

Molecular insights into insulin action and secretion

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Abstract

Tightly co-ordinated control of both insulin action and secretion is required in order to maintain glucose homeostasis. Gene knockout experiments have helped to define key signalling molecules that affect insulin action, including insulin and insulin-like growth factor-1 (IGF-1) receptors, insulin receptor substrate (IRS) proteins and various downstream effector proteins. β -cell function is also a tightly regulated process, with numerous factors (including certain signalling molecules) having an impact on insulin production, insulin secretion and β -cell mass. While signalling molecules play important roles in insulin action and secretion under normal circumstances, abnormal insulin signalling in muscle, adipose tissue, liver and pancreas leads to insulin resistance and β -cell dysfunction. In particular, the signalling protein IRS-2 may have a central role in linking these abnormalities, although other factors are likely to be involved.

Keywords diabetes mellitus, noninsulin-dependent; glucose/metabolism; insulin resistance; insulin/secretion; signal transduction

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Introduction

Insulin affects a wide range of physiological processes, although it is best known for its important regulatory role in glucose homeostasis. In response to elevations in plasma glucose, insulin secretion is increased and it stimulates glucose uptake and glycogen synthesis and inhibits glycogenolysis and gluconeogenesis, thus maintaining normoglycaemia. In addition to these well-established short-term actions, insulin exerts a number of other important metabolic effects, many of which are mediated via changes in the expression of more than 100 genes [1]. For example, insulin regulates the expression of genes involved in amino acid uptake, lipid metabolism in muscle and adipose tissue [2] and in cell growth, development and survival [3–7].

Maintenance of normal glucose metabolism requires tightly co-ordinated control of insulin action and secretion. In type 2 diabetes, loss of glycaemic control generally involves impairments in both insulin action (i.e. peripheral insulin resistance) and insulin secretion (i.e. β -cell dysfunction) [8,9]. However, a common underlying molecular mechanism has not yet been identified for the majority of cases of type 2 diabetes.

Our aim in this paper is to review recent studies of insulin signalling mechanisms and, in particular, to discuss how

alterations in the functioning of components of the signalling pathway contribute to the development of insulin resistance. Evidence is reviewed that certain key signalling molecules have a common role in both insulin action and production and that one or more of these is disrupted in type 2 diabetes. The identification of these molecular mechanisms and further understanding of their involvement in the pathogenesis of type 2 diabetes might drive the development of rational treatment strategies that effectively address the underlying defects of insulin resistance and β -cell dysfunction.

The insulin signalling pathway

The diverse effects of insulin are mediated through a multi-component signalling complex that is strongly conserved across a wide range of species [10]. Binding of insulin to its receptor triggers a cascade of signalling events that ultimately leads to modifications in a number of biological processes (Fig. 1). While more detailed reviews of the insulin signalling cascade are provided elsewhere [11,12], we present a brief overview below, describing the key steps in insulin action. Our understanding of insulin action and its relation to mammalian physiology is clarified greatly by the use of targeted gene disruption experiments (known as gene knockouts) in mice. This technique gives rise to animals lacking specific genes and is used to help elucidate the biological roles of particular proteins.

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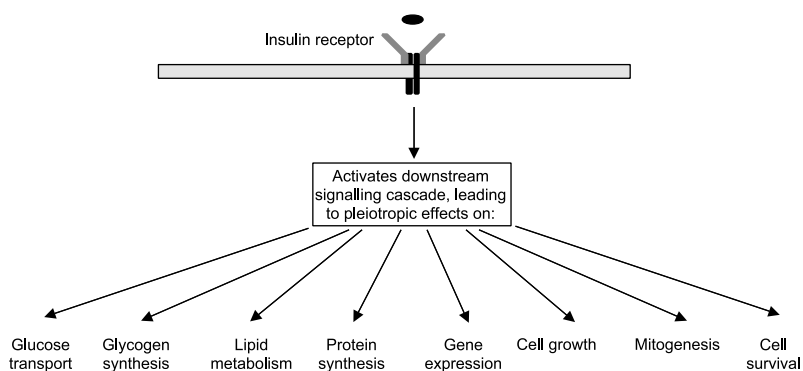


Figure 1 Diverse biological effects of insulin. When insulin binds to its receptor, resultant activation of the insulin signalling cascade leads to multiple effects on several biological processes, including glucose and lipid uptake/metabolism, gene expression/protein synthesis and cell growth, division and survival.

