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New Perspectives into the Molecular Pathogenesis and Treatment of Type 2 Diabetes

Review

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There is little doubt that we are in the midst of a worldwide epidemic of diabetes. There are an estimated 143 million people worldwide with the disease, almost five times more than estimates of ten years ago. This number will probably double by 2030 (Harris et al., 1998). Although diabetes is more prevalent in developed countries, it is likely that the developing world will bear the brunt of the epidemic in the future. In the U.S., almost 16 million people are thought to be afflicted, a third of whom are undiagnosed. The disease is considerably more common among the elderly and strikes African-, Mexican- and Native Americans at 1.7–3 times the rate for that of non-Hispanic whites. In patients with diabetes, the risk of heart disease and stroke are elevated 2–4 times. Moreover, diabetes is the leading cause of end stage renal disease, blindness, and nontraumatic limb amputation. While the human and economic costs of diabetes are difficult to calculate, the total medical costs incurred annually in the U.S. alone are close to $100 billion.

Diabetes is defined as a state in which carbohydrate and lipid metabolism are improperly regulated by insulin. This results in elevated fasting and postprandial serum glucose that leads to complications if left untreated. There are two major categories of the disease, Types 1 and 2. Patients with Type 1 diabetes are absolutely dependent on exogenous insulin. This form of the disease may account for 5%–10% of all cases and is thought to result from the autoimmune destruction of the insulin-producing β cells of the islets of Langerhans. Type 2 diabetes is far more common and results from a combination of defects in insulin secretion and action. While the complications that arise from these two forms of the disease are similar, the diseases are completely different entities in terms of pathophysiology. Because of these major differences, I will restrict the scope of this review to the exciting new insights into the underlying pathophysiology and genetics of Type 2 diabetes, as well as new prospects for therapeutic intervention.

Hormonal Regulation of Glucose Metabolism

A continuous supply of glucose is necessary to ensure proper function and survival of all organs. While hypoglycemia produces cellular death, chronic hyperglycemia also can result in organ damage. Therefore, the plasma glucose level is maintained in a narrow range around 5 mM, which is considered the physiological set point. Glucose homeostasis is regulated primarily by the liver and skeletal muscle. Following a meal, most glucose disposal occurs in skeletal muscle, whereas fasting plasma glucose levels are determined primarily by glucose output from the liver.

The balance between the utilization and production of glucose is maintained at equilibrium by two opposing hormones, insulin and glucagon. In response to an elevation in plasma glucose and amino acids (after consumption of a meal), insulin is released from the β cells of the islets of Langerhans in the pancreas. The glucose is transported into β cells via the Glut2 transporter, which has a relatively low affinity for glucose, such that the rate of glucose transport changes with fluctuations in blood glucose concentration. The β cells contain a specific glucokinase that exhibits a low affinity for glucose, and is not inhibited by product formation, thus enabling the enzyme to adjust its activity in response to a wide range of glucose concentrations. After phosphorylation, glucose is oxidized and ATP is generated. The increased ATP/ADP ratio closes potassium channels, and the cell becomes depolarized. This results in the opening of voltage-sensitive calcium channels, which increases intracellular calcium and thus stimulates the fusion of the insulin-containing secretory vesicles with the plasma membrane, resulting in the pulsatile release of insulin. When plasma glucose falls (during fasting or exercise), glucagon is secreted by α cells, which surround the β cells in the pancreas. Both α and β cells are extremely sensitive to glucose concentrations and can regulate hormone synthesis and release in response to small changes in plasma glucose levels. Insulin stimulates glucose uptake, utilization, and storage, while suppressing hepatic glucose production, thus reducing plasma glucose levels. Glucagon promotes the release of stored and newly synthesized glucose into the bloodstream. These two hormones act in concert to ensure that glucose homeostasis is maintained throughout a wide variety of physiological conditions.

The primary targets for insulin are skeletal and cardiac muscle, adipose tissue and liver. Glucose uptake is the rate-limiting step in glucose utilization and storage. Insulin stimulates the transport of glucose into muscle and fat cells by increasing the concentration of a specific glucose transporter isoform, Glut4, at the cell surface. This action of insulin causes a 10- to 40-fold increase in cellular glucose uptake. Upon entering the muscle cell, glucose is rapidly phosphorylated by hexokinase and either subsequently stored as glycogen due to the activation of glycogen synthase, or oxidized to generate ATP synthesis, via activation of enzymes such as pyruvate kinase. In adipocytes, glucose is stored primarily as lipid, due to increased uptake of glucose and activation of lipid synthetic enzymes. Insulin also profoundly inhibits lipolysis in adipocytes, primarily through inhibition of the enzyme hormone sensitive lipase. Most if not all of these insulin-dependent changes in enzyme activities are mediated by attenuation of their phosphorylation state, due to a combination of protein kinase inhibition and phophatase activation.

Insulin inhibits the production and release of glucose by the liver, due to the blockade of gluconeogenesis and

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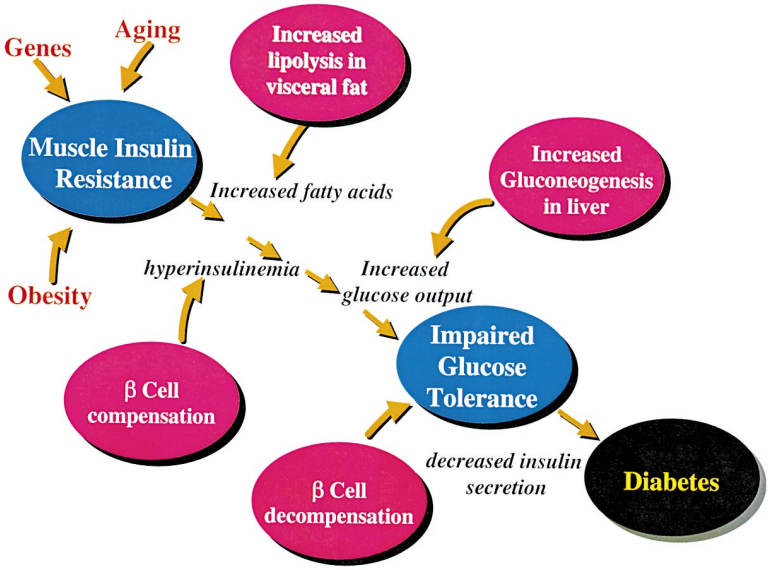

Figure 1. Metabolic Staging of Type 2 Diabetes

Type 2 diabetes is characterized by a progressive decrease in insulin action, followed by an inability of the β cell to compensate for insulin resistance. Insulin resistance is the first lesion, due to interactions among genes, aging, and metabolic changes produced by obesity. Insulin resistance in visceral fat leads to increased fatty acid production, which exacerbates insulin resistance in liver and muscle. The β cell compensates for insulin resistance by secreting more insulin. Ultimately, the β cell can no longer compensate, leading to impaired glucose tolerance, and diabetes.

