Cell signaling and animal disease model in glucose metabolism
포도당 대사에서 세포신호 전달과 동물질환 모델

B. C. Park / Research Center of Bioscience and Biotechnology, CTC BIO, Inc.

요 약

포도당 대사에서 중심적인 역할을 담당하고 있는 호르몬인 인슐린의 작용기전은 이전에 알려진 것보다 매우 다양하고 복잡한 세포내 신호전달 체계에 의해서 수행된다. 지난 10여년간 이러한 세포 신호전달 흐름내 중간물질인 여러 가지 단백질의 역할에 대해서 많은 연구가 진행되어 적지 않은 성과를 거두고 있다. 근래에는 해당 유전자에 대해 분자생물학적인 조작을 가하여 실험동물 체내에서 이러한 중간 단백질이 발현되지 못하도록 한 후, 각 조직에서의 인슐린 신호전달 체계를 더욱 심도 있게 연구하고 있다.

여러 연구방법 가운데서, 특히 조직 특이적으로 특정 유전자를 제거하여 태어난 개체를 이용하고, 또한 이들을 서로 교배시켜 나타나는 현상을 연구하여 비정상적인 포도당대사를 이해하는데 많은 도움을 받고 있다. 동물종에 따른 대사상의 차이점 등으로 인해 적지 않은 한계를 갖고 있지만, 동물 질환모델은 포도당 대사와 같은 생체 내에서 중요한 작용을 깊게 이해하고 더 나아가서는 비정상적인 대사를 밝히는데 핵심적인 역할을 수행하고 있다. 기초분야의 성과를 활용하는 응용학문인 축산분야에서도 이러한 접근방법과 연구 결과는 시사하는 바가 있을 것이다.

Introduction

Insulin acts through a cell surface receptor that belongs to a subfamily of growth factor receptor tyrosine kinases. There are two main pathways that propagate the signal generated through insulin. Research using mice bearing targeted gene mutations that affect insulin action and β-cell function has contributed important new information to our understanding of the pathogenesis of glucose metabolism. The phenotypes of these mutant mice have shed new light on mechanisms and sites of insulin resistance in vivo. Moreover, they have enabled investigators to analyze the genetic interactions underlying the complex mechanism of glucose abnormalities.

The abnormal glucose metabolism exerts its pathological condition in two forms, type 1 and type 2 diabetes. The type 1 diabetes is insulin-dependent, whereas type 2 is insulin-independent, so called non insulin-dependent diabetes mellitus(NIDDM). Type 2 diabetes is caused by genetic and environmental factors that affect the ability of the organism to respond to insulin. This impairment results from decreased insulin action in target tissues and insulin production in β-cells.

In this section, we will review recent progress of cellular signaling in insulin action and evaluate its relevance to the animal disease model of pathophysiological glucose metabolism in mice.

Overview of Insulin Action

Insulin acts through a cell surface tyrosine kinase receptor that belongs to a subfamily of growth factor receptor. The subfamily comprises three members: the insulin receptor (IR), the type 1 IGF receptor (IGF-1R) and the IR-related receptor (IRR) (Ullrich, et. al., 1986; Shier and Watt, 1989). Interestingly, although the three members of this subfamily are highly conserved from a structural standpoint, their functions are quite distinct, with IR regulating fuel metabolism and the IGF-1R regulating growth. No clear-cut function can be ascribed to the IRR, in part because it is unable to bind any of the ligands that are known to activate the other two receptors: insulin, insulin-like growth factor (IGF)-1 and IGF-2 (Accili, 1999). (Fig. 1).

Insulin binding activates the receptor, which phosphorylates a number of intracellular substrates and initiates the biological response to insulin (Fig. 2). There are two main pathways that propagate the signal generated through insulin and IGF-1Rs: the insulin receptor substrate (IRS)/phosphatidyl-inositol (PI) 3-kinase pathway, and the Ras/MAP kinase pathway (White, 1996). The IRS/PI 3-K pathway leads to the activation of a cascade of PI-dependent kinases. These include PDK1, PKC isoforms and the serine/threonine kinase AKT. AKT phosphorylates glycogen synthase kinase 3 (GSK3), cGMP-inhibitable phosphodiesterase and FKHR transcription factors, leading to the stimulation of glucose transport and glycogen synthesis, and the inhibition of lipolysis. (Kohn, et. al., 1996). The Ras/MAP kinase pathway can be activated by insulin through the formation of complexes between the exchange factor SOS and GRB2. GRB2 can be activated by IRS or SHC, which are direct substrates of the IR kinase. It appears that the acute metabolic effects of insulin require activation of the IRS/PI 3-K pathway, whereas the RAS/MAP kidnaps pathway may play a role in certain tissues to stimulate the actions of insulin on growth and proliferation. However, it is likely that pathways other than IRS/PI 3-kinase must be very important for insulin signaling, as indeed is becoming increasingly clear (Soni, et. al., 2000).

