletal muscle by use of transgenic and knockout mice. Early work by Moller and colleagues (Chang et al., Ref. 7) employed in
mice a dominant-negative IR transgene to inhibit IR function in muscle. In these mice, metabolic control was unaffected despite decreased IR signaling. Likewise, conditional knockout of Ir in skeletal muscle using the Cre/lox system leads to impaired insulin signaling without insulin resistance (5). A dominant-negative Ir transgene, bred onto an Ir heterozygous knockout background, impairs glucose tolerance but fails to cause diabetes (27). IR signaling in muscle appears to require IR substrate-1 (IRS-1), because ablation of IRS-2 has no effect on insulin-dependent glucose uptake (17). In view of the lack of insulin resistance in mice with a complete knockout of the main insulin-responsive glucose transporter GLUT4 (19), this body of work raised the question of whether skeletal muscle is indeed as pivotal a target of insulin action as it has been thought to be.

Several observations in the past two years have clarified this apparent discrepancy. First, analysis of glucose uptake in cultures of IR-deficient myoblasts and primary muscle cultures indicated that two alternative signaling pathways compensate for the lack of IRSs: insulin-like growth factor I (IGF-I) receptor (37) and contraction-activated signaling (40). The latter appears to be mediated through AMP-activated protein kinase (32). Moreover, shunting of glucose utilization from muscle to adipose tissue provides partial metabolic compensation in mice lacking IRSs in muscle (22). The latter findings are consistent with data showing that simultaneous ablation of IRSs in muscle and adipose tissue results in a more severe phenotype than muscle-restricted inactivation of IRSs (27). In contrast, selective disruption of the insulin-sensitive glucose transporter GLUT4 in muscle results in a profound reduction of both insulin- and contraction-stimulated glucose transport, with early-onset insulin resistance and glucose intolerance (43). These studies indicate that, although the presence of compensatory mechanisms enables mice lacking muscle IRSs to overcome the impairment of insulin signaling, a direct impediment to glucose uptake results in severe metabolic derangement. This explanation finds experimental support in a mouse model of combined ablation of insulin and IGF-I receptors in skeletal muscle. In this case, mice developed diabetes with metabolic changes typical of the insulin-resistant state (13).

In our attempt to reconcile these disparate data sets, we should be mindful of two basic truths. First, some of the phenotypic variations among these mouse models are due to the effects of genetic background (21); second, the milder phenotype of genetic alterations in muscle mirrors in part the different patterns of glycogen storage in rodents and humans. Whereas hepatic glycogen content is comparable in humans and mice, muscle glycogen content in mice is only ~10% of human muscle glycogen content as a percentage of total body glycogen (2). The phenotypes of mice with conditional knockouts of Ir and Glut4 in skeletal muscle confirm that muscle glucose disposal is central to fuel metabolism, but they indicate that IR signaling is only one of the pathways leading to GLUT4 translocation and glucose uptake (Fig. 1).

ROLES OF NONCANONICAL INSULIN TARGET TISSUES IN INSULIN RESISTANCE

It has been suggested that insulin has a direct effect on fuel metabolism in muscle and fat, whereas its effects on other organs are indirect and mediated in part by substrate fluxes (8). These findings have been challenged by the results of a combined ablation of IRSs in muscle and adipose tissue (27), in which mice develop impaired glucose tolerance without diabetes, suggesting that hepatic insulin resistance is required for the onset of overt diabetes. Consistent with this prediction, mice lacking hepatic IRSs develop insulin resistance and hyperglycemia, associated with increased hepatic glucose production (31). These data demonstrate that insulin exerts a direct effect on liver glucose metabolism. A broader implication of these findings is that noncanonical insulin target tissues play a central role in glucose homeostasis. Along these lines, the recent demonstration that neuronal IRSs regulate food intake and reproductive function (4) represents an important paradigm shift.

AKT AND GLUCOSE METABOLISM

Despite the large amount of information on mechanisms of insulin signaling, the sequence of molecular events leading to glucose transporter translocation and glucose uptake is still unknown (35). More specifically, the identity of the kinase(s) that couples activation of phosphatidylinositol (PI) 3-kinase to GLUT4 translo-

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**Fig. 1.** Converging pathways leading to GLUT4 translocation in muscle. In addition to insulin receptor (IR) activation, two other pathways appear to play important roles in GLUT4 translocation, leading to glucose uptake and utilization in muscle. Muscle contraction is a powerful trigger to GLUT4 translocation through activation of the AMP-activated kinase. In addition, muscle insulin-like growth factor 1 (IGF-I) receptors are able to signal through insulin receptor substrate (IRS) and phosphatidylinositol (PI) 3-kinase to stimulate GLUT4 translocation via activation of Akt and other inositol-triphosphate (PIP3)-dependent kinases, such as protein kinase C (PKC) isoforms. These pathways explain why, even though a muscle-specific GLUT4 knockout causes severe insulin resistance and diabetes, an isolated knockout of IR does not.
cation remains controversial. The serine/threonine kinase Akt is a critical mediator of many insulin actions. Although biochemical evidence for the involvement of Akt in glucose metabolism is generally strong, a genetic dissection of its contribution to glucose uptake has proved hard to obtain, in part because there are three closely related isoforms. Cho et al. (9) have recently shown that mice lacking Akt2 have mild diabetes associated with defects in insulin action on liver and skeletal muscle. These data provide the first genetic evidence that Akt is indeed a physiological mediator of insulin action, although its specific role in GLUT4 translocation remains unclear.