glycogenolysis. In addition to controlling the activities of metabolic enzymes by covalent modification through phosphorylation or dephosphorylation as stated above, insulin also regulates the expression of a number of genes encoding hepatic enzymes. For example, the hormone dramatically inhibits the transcription of the gene encoding phosphoenolpyruvate carboxylase (*PEPCK*), the rate-limiting step in gluconeogenesis. Just as glucagon reverses many of the covalent modifications of metabolic enzymes by insulin, the peptide also exerts opposing effects on gene transcription. Hepatic cAMP elevation produced by glucagon increases *PEPCK* gene expression during hypoglycemia. Therefore, the activity of many metabolic enzymes reflects the summation of signals from both insulin and glucagon receptors, and changes constantly in response to alterations in the concentration of plasma glucose.

Metabolic Staging of Type 2 Diabetes

Insulin resistance, the failure to respond to normal circulating concentrations of insulin, is a common state associated with obesity, aging, a sedentary lifestyle, as well as a genetic predisposition. The failure of insulin to stimulate glucose uptake in muscle appears to be a primary defect. Also, in certain fat depots, subsequent resistance to the antilipolytic effects of insulin causes increased lipolysis and fatty acid release. These fatty acids attenuate the ability of insulin to suppress glucose production in the liver, but allow a continual increase in insulin-stimulated fatty acid synthesis. Thus, this dysregulation of carbohydrate and lipid metabolism accelerates the progression of insulin resistance. β cells of the pancreas normally compensate for the insulin resistant state by increasing basal and postprandial insulin secretion, further aggravating insulin resistance. At some point, the β cells can no longer compensate, failing to respond appropriately to glucose. This ultimately leads to the deterioration of glucose homeostasis and the development of glucose intolerance, the inability to properly dispose of glucose (Figure 1). Approximately 5%–10% of glucose-intolerant patients per year progress to frank diabetes, which continues to worsen as insulin resistance increases. Adipose and liver cells generate more fatty acids, the liver produces more glucose in an unregulated fashion, and the β cells undergo progressive decompensation, resulting in the late stages of the disease, where high doses of exogenous insulin may be required.

Signal Transduction Defects in Insulin Action

Modifications of the Insulin Receptor

Insulin resistance results from a combination of genetic and environmental factors. It can arise from defects in insulin signal transduction, changes in the expression of proteins or genes that are targets of insulin action, cross talk from other hormonal systems, or metabolic abnormalities. One potential site of insulin resistance may involve the receptor itself. The insulin receptor is an $\alpha_2\beta_2$ heterotetrameric receptor tyrosine kinase that is disulfide-linked. Upon binding insulin, the receptor undergoes intramolecular transphosphorylation on specific tyrosine residues, leading to increased kinase activity toward cellular substrates. Alterations in insulin receptor expression, trafficking, ligand binding, phosphorylation, and/or kinase activity have been identified in rare cases of severe insulin resistance, such as the Type A syndrome, leprechaunism, and Rabson-Mendenhall syndrome (Taylor and Arioglu, 1998). These arise from point mutations in the receptor, many of which produce a dominant-negative phenotype. In mice, tissue-specific knockout of the insulin receptor in muscle failed to produce diabetes, instead generating only a muscle-specific insulin resistance, with little effect on glucose homeostasis (Bruning et al., 1998). In contrast, disruption of the gene in β cells (Kulkarni et al., 1999) or liver (Michael et al., 2000) produced a diabetic phenotype. Moreover, elimination of the insulin receptor in brain produced increased food intake and obesity, and associated insulin resistance, suggesting a coordinated regulation of brain insulin signaling and energy homeo-

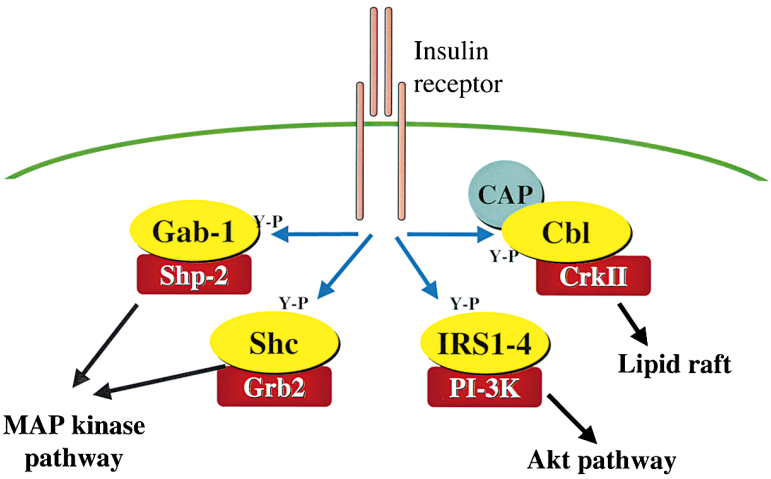

Figure 2. Tyrosine Kinase Substrates of the Insulin Receptor

Insulin stimulates the tyrosine kinase activity of its receptor, leading to the phosphorylation of a number of cellular substrates, including Gab1, Shc, IRS 1–4, and Cbl. Upon phosphorylation, each of these proteins can interact with SH2-containing proteins that direct a pathway of signal transduction. Gab1 appears to interact with the SH2 containing tyrosine phosphatase SHP2, leading to the activation of the MAP kinase pathway. Phosphorylation of Shc produces its binding to the adaptor protein Grb2, causing activation of the protooncogene *ras,* which is also upstream of the MAP kinase pathway. The IRS family of substrates can interact with a number of SH2 domain proteins. Notable among these is the regulatory subunit of PI 3-kinase, which leads to the activation of PIP_3-dependent protein kinases such as Akt. This pathway appears to be required for most of the cellular actions of insulin. The insulin receptor can also phosphorylate Cbl, via its recruitment to the receptor by the adaptor protein CAP. Upon phosphorylation, Cbl translocates to the lipid raft subdomain of the plasma membrane, where it interacts with the SH2 domain of the adaptor protein CrkII. This pathway is also necessary for the stimulation of glucose transport by insulin. IRS, insulin receptor substrate; PI-3K, phosphatidylinositol 3-kinase.

stasis (Bruning et al., 2000). Although care must be taken in interpretation of metabolic studies in rodent models and their extrapolation to human physiology, these findings challenge the current view of muscle as the primary site of insulin resistance and suggest an interaction between tissues in the regulation of glucose homeostasis more complex than previously anticipated. Thus, research concentrating only on muscle biopsies to measure the levels or function of genes or proteins involved in insulin action may miss critical defects that have indirect effects through other tissues.