IRS proteins mediate insulin signaling

IRSs represent a family of proteins that mediate insulin and IGF actions. Insulin, insulin-like growth factors and interleukins stimulate IRS phosphorylation. A key signaling complex in insulin action is formed between IRS and the regulatory subunit of the enzyme PI 3-kinase, leading to the activation of the p110 (catalytic) subunit of PI 3-kinase.

The IRS family is composed of four closely related members (IRS-1 to IRS-4). It is conceivable that the presence of a unique assortment of phosphotyrosines in each molecule would allow for differential signaling. In addition, there are distinctive patterns of tissue expression, such that each IRS protein may play a different role in individual tissues.

AKT and glucose metabolism

Despite the large amount of information on mechanisms of signaling, the sequence of molecular events leading to glucose transporter translocation and glucose uptake is still not clear (Saltiel, 2001). More specifically, the identity of the kinase(s) that couples activation of PI 3-kinase to GLUT4 translocation 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. (2001) 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 evidence that Akt is indeed a physiological mediator of insulin action, although its specific role in GLUT4 translocation remains unclear.

What is the role of insulin signaling in skeletal muscle?

In human tissues, skeletal muscle accounts for the largest fraction of insulin-dependent glucose disposal. Epidemiological data indicate that resistance of skeletal muscle to insulin-dependent glucose uptake and phosphorylation is an early step in the development of pathological glucose metabolism. 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., 1995) 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/*loxp* system leads to impaired insulin signaling without insulin resistance (Bruning, et. al., 1997). IR signaling in muscle appears to require IRS-1, because ablation of IRS-2 has no effect on insulin-dependent glucose uptake (Higaki, et. al., 1999). In view of the lack of insulin resistance in mice with a complete knockout of the main insulin-responsive glucose transporter GLUT4, this body of work raised the question of whether skeletal muscle is indeed as key 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 indicated that two alternative signaling pathways compensate for the lack of IRs: IGF-I receptor (Shefi-Friedman, et. al., 2001). Moreover, shunting of glucose utilization from muscle to adipose tissue provides partial metabolic compensation in mice lacking IRs in muscle (Kim, et. al., 2000). In contract, 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 (Zisman, et. al., 2000). These studies indicate that, although the presence

of compensatory mechanisms enables mice lacking muscle IRs 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 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 (Kido, et. al., 2000a); 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 about 10% of human muscle glycogen content as a percentage of total body glycogen. 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. 3).

In general, molecular studies of the glucose abnormalities manipulating and utilizing animal disease model are bothered with uncertainties and complicated by the considerable variations of the animal genetic pool and background. To dissect the complex of insulin deficiency and, investigators have generated transgenic and knockout mice bearing mutations in genes required for insulin action and/or insulin secretion (Table 1).

Mice lacking *Ir* die of diabetes

The key role of IR in insulin action is demonstrated by the observation that targeted ablation of its gene results in neonatal death from diabetic ketoacidosis (Accili, et. al., 1996). Mice heterozygous for a null *Ir* allele develop strain-dependent insulin resistance and are a valuable tool to study insulin metabolism (Park et. al., 1999; Kim, et. al., 2001).

Targeted inactivations of *Irs*-1, -2, -3 and -4 suggest that each molecule plays a distinct, but overlapping role in insulin action

The four members of the IRS family of proteins are important in insulin action (White, 1998). *Irs*-1 inactivation in mice leads to growth retardation (both intrauterine and postnatal) and insulin resistance without diabetes, suggesting that *Irs*-1 is more important for insulin-like growth factor (IGF)-1 function to stimulate growth than for insulin function to control fuel homeostasis (Fig. 4). However, lack of *Irs*-1 also impaired insulin secretion from β-cells, suggesting that insulin or IGF-1 signalling through IRS-1 is required for proper β-cell function. Moreover, combined heterozygosity for *Ir* and *Irs*-1 null alleles in mice causes a severe impairment of insulin action associated with a sharp rise in the incidence of diabetes in the resulting progeny (Kido,

et. al., 2000b), suggesting that IRS-1 has an important role in insulin action.

Mice lacking *Irs*-2 develop diabetes and, in some genetic backgrounds, die from nonketotic hyper-osmolar coma (Withers, et. al., 1998). The main abnormality caused by lack of *Irs*-2 is defective β-cell growth. However, moderate hepatic insulin resistance is also present. These data are consistent with an important role of *Irs*-2 in fuel homeostasis but also underline the conclusion that multiple substrates are required to mediate insulin action, as the phenotype resulting from the lack of *Ir* is substantially more severe than that resulting from the lack of *Irs*-2 (Rother, et. al., 1998).

Irs-3 is the main IRS in adipocytes (Smith-Hall, et. al., 1997). Lack of Irs-3 has no appear phenotype. However, this may result from compensation by *Irs*-1 in adipose cells, as a preliminary report indicates that combined knock-outs of Irs-1 and Irs-3 in adipose cells impair insulin action (Curtis, et. al., 2000).