Receptors

Insulin receptors are ubiquitous in vertebrate cells although expression varies significantly between cell types, from as few as 40 receptors per cell on erythrocytes to more than 200 000 on adipocytes and hepatocytes [11]. The receptor consists of two extracellular α -subunits containing the insulin-binding sites and two membrane-spanning β -subunits with intrinsic tyrosine protein kinase activity. The insulin-like growth factor-1 (IGF-1) receptor is structurally related to the insulin receptor, with more than 80% amino acid sequence homology in the kinase domains [11]. As such, insulin and IGF-1 share common signal transduction mechanisms [13]. In contrast, there is little homology between the extracellular domains of the insulin and IGF-1 receptors, consistent with the differing ligand preferences of these two receptors. However, some cross-talk occurs under certain conditions, for example, during fetal development [11].

It is well known that the intrinsic tyrosine kinase activity of the insulin receptor is essential for insulin action. Point mutations in the ATP-binding site that abolish kinase activity also eradicate insulin signalling in cultured cells [14,15], while mutations in humans causing partial kinase inhibition are associated with severe insulin resistance [16,17]. Without insulin receptors, mice die shortly after birth, while people survive for a short time with severe growth retardation and diabetes [7,18–21].

Tissue-specific knockout models

Further insight into the multiple roles of insulin is provided by tissue-specific receptor knockouts (summarized in Table 1). Mice with a muscle-specific insulin receptor knockout (MIRKO) have impaired insulin action in skeletal muscle and abnormalities in lipid metabolism that are reminiscent of the Metabolic Syndrome [22]. Since many believe that muscle represents the primary site of insulin resistance in diabetes, it is surprising that glucose tolerance and plasma insulin levels remain normal in these animals. Clearly other tissues besides muscle are important in insulin-stimulated glucose homeostasis. Liver insulin receptor knockout (LIRKO) mice, on the other hand, are severely insulin resistant and glucose intolerant, confirming that

hepatic insulin signalling has a significant role in the regulation of glucose homeostasis and whole body insulin sensitivity, at least in mice [23]. However, these mice do not develop diabetes in the long term owing to compensatory hyperinsulinaemia, indicating once more that defects in the β cell are required for the onset of diabetes.

Type 2 diabetes might be the consequence of insulin resistance in multiple target tissues, including the β cell itself. The β -cell-specific insulin receptor knockout (β IRKO) mouse exhibits a progressive loss of first-phase insulin secretion in response to glucose that causes glucose intolerance [24]. However, it is unclear whether the glucose-intolerant phenotype of the β IRKO mouse is entirely due to eliminating an insulin feedback effect on the β cell itself. Indeed, the notion of an insulin feedback action on the β cell remains controversial [25,26]. The β IRKO mice also develop insulin resistance with obesity and hyperinsulinaemia [27]. Some of the metabolic changes that occur in the β IRKO mice might be a consequence of unintended disruption of the insulin gene in neuronal tissues. The truncated rat insulin promoter used to drive Cre-recombinase expression (an enzyme used to generate tissue-specific gene knockout mice) in pancreatic β cells in order to generate the β IRKO mice [24] also gives marked developmental expression of Cre in certain regions of the brain [28]. Consequently, β IRKO mice might also lack the insulin receptor in parts of the brain that control body weight and energy homeostasis [29]. Intriguingly, a transgenic mouse brain-specific knockout of the insulin receptor (NIRKO) also develops obesity, insulin resistance and glucose intolerance [30]. Thus, some of the β IRKO phenotype might arise from an unintended NIRKO phenotype. Nevertheless, these tissue-specific insulin receptor knockout studies highlight dramatically the contribution of multiple tissue defects to abnormal carbohydrate metabolism and the pathogenesis of type 2 diabetes.

Insulin receptor substrate proteins

As described above, binding of insulin to its receptor activates tyrosine kinase, resulting in autophosphorylation of tyrosine residues on the receptor β -subunit. This in turn leads to phosphorylation of several protein substrates,