Insulin Signaling Pathways

Although studies on the genetic forms of extreme insulin resistance in humans and in mouse knockouts have provided a conceptual framework for our understanding of insulin action, mutations in the insulin receptor are not common in the general population, suggesting that postreceptor defects represent the primary failures of insulin action in diabetes. Once activated, the insulin receptor phosphorylates a number of intracellular substrates on tyrosine, including members of the insulin receptor substrate family (IRS1/2/3/4), the Shc adaptor protein, Gab-1 and Cbl (Figure 2). Tyrosine phosphorylation of these proteins creates recognition sites for additional effector molecules containing Src Homology 2 (SH2) domains. Mice bearing a homozygous disruption of the *IRS1* gene develop a moderate state of insulin resistance (Araki et al., 1994; Tamemoto et al., 1994). These mice do not become diabetic, presumably due to β cell compensation. In contrast, homozygous disruption of the *IRS2* gene generates diabetes, due to insulin resistance coupled with impaired insulin secretion (Withers et al., 1998). Since skeletal muscle IRS2 does not appear to be necessary for insulin- or exercise-stimulated glucose transport, the insulin resistance observed in the *IRS2* knockout mice might reflect changes in hepatic insulin action plus alterations in β cell function or survival.

Tyrosine phosphorylation of the IRS proteins induces their binding to the SH2 domains of p85, the regulatory subunit of phosphatidylinositol (PI) 3-kinase. Activated PI 3-kinase generates the lipid phosphatidylinositol 3,4,5 trisphosphate (PIP_3). Increased PIP_3 leads to the activation of a protein kinase cascade, first stimulating the protein kinase PDK, which phosphorylates and activates two classes of serine/threonine kinases, Akt (also known as protein kinase B [PKB]), and the atypical protein kinase C isoforms ζ and λ (PKCζ/λ) (Figure 2). Studies using pharmacological inhibitors, microinjection of blocking antibodies, and expression of dominant-interfering mutants indicate a necessary role for PI 3-kinase activity in insulin-stimulated glucose uptake in fat and muscle cells (Czech and Corvera, 1999). To determine the in vivo role of PI 3-kinase in glucose homeostasis, mice were generated with a targeted disruption of the gene encoding the p85 regulatory subunit of PI 3-kinase (Terauchi et al., 1999). Paradoxically, these mice exhibited increased insulin sensitivity and hypoglycemia due to increased glucose transport in skeletal muscle and adipocytes. Although the significance of these findings is uncertain, it is possible that other isoforms of the regulatory protein are increased in compensatory fashion, or that p85 exists in excess to the catalytic subunit of the enzyme, so that a reduction in the expression of the former protein might lead to enhanced activation of the latter.

There may be a relative decrease in insulin-stimulated association of IRS proteins with PI 3-kinase and in activation of Akt in insulin-resistant skeletal muscle (Krook et al., 1997; Cusi et al., 2000). However, in one study, patients with reduced insulin-stimulated PI 3-kinase maintained normal activation of Akt, suggesting that only a relatively small activation of PI 3-kinase is necessary for the full expression of downstream signaling (Kim et al., 1999). These data imply that defects in the pathway leading from IRS tyrosine phosphorylation to Akt activation may not be responsible for insulin resistance in all patients with Type 2 diabetes, although more studies are needed.

Despite the general agreement that PI 3-kinase activ-

ity is necessary for insulin-stimulated glucose uptake, additional signals are required. For example, activation of PI 3-kinase by PDGF, or interleukin-4, or through engagement of integrin receptors does not stimulate glucose transport (Pessin and Saltiel, 2000). In addition, two naturally occurring insulin receptor mutations were fully capable of activating PI 3-kinase, yet were unable to mediate insulin action (Krook et al., 1997). Moreover, addition of a PIP_3 analog had no effect on glucose transport (Jiang et al., 1998). Thus, although PI 3-kinase activation is necessary, there is at least one additional pathway involved.

The PI 3-kinase-independent pathway might involve the tyrosine phosphorylation of the Cbl protooncogene (Ribon and Saltiel, 1997). This phosphorylation requires the presence of the adaptor protein CAP, which associates with a proline-rich domain in Cbl through its carboxy-terminal SH3 domain (Figure 2). CAP expression correlates well with insulin responsiveness; it is expressed in insulin-sensitive tissues, markedly induced during adipocyte differentiation, and is transcriptionally regulated by the thiazolidinedione family of insulin-sensitizing PPARγ agonists (Ribon et al., 1998) (see below). Upon phosphorylation, the cbl/CAP complex translocates to a lipid raft domain of the plasma membrane (Mastick and Saltiel, 1997). Expression of a dominant-interfering CAP mutant completely inhibited cbl translocation and insulin-stimulated glucose uptake (Baumann et al., 2000). The translocation of phosphorylated cbl recruits additional signaling proteins to the lipid raft, resulting in the activation of the G protein TC10. This molecular switch appears to provide a second signal to the Glut4 protein that functions in parallel with the activation of the PI 3-kinase-dependent signaling pathway (Chiang et al., 2001). However, there is no data yet available as to whether this pathway is attenuated in states of insulin resistance.

Glut4 and Glucose Transport

Insulin increases glucose transport in fat and muscle cells by stimulating the translocation of the transporter Glut4 from intracellular sites to the plasma membrane. While it is known that insulin-stimulated glucose transport is attenuated in insulin resistance (Shulman, 2000), our understanding of the precise mechanisms by which insulin directs the translocation of Glut4-containing vesicles to the cell surface remains incomplete. In the basal state, Glut4 continuously recycles between the cell surface and various intracellular compartments. Insulin markedly increases the rate of Glut4 vesicle exocytosis, and slightly decreases the rate of internalization. Although the mechanisms are unknown, it is likely that the Glut4-containing vesicle is tethered to intracellular sites, perhaps defined by a microtubule network (Guilherme et al., 2000). Phosphorylation events such as those catalyzed by PIP_3-dependent kinases might release the vesicle from these sites, allowing for trafficking of Glut4 to the cell surface.

The v-SNARE protein VAMP2 on Glut4-containing vesicles physically interacts with its t-SNARE counterpart syntaxin 4 during Glut4 vesicle docking and fusion with the plasma membrane (Pessin et al., 1999). One interesting possibility is that the PI 3-kinase-independent arm of insulin action may be directed at the docking and fusion step of Glut4 regulation. Although these SNARE interactions are essential, neither SNARE protein appears to be a direct target of insulin action. However, the SNARE accessory protein Synip may be involved in the control of Glut4 docking and fusion in an insulin-dependent, PI 3-kinase-independent manner (Min et al., 1999).