Ablation of *Irs*-4 results in modest growth retardation, impaired glucose tolerance and reproductive abnormalities (Fantin, et. al., 2000). This phenotype is reminiscent of the *Irs*-1 phenotype, albeit considerably milder. It remains to be seen whether this subtle phenotype is caused by compensation by other family members.

Role of *Igf1r* in metabolic control

Lack of *Igf1r* impairs fetal growth, resulting in mice that weigh 45% of the normal at birth, and is incompatible with postnatal survival (Efstratiadis, 1998). Interestingly, this phenotype can be rescued by combined lack of *Igf2r*, suggesting that *Ir* can compensate for the growth-promoting actions of *Igf1r* when circulating IGF-2 levels rise. Recently, Withers and co-workers have proposed that *Igf1r* promotes β-cell growth through *Irs*-2 (1999).

Pathogenesis of insulin resistance : tissue-specific *Ir* knock-outs

The role of specific tissues in the pathogenesis of insulin resistance has been addressed by using dominant negative mutants and tissue-specific recombination (Bruning, et. al., 1998). These results have contributed to a reassessment of the pathophysiology of glucose metabolism (Fig. 5).

Muscle and fat-specific *Ir* knock-out

It has been proposed that combined insulin resistance in muscle and adipose tissue is sufficient to cause type 2 diabetes through a direct effect to impair glucose uptake and an indirect effect mediated by free fatty acids to impair glucose oxidation and insulin secretion (Kadowaki, 2000). Lauro and co-workers have shown, by using a dominant negative mutant *Ir*, that selective insulin resistance in skeletal muscle and fat is associated with impaired glucose tolerance and insulin resistance, without overt type 2 diabetes (1998). These findings suggest that, in mice, compensation by the liver for the

impairment of insulin action in muscle and fat protects against the development of diabetes. Nevertheless, it should be considered that the liver accounts for a much larger fraction of glucose storage in rodents than it dose in humans.

Muscle-specific *Ir* and Glut4 knock-outs

Skeletal muscle is thought to be the primary site of insulin resistance in mammalian tissues (Reaven, 1996). Bruning and co-workers have generated mice that lack *Ir* in muscle by crossing mice with a 'floxed' *Ir* allele with transgenic mice expressing the Cre recombinase in skeletal muscle (1998). *Ir* inactivation failed to cause insulin resistance, a rather unexpected finding that can be explained by the compensatory role of contraction or *Igf1r* to stimulate glucose uptake. In fact, when a similar mutation was introduced into the gene encoding the insulin responsive glucose transporter, *GLUT4*, mice developed insulin resistance and diabetes, consistent with the notion that a 'distal' alteration in the insulin signalling pathway in muscle does indeed cause an impairment of glucose homeostasis (Zisman, et. al., 2000).

Lack of *Ir* in liver impairs glucose homeostasis

The prediction of Lauro and co-workers is confirmed by a study of liver-specific *Ir* inactivation, obtained through Cre-loxP *in vivo* recombination. Mice lacking hepatic *Ir* exhibit severe insulin resistance, glucose intolerance and dysregulated hepatic glucose production. The phenotype improves with age, as liver function deteriorates and glycogen stores decrease (Michael, et. al., 2000). These data also indicate that insulin has a direct effect in hepatocytes, in addition to its indirect effect through peripheral substrate utilization.

β-cell-specific *Ir* knock-out

Ir inactivation in β cells (Kulkarni, et. al., 1999) causes defective glucose-induced insulin secretion, similar to the defect seen in type 2 diabetes, as well as *Irs*-1 knock-out mice. This observation suggests that insulin signalling plays a physiological role in insulin secretion. If this novel hypothesis finds further experimental confirmation, it would indicate that defective insulin signalling may account for both metabolic abnormalities of diabetes, ie peripheral insulin resistance and impaired β-cell function.

Brain-specific *Ir* inactivation

The central nervous system expresses high levels of *Ir* and *Igf1r*, which have been thought to regulate food intake and satiety. *Ir* inactivation in the brain by using a neurone-specific *Cre* recombinase did not affect brain development and structure, but resulted in increased food intake in female mice, diet-sensitive obesity in both sexes, and subfertility caused by hypothalamic hypogonadotrophic hypogonadism. These data

indicate an important function of neuronal *Ir* in energy homeostasis and reproduction (Bruning, et. al., 2000).