NEGATIVE REGULATORS OF INSULIN SENSITIVITY

In addition to mutations affecting insulin action, mutations in genes required to terminate or modulate insulin signaling have proved very informative in examining the pathophysiology of insulin resistance. The SH2 domain-containing inositol 5-phosphatase SHP2 can dephosphorylate phosphoinositol, thus dampening insulin signaling. Mice lacking SHP2 show increased insulin sensitivity, with severe neonatal hypoglycemia, decreased gluconeogenesis, and perinatal death. SHP2 heterozygotes show improved glucose tolerance and insulin sensitivity (10). These data demonstrate both the involvement of phosphoinositides in insulin signaling and the specific role of this class of phosphatases in its negative control.

A similar, but milder, phenotype has been described in mice lacking protein-tyrosine-phosphatase-1B (PTP-1B). These mice show enhanced insulin sensitivity, with severe neonatal hypoglycemia, decreased gluconeogenesis, and perinatal death. SHP2 heterozygotes show improved glucose tolerance and insulin sensitivity (10). These data demonstrate both the involvement of phosphoinositides in insulin signaling and the specific role of this class of phosphatases in its negative control.

What is the role of insulin signaling in pancreatic β-cells?

Whether as a result of autoimmune destruction or impaired function, diabetes is inevitably associated with β-cell failure. Patients with type 2 diabetes tend to have increased β-cell mass. However, they do not have sufficient β-cells to compensate for insulin resistance. We do not know with certainty what the central defect in β-cells of type 2 diabetics is. However, they show an impairment of glucose-stimulated insulin secretion, which, at least in part, is genetically determined (34).

Classically, insulin secretion is thought to be regulated by the products of glucose metabolism in the β-cell (30). However, the possibility that signaling through receptor tyrosine kinases participates in insulin synthesis and release has been raised on the basis of several observations. Conditional knockout of Ir in β-cells (25) or complete knockout of Irs1 (26) leads to defective insulin secretion in response to glucose and amino acids. On the other hand, inactivation of Irs2 leads to impaired β-cell growth (39), presumably for lack of neogenesis. These data are supported by evidence that insulin promotes its own synthesis, as well as glucokinase mRNA transcription, through its receptor (29). Overexpression of Akt in β-cells increases neogenesis and results in increased β-cell mass and size, without affecting insulin secretion (3, 38), whereas ablation of its substrate protein p70 S6 kinase 1 (p70s6k1) results in decreased cell size and hypoinsulinemia (32a). In contrast, mutations of the eukaryotic translation initiation factor 2α (eIF2α) (36) and its kinase Perk (16) impair the metabolic stimulus/secretion coupling and result in frank diabetes. From these data, it appears that signaling from receptor tyrosine kinases through PI 3-kinase regulates both β-cell proliferation and insulin secretion. However, the two signals appear to diverge downstream of PI 3-kinase, possibly because of the activation of different enzyme pools (29).

Other workers have reached opposite conclusions. Inhibition of PI 3-kinase signaling has been shown to increase insulin release (41), and IGF-1 has been shown to inhibit insulin secretion from β-cells (42) and perfused rat pancreas (28).
How does insulin control its own secretion? The pancreatic portal system is designed to provide for insulin control of glucagon secretion, but it is less clear how insulin would control its own secretion, given that IRs on β-cells are presumably exposed to high insulin concentrations and would thus be constantly down-regulated. Thus, although the idea of IR signaling controlling insulin production is teleologically attractive, in that it would provide a unifying mechanism for insulin resistance and impaired β-cell function, mechanistically it remains to be shown how this is accomplished. Our laboratory had set much stock by the hypothesis that an accessory receptor of the insulin family (insulin receptor-related receptor, Irr) played an important role in β-cell function. In fact, because this receptor does not bind insulin but can form heterotetrameric receptors with insulin and IGF-I receptors, it could provide a signaling mechanism that would be resistant to insulin-induced receptor downregulation. However, metabolic analyses and insulin release studies from perfused islets of Irr knockouts have failed thus far to demonstrate a role for this receptor in β-cell function (23).

We are far from having reached a consensus on this contentious issue. However, a tentative conclusion is thus far to demonstrate a role for this receptor in insulin resistance and impaired glucose uptake and release by the liver in vivo. Diabetes 48: 1198–1214, 1999.

CONCLUSIONS

This rapid overview of insulin signaling in transgenic and knockout mice is intended to convey the daunting challenge of untangling this complex problem. Thanks in no small measure to technical advances in manipulating the mouse genome, our understanding of the different systems that determine insulin sensitivity has been broadened substantially. As our scientific challenge grows in scope, so do the possibilities of therapeutic intervention by identifying new targets among the vast array of molecular effectors.

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