Regulation of Glycogen Synthesis Involves Targeted Signaling

Studies in cases of early onset insulin resistance (Warram et al., 1990) revealed that the first detectable lesion in insulin action lies in the uptake and storage of glucose as glycogen in muscle. Although it is likely that these observations were reflective of decreased glucose uptake in insulin-resistant subjects, glycogen storage is typically diminished in patients with Type 2 diabetes, and may represent a critical event in the pathophysiology of the disease. Insulin modulates glycogen accumulation through a coordinated increase in glucose transport and glycogen synthesis (Figure 3). The hormone activates glycogen synthase by promoting its dephosphorylation, via the inhibition of kinases such as PKA or GSK3 (Cross et al., 1995), and activation of protein phosphatase 1 (PP1) (Brady et al., 1997). Upon its activation downstream of PI 3-kinase, Akt phosphorylates and inactivates GSK-3, decreasing the rate of phosphorylation of glycogen synthase. However, inhibition of GSK-3 is not sufficient for glycogen synthase activation, since the kinase does not phosphorylate several residues of the synthase that are dephosphorylated by insulin signaling (Lawrence and Roach, 1997). Moreover, in certain model systems, GSK-3 inhibition can be dissociated from synthase activation, whereas activation of PP1 correlates well with changes in glycogen synthase activity (Brady et al., 1998).

Insulin does not globally activate PP1, but rather specifically targets discrete pools of the phosphatase, primarily increasing PP1 activity localized at the glycogen particle. The compartmentalized activation of PP1 by insulin is due to glycogen-targeting subunits which serve as "molecular scaffolds." bringing together the enzyme directly with its substrates glycogen synthase and glycogen phosphorylase in a macromolecular complex, and in the process exerting profound effects on PP1 activity in a substrate-specific manner (Newgard et al., 2000). Four different proteins with some overlap in tissue distribution, G_M, G_L, PTG, and R_6, have been reported to target PP1 to the glycogen particle. Despite a proposed common function, no two targeting subunits share more than 50% sequence homology, which is largely confined to the PP1- and putative glycogen binding regions. Overexpression of these scaffolding proteins in cells or in vivo by adenoviral-mediated gene transfer resulted in a dramatic increase in basal cellular glycogen levels, even when the extracellular media was supplemented with only amino acids in the absence of glucose (Berman et al., 1998). Furthermore, glycogen stores in the cells infected with PTG were refractory to breakdown by agents that raise intracellular cAMP levels. These results suggested that PTG overexpression locks the cell into a glycogenic mode (Newgard et al., 2000). Thus, although glucose transport is rate-limiting for glycogen synthesis, manipulation of proteins involved in the regulation of synthase and its regulators can have profound effects on glucose metabolism.

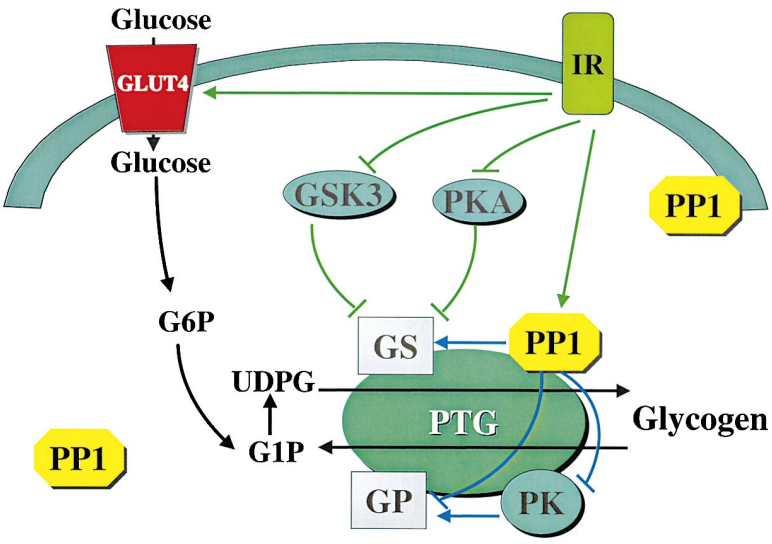

Figure 3. The Coordinated Regulation of Glucose Uptake and Glycogen Synthesis by Insulin

Insulin stimulates the uptake and storage of glucose in fat and muscle cells. The hormone stimulates transport of glucose, which is then phosphorylated by hexokinase, and converted into a substrate for glycogen synthase. This enzyme is regulated by insulin through allosteric and covalent modifications. Insulin produces a net reduction in the phosphorylation state of the enzyme, via inhibition of kinases such as PKA and GSK3, and activation of the phosphatase PP1. Glucose 6-phosphate and UDP-Glucose also allosterically activate the enzyme, and make it a better substrate for dephosphorylation. Glycogen phosphorylase is activated by the calcium-dependent phosphorylase kinase, and inactivated by dephosphorylation via PP1. These dephosphorylation events are coordinated by targeting proteins such as PTG, which assembles these proteins at the glycogen pellet into a signal reception module for regulation by insulin. IR, insulin receptor; PP1, protein phosphatase 1; PKA, protein kinase A; GSK3, glycogen synthase kinase 3; GS, glycogen synthase; GP, glycogen phosphorylase; PK, phosphorylase kinase; PTG protein targeting to glycogen; G6P, glucose 6-phosphate; G1P, glucose 1-phosphate; UDPG, UDP-glucose.

In addition to a primary defect in glucose transport, evidence exists for reduced glycogen synthase activity in certain populations (Shulman et al., 1990). A point mutation in G_M has been associated with altered rates of glucose metabolism in Danish subjects in vivo (Hansen et al., 1995) and increased sensitivity to glycogenolytic agents in vitro, although a linkage was not found in Japanese patients (Shen et al., 1998). Another mutation in the 3′ noncoding region of this gene may cause mRNA instability in Native American populations (Xia et al., 1998). While it is tempting to speculate about lesions in PP1 or synthase targeting in diabetes, there have been no reports of widespread changes in any of these genes that might explain reductions in nonoxidative glucose disposal in insulin resistance.

Obesity and Insulin Resistance

As described in an accompanying article (Spiegelman and Flier, 2001 [this issue of *Cell*]), a majority of individuals suffering from type 2 diabetes are obese, with central visceral adiposity, and an imbalance in energy intake and expenditure that leads to numerous metabolic abnormalities. Insulin resistance might be the result of obesity, but might also contribute to its development. Recent insights into the biology of the adipocyte as an endocrine organ have supported this latter idea. It is now known that this cell type is more than a storage site for lipids; it also secretes a number of important circulating factors, including leptin, TNFα, angiotensin, and PAI-1, and is the major source for endogenous production of nonesterified fatty acids (NEFA) via lipolysis (Kahn and Flier, 2000). Indeed, the dynamic interactions between the adipocyte, certain nuclei of the ventromedial hypothalamus and the organs of insulin synthesis and action insure the coordinated regulation of insulin sensitivity, as well as energy intake and expenditure.