Irs-1 and *Irs*-2 mutations cause tissue-specific insulin resistance

Mice with combined heterozygous null mutations in *Ir*, *Irs-1* and/or *Irs*-2 have indicated that *Irs*-1 and *Irs*-2 mediate insulin action in different tissues (Bruning, et. al., 1997), thus providing a further explanation for the genetic heterogeneity of type 2 diabetes. Combined hetero-zygosity for *Ir/Irs*-$1^{+/-}$ and *Ir/Irs*-$2^{+/-}$ results in a similar prevalence of diabetes in mice. However, *Ir/Irs*-$1^{+/-}$mice are primarily insulin resistant in skeletal muscle, whereas *Ir/Irs*-$2^{+/-}$ mice are primarily insulin resistant in liver (Kido, et. al., 2000b). Similar data been obtained by analysing glucose disposal rates during glucose clamps in mice lacking either *Irs*-1 or *Irs*-2 and in studies of hepatocytes (Rother, et. al., 1998) and isolated muscle (Higaki, et. al., 1999) lacking *Ir*.

PI 3-kinase subunit p85α inactivation

p85α-deficient mice are insulin sensitive and present with hypoglycemia due to increased glucose transport in skeletal muscle and adipocytes (Terauchi, et. al., 1999). This evidence suggests that the regulatory subunit p85α behaves as an inhibitor of PI 3-kinase activity under basal conditions, whereas insulin acts to remove this inhibition via IRS proteins.

Glucokinase knock-out

Glucokinase is the low-Km glucose-phosphorylating enzyme expressed in pancreatic β-cells and liver. Mutations of glucokinase are associated with impaired insulin secretion in humans. Tissue-specific glucokinase inactivation in β-cells and liver reveals that β cell glucokinase plays a critical role in metabolic control and survival, and the hepatic enzyme is important to regulate glycogen synthesis and insulin secretion (Postic, et. al., 1995).

Glut2 inactivation

Glut2 is a low-affinity glucose transporter of β cells, liver, kidney and intestine and participates in on to become exocrine pancreatic cells, whereas cells in which Notch signaling is inhibited are committed to an endocrine fate (Guillam, et. al., 1997).

Summary

We have begun to define the role of various signaling proteins in insulin action. Targeted mutagenesis targeting the various signaling molecules in animals has enabled us to define the role of insulin signalling pathways and the function of different tissues in the pathogenesis of insulin action. Moreover, our knowledge of the abnormal glucose

metabolism has rapidly changed as a result of tissue-specific mutations. In addition, crosses among the various mutant animals have provided important evidence as to the mechanisms of pathophysiology of glucose metabolism. The use of animal disease models, with significant limitations due to species-specific metabolic differences, continue to play an important part in the process of novel discovery of the abnormalities in glucose metabolism.

〈 Literature cited 〉

- Abel ED, Kaulbach HC, Tian R, et al. 1999 Cardiac hypertrophy with preserved contractile function after selective deletion of GLUT4 from the heart. J Clin Invest. 104:1703-1714.
- Accili D, Kido Y, Nakae J, Lauro D, and Park BC 2001 Genetics of type 2 diabetes: insights from targeted mouse mutants. Curr Mor Med 1:9-23.
- Accili, D, Nakae, J, Kim, JJ, Park, BC and Rother, KI 1999 J. Pediatr. Endocrinol. Metab., 12, 475-485.
- Accili D, Drago J, Lee EJ, Johnson MD, Cool MH, Salvatore P, et al. 1996 Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 12: 106-109.
- Araki E, Lipes MA, Patti ME, et al. 1994 Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 372:182-186.
- Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. 2000 Role of brain insulin receptor in control of body weight and reproduction. Science: 289:2122-5.
- Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, and Kahn CR 1998 A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell 2: 559-569.
- Bruning JC, Winnay J, Bommer WS, Taylor SI, Accili D, Kahn CR 1997 Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. Cell 88:: 561-72.
- Chang PY, Goodyear LJ, Benecke H, Markuns JS, and Moller DE 1995 Impaired insulin signaling in skeletal muscles from transgenic mice expressing kinase-deficient insulin receptors. J Biol Chem 270: 12593-12600.
- Cho H, Mu J, Kim JK, Thorvaldsen JL, Chu Q, Crenshaw EB III, Kaestner KH, Bartolomei MS, Shulman GI, and Birnbaum MJ 2001 Insulin resistance and diabetes mellitus-like syndroms in mice lacking the protein kinase Akt2 (PKB beta). Science 292: 1728-1731.
- Curtis SE, Michael MD, Crute BE, Keller SR, Lienhard GE 2000 Double knockout of IRS proteins reveals critical roles of IRS-1 and IRS-3 in the maintenance of glucose homeostasis. Diabetes: 49 Suppl 1: A5.
- Efstratiadis A 1998 Genetics of mouse growth. Int J Dev Biol 42: 955-76.
- Eto K, Tsubamoto Y, Terauchi Y, et al. 1999 Role of NADH shuttle system in glucose-induced activation of mitochondrial metabolism and insulin secretion. Science 283:981-985.
- Fantia VR, Wang Q, Lienhard GE, et al. 2000 Mice lacking insulin receptor substrate 4 exhibit

mild defects in growth, reproduction, and glucose homeostasis. Am J Physiol Endocrinol Metab 278: E127-E133.

▶ Guillam MT, Hummier E, Schaerer E, et al. 1997 Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. Nat Genet 17:327-330.

▶ Higaki Y, Wojtaszewski JF, Hirshman MF, Withers DJ, Towery H, White MF, and Goodyear LJ 1999 Insulin receptor substrate-2 is not necessary for insulin-and exercise-stimulated glucose transport in skeletal muscle. J Biol Chem 274: 20791-20795.

▶ Kadowaki T 2000 Insights into insulin resistance and type 2 diabetes from knockout mouse models. J Clin Invest 106: 459-65.

▶ Katz EB, Stenbit AE, Hatton K, et al. 1995 Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. Nature 377:151-155.

▶ Kido Y, Philippe N, Schaeffer AA, and Accili D 2000a Genetic Modifiers of the insulin resistance phenotype. Diabetes 49: 589-596.

▶ Kido Y, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF, et al. 2000b Tissue-specific insulin resistance in mice with combined mutations of Insulin Receptor, IRS-1 and IRS-2. J Clin Invest 105: 199-205.

▶ Kim JJ, Park BC, Kido Y, Accili D 2001. Mitogenic nad metabolic effects of type 1IGF receptor overexpression in insulin receptor-deficient hepatocytes. Endocrinology 142: 3354-3360.

▶ Kim JK, Michael MD, Previs SF, Peroni OD, Mauvais-Jarvis F, Neschen S, Kahn BB, Kahn CR, and Shulman GI 2000 Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. J Clin Invest 105: 1791-1797.

▶ Kohn, AD, Summers, SA, Bimbaum, MJ and Roth, RA 1996 J. Biol. Chem., 271, 31372-31378.

▶ Kulkarni RN, Bruming JC, Winnay JN, Postic C, Magnuson MA, Kahn CR 1999 Tissue-specific knockout of the insulin receptor in pancreatic beta cells creates an insulin secretory defect similar to that in type 2 diabetes. Cell 96: 329-39.

▶ Lauro D, Kido Y, Castle AL, Zarnowski MJ, Hayashi H, Ebina Y, et al. 1998 Impaired glucose tolerance in mice with a targeted impairment of insulin action in muscle Liu SCH, Wang Q, Lienhard GE, et al. 1999 Insulin receptor substrates 3 is not essential for growth or glucose homeostasis. J Biol Chem 274:18093-18099.

▶ Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, et al. 2000 Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 6: 87-97.

▶ Park, BC, Kido Y, Accili D 1999 Differential signaling of insulin and IGF-1 receptors glycogen synthesis in murine hepatocytes Biochemistry 38: 7511-7523.

▶ Postic C, Shiota M, Niswender KD, et al., 1999. Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. J Biol Chem 274:305-315.

▶ Reaven GM 1996 Insulin resistance and its consequences: non-insulin-dependent diabetes millitus and coronary heart disease. In: LeRoith D, Taylor SI,Olefsky JM, eds. Diabetes mellitus: a

fundamental and clinical text. Philadelphia, PA: Lippincott-Raven 509-18.

- Rother KI, Imai Y, Caruso M, Beguinor F, Formisano P, Accili D 1998 Evidence that IRS-2 phosphorylation is required for insulin action in hepatocytes. J Biol Chem 273: 17491-7.
- Saltiel AR. 2001 New perspectives into the molecular pathogenesis and treatment of type 2 diabetes. Cell 104: 517-529.
- Shier, P and Watt, VM 1989 J. Biol. Chem., 264, 14605-14608.
- Shefi-Friendman L, Wertheimer E, Shen S, Bak A, Accili D, and Sampsom SR. 2001 Increased IGFR activity and glucose transport in cultured skeletal muscle from insulin receptor null mice. Am J Physiol Endocrinol Metab 281: E16-E24.
- Soni P, Lakkis M, Poy MN, Fernstrom MA, Najjar SM 2000 The differential effects of pp120 (Ceacam 1) on the mitogenic action of insulin and insulin-like growth factor 1 are regulated by the nonconserved tyrosine 1316 in the insulin receptor. Mol Cell Biol 20:3896-3905.
- Stenbit AE, Tsao TS, Li J, et al. 1997 GLUT4 heterozygous knockout mice develop muscle insulin resistance and diabetes. Nat Med 3:1096-1101.
- Terauchi Y, Tsuji Y, Saton S, et al. 1999 Increased insulin sensitivity and hypo-glycemia in mice lacking the p85 alpha subunit of phosphoinositide 3-kinase. Nat Genet 21:230-235.
- Ullrich, A, Gray, A, Tam, AW, Yang, FT, Tsubokawa, M, Collins, C, Henzel, W, Le, BT, Kathuria, S, Chen, E and et, al. 1986 EMBO J, 5, 2503-12.
- White, MF Philos. Trans. R Soc. Lond. B 1996 Biol. Sci., 351, 181-9.
- White MF 1998 The IRS-signalling systems: a network of docking proteins that mediate insulin and interleukin signalling. Mol Cell Biochem 182:3-11.
- Withers DJ, Sanchez-Gutierrez J, Towery H, et al. 1998 Disruption of IRS-2 causes type 2 diabetes in mice. Nature 391:900-904
- Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF 1999 Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat Genet 23:32-40.
- Zisman A, Peroni OD, Abel ED, Michael MD, Mauvais-Jarvis F, Lowell BB, Wojtaszewski JF, Hirshman MF, Virkamaki A, Goodyear LJ, Kahn CR, and Kahn BB 2000 Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. Nat Med 6: 924-928.