The Central Regulation of Insulin Sensitivity

Prior to the development of transgenic and knockout technologies, most rodent studies on the insulin resistance of type 2 diabetes were performed with monogenic models of obesity, especially the *ob/ob* and *db/db* mice. Investigations by Friedman and others (Friedman, 2000) uncovered the genetic pathways responsible for obesity in these strains, defects in the adipocyte-derived hormone leptin (*ob/ob*) or its receptor (*db/db*). In addition to studies on the regulation of food intake and energy expenditure by this hormone, investigators have focused on the mechanism by which this system might impact on insulin sensitivity. Leptin acts primarily in the ventromedial hypothalamus, and lesions in this region of the brain are known to produce hyperinsulinemia and insulin resistance, implicating leptin or other centrally acting peptides in the control of insulin sensitivity. Moreover, leptin improves insulin sensitivity in normal rodents independent of effects on food intake and profoundly impacts different neuroendocrine pathways that modulate insulin action (Halaas et al., 1995; Shimomura et al., 1999b). However, leptin receptors are also found in many insulin target tissues, and the hormone has been reported to increase fatty acid oxidation, suggesting the possibility of a direct effect on the periphery (see accompanying article by Spiegelman and Flier, 2001, for more discussion).

The Randle Hypothesis Revisited

Evidence now suggests that visceral adipocytes may be relatively resistant to the antilipolytic actions of insulin (Kissebah and Krakower, 1994), and thus might preferentially release NEFAs into the circulation. Indeed, patients with uncontrolled diabetes typically have abnormally high levels of circulating lipids, especially NEFAs. How elevated NEFAs contribute to the dysregulation of glucose homeostasis, or directly impair insulin sensitivity or insulin secretion remains uncertain, but evidence is emerging that muscle, liver, and the β cell might be direct targets.

The idea that NEFAs might interfere with insulin action in muscle or liver was introduced by Randle in the 1960s (Randle et al., 1963). His group observed that increased

fatty acid flux would lead to increased acetyl CoA in mitochondria, which in turn could reduce pyruvate dehydrogenase activity, producing an elevation in citrate concentration. Citrate allosterically inhibits the activity of phosphofructokinase, leading to an accumulation of glucose 6-phosphate which in turn inhibits glucose phosphorylation by hexokinase, thus reducing glucose utilization, a common feature of insulin resistance and diabetes. Later studies in humans confirmed the idea, since lipid infusion significantly lowered glucose utilization (Roden et al., 1996). However, Randle might not have accurately predicted the site at which the effect is exerted. Petersen et al. (1998) showed that fatty acids could interfere with insulin signaling, resulting in decreased muscle glucose transport. This reduction in glucose transport produced parallel defects in glycogen synthesis and glucose oxidation in muscle. Since glycogen synthesis accounts for a much larger percentage of the flux, it accounts for a larger percentage of the defect. Additionally, recent studies in both rodents and humans using infusion of lipid while directly measuring insulin action by hyperinsulinemic clamp have reproduced the findings observed in patients with insulin resistance, showing a defect in glucose transport and glycogen synthesis, rather than a direct attenuation of glucose oxidation (Dresner et al., 1999).

How does increased NEFA exposure lead to an inhibition of insulin action in muscle? The primary site may lie just downstream from the insulin receptor itself. Elevations in plasma NEFAs inhibited the insulin-dependent tyrosine phosphorylation of IRS1 and its association with PI 3-kinase (Dresner et al., 1999). The diminished activation of this pathway might result from a direct interaction of fatty acids or metabolites with components of the phosphorylation pathway. In that regard, recent data indicate a strong correlation between accumulation of intramuscular lipids and insulin resistance, not only in diabetics, but across a wide range of glucose tolerant and intolerant subjects with or without obesity (Perseghin et al., 1999). Transgenic mice that are devoid of fat are severely insulin resistant, recapitulating the phenotype observed in patients with lipodystrophy (Gavrilova et al., 2000). These mice also have a 2-fold increase in intramuscular and intrahepatic triglycerides (Kim et al., 2000), due to the forced partitioning of lipid into muscle and liver. Earlier studies (Boden et al., 1994) reported a 3 hr lag in the inhibitory effect of lipid infusion, suggesting that some metabolism or accumulation of lipid to threshold levels was required. Although all of the studies reported thus far are correlative in nature, one interesting possibility is that fatty acid influx or accumulation leads to the activation of a serine kinase which phosphorylates IRS1, reducing its tyrosine phosphorylation by the insulin receptor, as discussed above. Schmitz-Peiffer et al. (1997) have proposed that specific isoforms of protein kinase C might serve this function, although there are several other possibilities.

The Single Gateway Hypothesis of Hepatic Metabolic Regulation by Fatty Acids

Much attention has also focused on the role of NEFAs in regulating hepatic glucose metabolism. NEFAs can stimulate gluconeogenesis in the liver, possibly by increasing expression of subunits of the glucose 6-phosphatase system (Rebrin et al., 1996). These findings led Bergman and coworkers to propose the "single gateway hypothesis" in which hormonal regulation of lipolysis in the adipocyte indirectly controls hepatic glucose output (Bergman and Ader, 2000). These investigators cite the observation that the visceral fat is less sensitive to insulin than subcutaneous fat, such that even after a meal, there is little suppression of lipolysis by the hormone in this fat depot. The resulting direct flux of fatty acids derived from these fat cells through the portal vein to the liver can directly stimulate glucose production, thus providing a single signal for insulin action and insulin resistance to the liver, via a visceral fat axis.