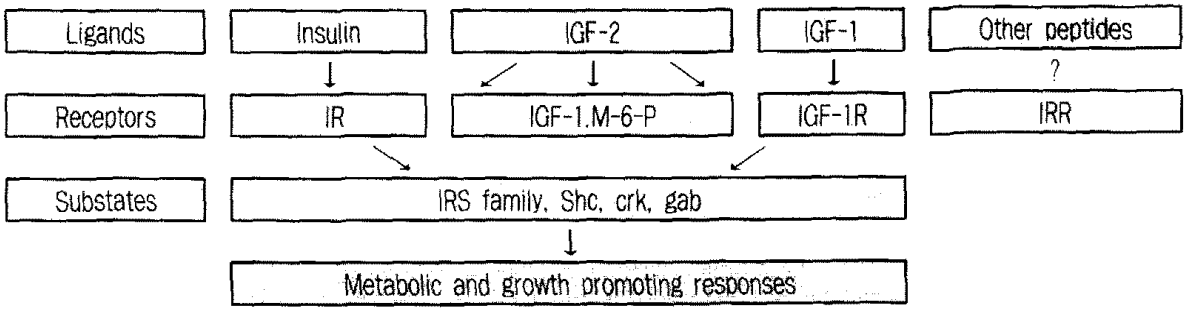

Figure 1. Interactions among ligands and receptors of the insulin family

This diagram the interaction of the known ligands of the insulin/insulin-like growth factor family with their receptors. Of note, both insulin and IGF-1 bind poorly to the cognate receptor, whereas IGF-2 binds to both receptors with equal affinities. In addition, IGF-2 binds to the IGF-2/mannose-6-phosphate receptor, which acts to remove IGF-2 from the circulation, but does not mediate IGF-2 signaling. The IR-related receptor (IRR) is indicated at the right end of the diagram. The ligand for this receptor is unknown. None of the known peptides that have been shown to bind to insulin or IGF receptors binds IRR.

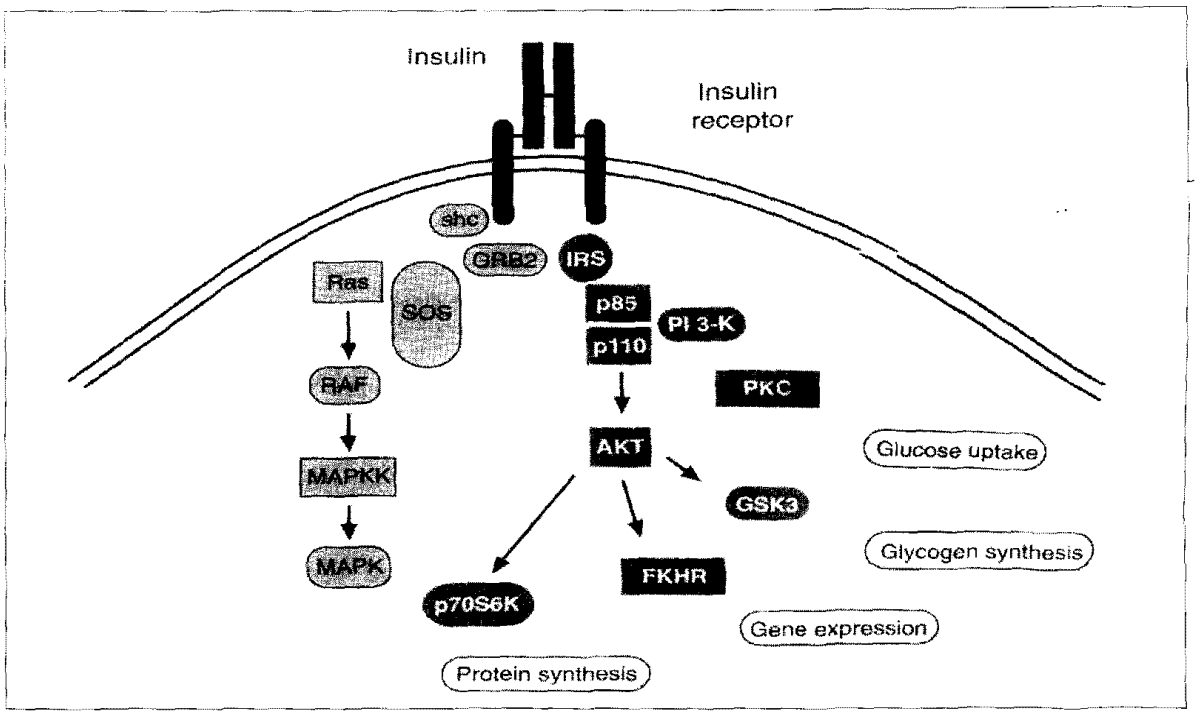

Figure 2. Summary of the main insulin signalling pathways

The diagram depicts the main signalling pathways utilized by the insulin receptors (IR). For a discussion of these findings, see text. Additional pathways are not indicated in this diagram, but there is increasing evidence that these pathways are also important for insulin signalling. Akt, product of the v-Akt proto-oncogens; FKHR, forkhead in human rhabdomyosarcoma; GRB-2, growth factor receptor binding protein-2; GSK3, glycogen synthase kinase 3; MARK, mitogen activated protein kinase; PI 3-K, phosphatidyl-inositol 3 kinase; PKC, protein kinase C; RAF, product of the v-raf proto-oncogene; RAS, product of the v-ras proto-oncogene; shc, src homology- and collagen homology-containing protein; SOS, Son-of-sevenless.

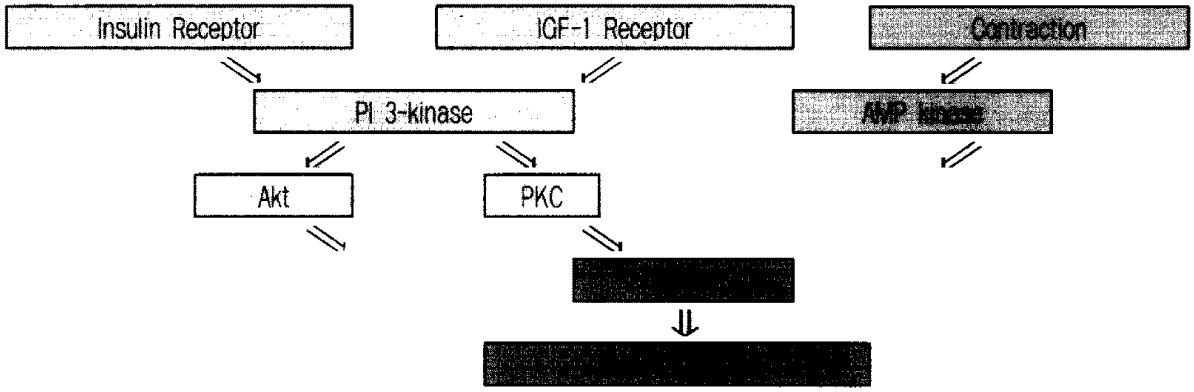

Figure 3. Converging pathways leading to GLUT4 translocation in muscle
In addition to insulin receptor (IR) activation, two other pathways appear to play important roles in GLUT4 translocation, leading to glucose uptake and utilization in muscle. Muscle contraction is a powerful trigger to GLUT4 translocation through activation of the AMP-activated kinase. In addition, muscle insulin-like growth factor I(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 (PIPa)-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 disbetes, an isolated knockout of IR does not.