While this mechanism might help explain the coordinated regulation of glucose and lipid metabolism, there are also clear direct effects of insulin on hepatic gluconeogenic and glycogenic enzymes, both through transcriptional and posttranslational mechanisms (O'Brian and Granner, 1996). Insulin decreases transcription of the genes encoding gluconeogenic enzymes such as PEPCK, fructose 1,6 bisphosphatase, and glucose 6-phosphatase, and increases transcription of those encoding glycolytic enzymes such as glucokinase and pyruvate kinase, and lipogenic enzymes such as fatty acid synthase and acetyl CoA carboxylase. Recent studies suggest that many of these changes might be mediated by an increase in levels of the transcription factor SREBP1-c, whose mRNA levels are increased by insulin (Kim et al., 1998; Foretz et al., 1999; Shimomura et al., 1999a). Dominant-negative forms of SREBP1 can block expression of these gluconeogenic and lipogenic genes (Foretz et al., 1999), while overexpression can increase their expression (Shimomura et al., 1999a). Interestingly, hepatic SREBP levels are increased in rodent models of lipodystrophy, along with coordinated increases in fatty acid synthesis and gluconeogenesis, the exact phenotype observed in genetic models of obesity-induced diabetes (Shimomura et al., 2000). These observations led Shimomura et al. (2000) to speculate that increased expression of SREBP-1c might lead to the mixed insulin resistance observed in liver of diabetic rodents, with increased rates of both gluconeogenesis and lipogenesis. The pathways that account for the changes in SREBP1-c expression in response to insulin or other metabolic changes are not known, but probably lie downstream of the IRS/PI 3-kinase pathway.

Cytokines and Insulin Resistance

In addition to generating increases in fatty acids, the development of obesity may provide other critical signals that contribute to insulin resistance. While the central nervous system plays an important role in regulating insulin sensitivity, there is also growing evidence that adipocyte-derived cytokines, particularly TNFα, might block insulin action. Some, but not all studies have indicated that TNFα may be elevated in states of obesity, perhaps reflecting increased adiposity (Peraldi and Spiegelman, 1998). The cytokine exerts catabolic effects on the adipocyte, blocking lipid synthesis and lipoprotein lipase expression, while activating lipolysis, and can block or reverse differentiation of fibroblastic precursors into adipocytes (Hotamisligil, 1999). These data suggest that TNFα might normally function in a feedback pathway to limit adipocyte number or lipid storage.

Hotamisligil (1999) has suggested that excessive TNFα, perhaps at a local level, might mediate obesity-

induced insulin resistance, although studies with blocking antibodies or knockout of TNFα or its receptors have not been consistent (Ofei et al., 1996; Ventre et al., 1997; Schreyer et al., 1998). In vitro studies revealed that TNFα can impair insulin signaling (Hotamisgil et al., 1996). Although the mechanisms have not been fully elucidated, TNFα may stimulate the serine phosphorylation of IRS proteins, leading to decreased tyrosine phosphorylation and PI 3-kinase association. Although the kinases that catalyze these phosphorylations remain unknown, there are a number of candidates that are suggested in the cytokine literature, including the MAP kinases, ceramide-activated kinases, and IKB kinase. Studies using inhibitors and dominant-negative forms of these enzymes may help to clarify the potential role of this pathway. However, interpretation of these in vitro approaches is difficult, since the cytokine can reverse the adipogenic phenotype in cultured cells, thus obscurring the data.

Defective Glucorecognition in the β Cell Is Critical to the Development of Diabetes

As described above, insulin is released from the β cell as a consequence of glucose metabolism, and the development of Type 2 diabetes occurs upon the failure of the β cell to compensate adequately for insulin resistance by secreting more insulin. Studies in monogenic obese rodent models of Type 2 diabetes have shown a general pattern of expansion of β cell mass to compensate for increased insulin demand, followed by decreased glucose recognition, islet degeneration, degranulation, and loss of β cells (Cavaghan et al., 2000). There have been few morphological examinations of pancreata in patients with Type 2 diabetes, yet it is likely that a similar sequelae occurs. Although the mechanisms underlying this phenomenon remain unknown, both genetic and environmental factors are thought to be involved.

Failure of β Cell Compensation

In addition to an increase in β cell mass, the compensatory increase in insulin secretion during states of insulin resistance is the result of elevated expression of certain genes encoding proteins of glucose metabolism. Both mechanisms are evident in the Zucker fatty rat, which develops insulin resistance and hyperinsulinemia, without progressing to hyperglycemia (Tokuyama et al., 1995). In this rat, the increase in islet mass seems to reflect β cell proliferation, perhaps with some changes in matrix proteins surrounding the β cells. Increased expression of glucokinase and hexokinase also appears to contribute to the hypersecretion of insulin, by increasing glucose phosphorylation, making more substrate available for glycolysis. The signal that produces these changes remains uncertain, but may be glucose itself, or perhaps even insulin, generating a positive feedback loop.

Unlike the Zucker fatty rat, the male Zucker diabetic fatty rat progresses to frank diabetes due to failure to compensate adequately for insulin resistance, suggesting a modifier gene present in one or the other strain that directly impacts β cell function. Studies on cell turnover in these animals suggest that the primary defect lies not in an inability of β cells to proliferate but rather in an enhanced rate of apoptosis (Pick et al., 1998). A similar phenotype has been observed in mice in which IRS2 or the insulin or IGF1 receptors have been deleted in a β cell–specific manner (Withers et al., 1998; Kulkarni et al., 1999), suggesting that the insulin or IGF1 receptors signal in a critical way for β cell function or survival.

Lipid Metabolism and β Cell Function

In vitro and in vivo studies have shown that NEFAs can acutely enhance insulin secretion and support the glucose response (Stein et al., 1996). Suppression of lipolysis with nicotinic acid inhibits insulin secretion, although the mechanism for this action remains unknown. However, prolonged exposure to NEFAs appears to reduce insulin secretion, and prevent β cell compensation in Zucker rats (Carpentier et al., 1999), although it remains unclear whether a similar phenomenon occurs in humans. The mechanism of "lipotoxicity" in the β cell remains uncertain. Unger and colleagues have suggested that accumulation of triglycerides increases nitric oxide, which causes oxidative damage and apoptosis in the cells (Shimabukuro et al., 1997; Unger et al., 1999).

Genetics of Human Type 2 Diabetes

Genetic factors profoundly influence insulin sensitivity. Insulin resistance occurs commonly in relatives of type 2 diabetics, and offspring of two type 2 diabetic parents are invariably insulin resistant. In addition, studies in monozygotic twins reveal a high heritability of diabetes, with a 50%–75% estimate in the heritability of insulin resistance. Given that genes influence glucose homeostasis, the major question arises as to whether these traits are the consequence of one or two "major' genes, or a large number of "minor" genes, that may be expressed only under the appropriate environmental insults. While this issue remains unresolved, a number of groups have looked at obvious candidate genes, attempting to associate clear differences in metabolic phenotype with mutations or polymorphisms in known genes. Genes encoding proteins involved in insulin signaling, glucose transport, glycogen synthesis, fatty acid uptake and synthesis, and adipocyte differentiation have all yielded associations with diabetes, but in only perhaps 2%–5% of patients, suggesting that such syndromes are relatively rare.