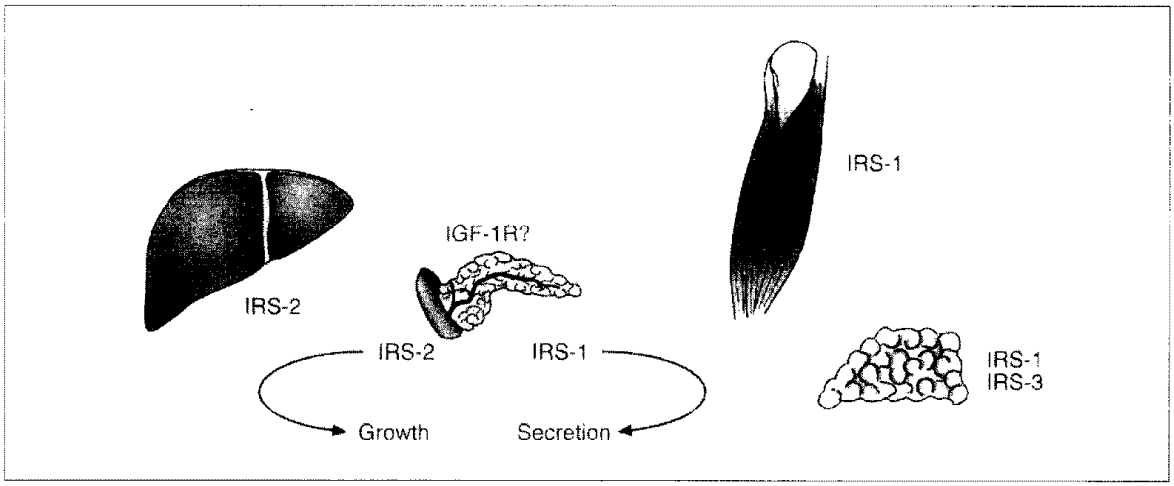

Figure 4. Mutations in Irs genes affect insulin action in different tissues
Gene knock-out experiments have indicated that different insulin receptor substrate (IRS) proteins are employed to transduce insulin signal in different tissues. IRS-1 is the primary substrate in muscle and adipose tissue and appears to play a role in insulin secretion. IRS-2 is the main signalling substrate in liver and is required for β -cell growth. IRS-3 is also important in adipose tissue. It is yet unclear whether this tissue-specific role reflects preferential coupling of the various substrates to different receptors, different subcellular localization, or other unidentified mechanisms. IGF, insulin-like growth factor.

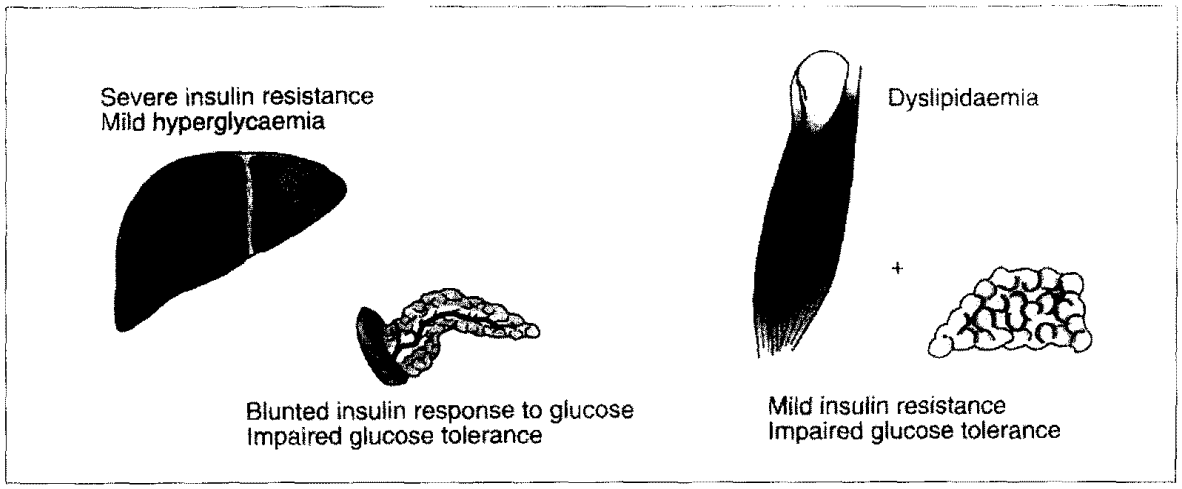

Figure 5. Effects of targeted *Ir* inactivation in different tissues
Mutations inactivating *Ir* by way of Cre-loxP recombination or through dominant-negative constructs have contributed to dissection the role of this key mediator of insulin action in different tissues. The relative phenotype are indicated next to each tissue.

Table 1. Knockouts in the insulin action/secretion and β -cell differentiation pathways

Gene	Phenotype	Reference
IR	Ketoacidotic diabetes	Accili, et.al., 1996
Muscle/fat-specific IR	Impaired glucose tolerance	Lauro, et.al., 1998
Muscle-specific IR	Dyslipidemia	Bruning, et.al., 1998
β -cell-specific IR	Impaired glucose tolerance	Kulkarni, et.al., 1999
Liver-specific IR	Insulin resistance, diabetes	Michael, et.al., 2000
Brain-specific IR	diet-sensitive obesity, subfertility	Bruning, et.al., 2000
IGF-1 receptor	Impaired β -cell growth	Withers, et.al., 1998
IRS-1	Growth retardation, insulin resistance	Araki, et.al., 1994
IRS-2	β -cell dysfunction	Withers, et.al., 1998
IRS-3	None apparent	Liu, et.al., 1999
IRS-4	Modest growth retardation and insulin resistance	Fantin, et.al., 2000
PI 3-kinase(P85α)	Hypoglycemia	Terauchi, et.al., 1999
GLUT2	Impaired insulin secretion, diabetes	Guillam, et.al., 1997
GLUT4	Cardiac hypertrophy	Katz, et.al., 1995
GLUT4(heterozygous)	Insulin resistance, diabetes	Stenbit et.al., 1997
GLUT4(heart-specific)	Cardiac hypertrophy	Abel, et.al., 1999
GLUT4(muscle-specific)	Insulin resistance, diabetes	Zisman, et.al., 2000
Glucokinase	Impaired insulin secretion, hepatic glycogen synthesis	Postic, et.al., 1995