In recent years, most investigators in this area have focused on identifying diabetes genes via positional cloning methods, looking for linkage between regions of chromosomal DNA that are shared in affected family members. A number of relatively uncommon monogenic forms of the disease, known as maturity-onset diabetes of the young (MODY), have been attributed to mutations resulting in β cell dysfunction. These patients usually present in adolescence to early adulthood, with defective insulin secretion in response to glucose. Five of these genes include the glucose metabolizing enzyme glucokinase, transcription factors HNF1α and β, HNF4α, and insulin promoter factor 1 (IPF1) (Hattersley, 1998; Permutt and Hattersley, 2000). These mutations can lead to moderate to significant reductions in insulin secretion, with consequent development of hyperglycemia. While the incidence of these monogenic forms of diabetes is not precisely known, it may represent up to 5% of all patients with Type 2 diabetes.

Table 1. Some Therapeutic Targets in Type 2 Diabetes

Protein Class	Target	Action	Aim
Cell Surface Receptors	Insulin receptor	Agonist	Insulin mimetic
	Glucagon receptor	Antagonist	Lower fasting glucose
	β-3 Adrenergic receptor	Agonist	Increase lipolysis
	GLP receptor	Agonist	Increase insulin secretion
Protein kinases	AMP-activated kinase	Activator	Increase glucose transport
	Protein kinase C	Inhibitor	Block receptor desensitization
	MAP kinase	Inhibitor	Block receptor desensitization
	Ceramide activated kinase	Inhibitor	Block receptor desensitization
	IκB kinase	Inhibitor	Block receptor desensitization
	GSK-3	Inhibitor	Activate glycogen synthase
Protein phosphatases	PTP1b	Inhibitor	Block receptor dephosphorylation
	LAR	Inhibitor	Block receptor dephosphorylation
	PP1	Activator	Activate glycogen synthase
Lipid Phosphatases	SHIP2	Inhibitor	Increase PIP_3-stimulated
	PTEN		glucose transport
Adaptor proteins	Synip	Inhibitor	Increase glucose transport
Transcription factors	PPARγ	Selective modulator	Insulin sensitizer
	HNF4	Selective modulator	Increase insulin secretion
Ion channels	Sulfonylurea receptor	Inhibit K channel	Increase insulin secretion

A number of genomic scans have been carried out to define the more common polygenic forms of the disease. Studies concentrating on several ethnic and racial groups have identified a series of linkages across several regions of the genome. The first of these studies to be completed focused on 170 Mexican-American families in Starr County, Texas, identifying a linkage on Ch2p37 (Hanis et al., 1996). This locus was shown to require another locus on Ch15 for its effects. The recently cloned gene encodes for a novel calpain, called calpain10 (CAPN10). A pair of haplotypes was found that were defined by a polymorphism in an intron, at a site that may possibly influence transcription of the *CAPN10* gene (Horikawa et al., 2000). While the genotype is associated with increased incidence of insulin resistance in Pima Indians, along with a reduction in CAPN10 mRNA levels (Baier et al., 2000), it has yet to be proven that a genetic variation in CAPN10 can cause diabetes. The function of the CAPN10 protein remains unclear, as does the mechanism by which it might impact on glucose metabolism. Other isoforms of calpain may be involved in signaling or fat cell differentiation. However, there is as of yet no evidence that CAPN10 has enzyme activity, and its role in metabolism or signal transduction remains unknown.

The elucidation of the human genomic sequence is expected to accelerate the identification of diabetes genes. However, finding positional candidates will require strong evidence of linkage between a chromosomal region and a metabolic abnormality that predisposes to a clinically relevant phenotype, and then proof that the genetic variant causes meaningful changes in function. A major challenge will entail understanding the gene/environment or gene/gene interactions that are likely to be required for the development of diabetes.

New Molecular Targets for Antidiabetic Therapy

The advances in our understanding of the molecular pathways that underlie insulin action and secretion and intermediary metabolism point to a number of new opportunities for therapeutic intervention. Recent advances in mechanisms of signal transduction, cell differentiation, and membrane trafficking have led to new approaches (Table 1). A major challenge of these efforts will include the need to specifically target molecules that impact on metabolism, without interfering with or activating other pathways that might lead to unacceptable side effects. Thus, it will be necessary to keep in mind tissue distribution, cellular location, isotype selectivity and kinetics when designing or searching for modulators of enzymes, receptors, or macromolecular interactions.

PPARγ Modulators Improve Insulin Sensitivity

The thiazolidinedione (TZD) drugs improve insulin resistance in a variety of obese and diabetic animal models and patients (Saltiel and Olefsky, 1996). These drugs reduce plasma glucose levels and concomitantly lower hyperinsulinemia by improving the stimulation of glucose disposal and the inhibition of hepatic glucose production by insulin. It is now clear that the antidiabetic actions of TZDs are due to the activation of the peroxisome proliferator activated receptor gamma (PPARγ) (Lehmann et al., 1995), a member of the nuclear receptor superfamily of transcription factors (Braissant et al., 1996). While the identities of endogenous ligand(s) for PPARγ remain uncertain, various lipid or prostaglandin molecules have been proposed (Forman et al., 1995; Kliewer et al., 1997). PPARγ exists as a heterodimer with the nuclear receptor RXR. The heterodimer binds to PPAR response elements (PPREs) in the promoters of target genes and recruits a histone acetylase, activating transcription (Glass and Rosenfeld, 2000).

PPARγ activation by ligands correlates well with the antidiabetic actions of these agents, suggesting that regulated expression of PPAR-responsive genes accounts for the insulin-sensitizing effects. However, it has been difficult to identify precisely the genes that are responsible for the reversal of insulin resistance. Thus far, most of the genes known to change in response to TZDs encode lipogenic proteins that are associated with

differentiation of adipocytes; few have been found that are regulated in terminally differentiated cells. The discovery that the gene encoding the signaling protein CAP (see above) is upregulated by PPARγ in mature fat cells, both in vitro and in vivo, suggests a mechanism for increased insulin-stimulated glucose uptake by PPARγ activators (Ribon et al., 1998). Another potential target is the adipocyte-derived protein resistin, the expression of which is reduced after PPARγ activation (Steppan et al., 2001). Resistin may act as a negative regulator of insulin action, although more characterization of this interesting protein is needed. Moreover, it is possible and perhaps likely that the PPARγ activators operate through secondary mechanisms, including a decrease in circulating free fatty acid levels or increased oxidation of lipids in muscle (Saltiel and Olefsky, 1996).

While the search for new activators of PPARγ represents an obvious target of antidiabetic drug discovery, genetic variations and knockouts of the receptor have complicated the issue. Although homozygous PPARγ null animals were not viable due to placental lethality, heterozygous PPARγ$^{+/-}$ mice surprisingly exhibited normal glucose tolerance in the face of reduced insulin levels, suggesting a state of enhanced insulin sensitivity (Barak et al., 1999; Miles et al., 2000). This paradoxical finding was confirmed by studies that revealed a substantial increase in insulin-stimulated glucose disposal (Miles et al., 2000), and in separate studies that showed a resistance to high fat diet–induced insulin resistance (Kubota et al., 1999).

Although it is possible that these results reflect an indirect modulation of insulin resistance through changes in the metabolic milieu, they suggest that PPARγ activators might not act purely as agonists for the receptor, but may also antagonize effects of natural ligands at some sites (Camp et al., 2000). Indeed, some TZDs are partial agonists of PPARγ, and different TZDs induce an overlapping but distinct set of genes, suggesting that the conformation and activity of the TZD/PPARγ complex might depend upon ligand structure or promoter context (Camp et al., 2000). These data have led to the concept of the selective PPAR modulator (Olefsky and Saltiel, 2000). This model expands the signaling repertoire of a specific nuclear receptor, since it would allow a response to a given endogenous ligand in a way that is gene context specific. Moreover, different endogenous ligands, working through the same nuclear receptor, could hypothetically lead to different responses in different tissues. Thus, identification of the appropriate "molecular signature" for a ligand, based on binding properties, molecular structure, cataloged changes in gene expression in target tissues, coactivator and corepressor binding patterns, and in vivo phenotype regarding efficacy and side effect profile, might lead to the development of rationally designed antidiabetic drugs that are greatly improved over the TZDs.

Targeting Insulin Signaling

The insulin receptor and its signaling pathways are also a fertile area for drug development. Efforts are underway to identify small molecule mimics of the receptor (Zhang et al., 1999), although whether these will lead to effective treatments of insulin resistance remains uncertain. The receptor might also be negatively regulated by protein tyrosine phosphatases. Elevated expression of PTP1b and LAR has been reported in insulin-resistant patients (Drake and Posner, 1998). Overexpression of these enzymes in cultured cells prevents insulin receptor kinase activation. A PTP1b knockout mouse was more insulin sensitive than control littermates, although it remains uncertain whether this resulted from a direct improvement in insulin signaling in all affected tissues (Elchebly et al., 1999), or an indirect effect due to changes in energy expenditure. In either case, inhibition of PTP1b represents an obvious target for drug discovery.

Since there is evidence that serine kinases may phosphorylate and thus inhibit the tyrosine phosphorylation of IRS1 in a variety of experimental paradigms of insulin resistance, identification of these kinases and specific inhibitors represents another rich area for antidiabetic therapy. Along the same lines, the products of PI 3-kinase play a critical role in insulin action, and might be reduced in states of insulin resistance. In this regard, the pathway could also be negatively regulated by polyphosphoinositide phosphatases, such as PTEN or SHIP2. SHIP2 has been implicated in regulating insulin sensitivity (Wada et al., 1999), and might be an attractive target for inhibition, especially considering its restricted expression in insulin target tissues.

Targeting Glucose Utilization

As described above, the earliest detectable lesion in insulin resistance involves the stimulation of nonoxidative glucose metabolism by insulin, including glucose transport, phosphorylation, and storage as glycogen. While the mechanics of Glut4 translocation remain largely unexplained, there are clues about some therapeutic targets, including proteins involved in the regulation of Glut4 vesicle docking described above. Another approach emerges from insights into glucose transport in exercise. Recent studies suggest that the stimulation of Glut4 translocation in response to acute exercise or hypoxia utilizes an insulin-independent pathway, possibly involving activation of AMP-activated kinase (Hayashi et al., 1998). Although the protein substrates of this kinase are unknown at present, this enzyme may represent an attractive target for development of activators, which might also produce beneficial effects on lipids (Kemp et al., 1999).

Although untested, it is also possible that direct stimulation of glycogen synthesis might mimic or enhance insulin action. As described above, the phosphorylation state of glycogen synthase and phosphorylase are controlled by the kinases GSK3 and PKA, and the phosphatase PP1, all of which are potential therapeutic targets. Although glucose transport is clearly the rate-limiting step in glucose utilization, it is possible that the stimulation of glycogen synthesis might sensitize cells to insulin, via a "pull" mechanism. Appropriate targeting of molecules that modulate the activities of these enzymes is critical, since they are involved in a number of other processes that critically control cell growth and apoptosis. In this regard, it will be important to take into consideration the scaffolding roles played by the glycogen-targeting subunits.

β *Cell Targets*

The most widely used antidiabetic drugs stimulate insulin secretion and are typified by the sulfonylurea class, which directly inhibit the β cell isoform of a specific K channel. However, while these drugs are effective in

patients with Type 2 diabetes, they have significant side effects, and might accelerate the progression of the disease, as well as some of the cardiovascular liabilities associated with hyperinsulinemia. On the other hand, restoration of normal glucose responsiveness of the β cell, or prevention of the events leading to decompensation is an important goal of antidiabetic therapy. While breakthrough therapies will require more insight into the molecular basis of β cell dysfunction and apoptosis, there are a number of candidate targets known from studies on apoptosis in other systems and glycolytic stimulus secretion coupling in the β cell.

Summary and Future Directions

It is clear that research into diabetes and its related syndromes is a complex topic. While I have attempted to highlight some of the important areas, there are other subjects that also deserve mention. Some of these include the important role of endothelial function and nitric oxide synthase in insulin action (Shankar et al., 2000), counterregulatory hormones such as glucagon and amylin (Shah et al., 2000), steroid-induced diabetes (Strack et al., 1995), gestational diabetes (Kjos and Buchanan, 1999), transcription of the insulin gene (Ohneda et al., 2000), developmental biology of β cells (Schuldiner et al., 2000), degradation of insulin (Duckworth et al., 1998), peptides that control insulin secretion (Drucker, 1999), other central regulators besides leptin (Friedman, 2000), and the major influence of diabetes on lipoprotein and lipid metabolism (Ginsberg, 2000), to name a few. I have also not discussed advances in gene therapy or additional therapeutic targets that are under investigation, nor have I touched upon many of the animal models that have taught valuable lessons about genetics, metabolism, and the complex interactions between tissues (Leiter et al., 1998).

The progress in understanding the metabolic staging of diabetes over the past few years has led to significant advances in the regimen of treatment of this devastating disease. Indeed, there are now tools in the therapeutic armamentarium that allow for the targeting of both insulin resistance and secretion. However, it remains unclear whether current therapeutic approaches truly tackle the underlying metabolic defects. Improvements in the treatment or prevention of the disease will depend on understanding the underlying molecular pathophysiology in more detail. Indeed, advances in genetics, signal transduction, and the neurobiology of energy intake and metabolism should permit a more precise and perhaps individualized approach to therapy, allowing us to focus the attack where the problem lies. This alone is reason for optimism.

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