Table 1 Summary of key knockout mouse models

Mutant	Phenotype	Reference
Insulin receptor knockouts		
Complete	Normal intrauterine growth and development Severe hyperglycaemia and hyperketonaemia develops shortly after birth, leading to death after 48–72 h	[7]
Muscle (MIRKO)	Elevated fat mass, serum triglycerides and free fatty acids (FFA) Normal blood glucose, serum insulin and glucose tolerance	[22]
Liver (LIRKO)	Insulin resistance, severe glucose intolerance, insulin fails to suppress hepatic glucose output Marked hyperinsulinaemia caused by increased insulin secretion/decreased insulin clearance Metabolic phenotype improves with ageing	[23]
β cell (β IRKO)	Reduced insulin secretion in response to glucose Progressive impairment of glucose tolerance and mild obesity	[24] [27]
Brain (NIRKO)	Develop diet-sensitive obesity and insulin resistance Hyperinsulinaemia and hypertriglyceridaemia Impaired spermatogenesis and ovarian follicle maturation	[30]
IGF-1 receptor knockout		
Complete	Lethal at birth owing to respiratory failure Severe growth deficiency and widespread developmental defects	[110]
IRS protein knockouts		
IRS-1	Significant growth inhibition Mild insulin resistance and glucose intolerance but diabetes does not develop owing to compensatory hyperinsulinaemia	[36] [35]
IRS-2	Insulin resistance in muscle and liver coupled with abnormal β -cell function lead to diabetes Males develop dehydration and hyperosmolar coma leading to death	[40]
IRS-3	Body weight and plasma glucose/insulin levels comparable to wild type Insulin-stimulated glucose uptake in adipocytes from IRS-3 knockout mice similar to wild type	[44]
IRS-4	Mild defects in growth in male mice Mild defects in reproduction and slight impairments in glucose homeostasis	[47]
Insulin/IGF-1 signalling protein knockout		
Knockout of PI 3-kinase p85 regulatory subunit	Increased insulin sensitivity, hypoglycaemia and increased glucose transport caused by switch to alternative pathway (p50) Demonstrates role for PI 3-kinase in glucose homeostasis	[111]
Akt/PKB-2 (Complete)	Insulin resistance in muscle and liver coupled with increased pancreatic islet mass Glucose intolerant and hyperinsulinaemic	[52]
p70 ^{S6K} -1 (Complete)	No insulin resistance Reduced β -cell size coupled to decreased β -cell mass, insulin content and secretion	[88]
Glucose transporter knockouts		
GLUT4 (Complete)	Insulin resistant with mild impairment of glucose tolerance, growth retardation and decreased fat tissue deposition Hyperinsulinaemia, cardiac hypertrophy, decreased levels of lactate and FFA	[56]
GLUT4 (Muscle)	Insulin resistant, fasting hyperglycaemia, glucose intolerance – effects more severe than in muscle-specific insulin receptor knockout	[59]
GLUT4 (Adipose)	Markedly impaired insulin-stimulated glucose uptake in adipocytes Insulin resistance in muscle and liver leading to glucose intolerance and hyperinsulinaemia	[60]

primarily the insulin receptor substrate (IRS) proteins (Fig. 2). These proteins have an important regulatory role, providing an interface between insulin receptors and downstream effector molecules. To date, four mammalian IRS proteins have been identified [31–34]. Based on work with transgenic mice, IRS-1 is primarily involved in somatic cell growth and insulin action in muscle and adipose tissue [35–38], whereas IRS-2 plays important roles in β -cell survival/growth, insulin action in the liver, brain growth, reproduction and food intake [39–43]. IRS-3 and IRS-4 are predominantly expressed in adipose and neuroendocrine tissue,

respectively [44,45], although their precise roles are still under investigation.

Gene knockout experiments demonstrate the critical roles that IRS-1 and IRS-2 play in activating multiple signalling pathways. Although mice deficient in IRS-1 are viable, they exhibit marked defects in both embryonic and postnatal growth [35,36]. These mice are also insulin resistant, with impaired glucose tolerance coupled with other features of the Metabolic Syndrome, such as hypertriglyceridaemia and hypertension [35,36,46]. However, despite insulin resistance, diabetes never develops in

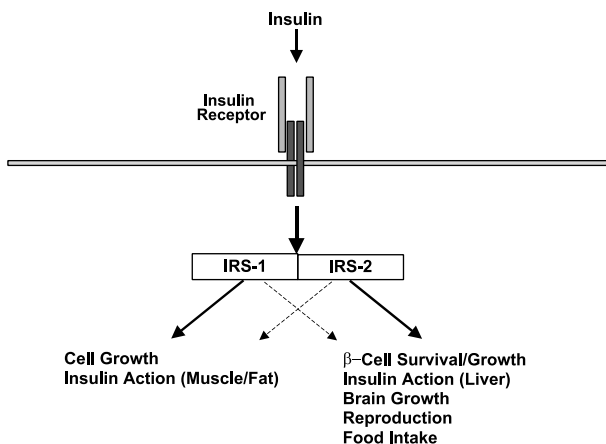


Figure 2 Interaction between insulin and the insulin receptor substrate (IRS) proteins. Insulin activates the insulin signalling cascade largely through its interaction with the IRS proteins. Phosphorylation of IRS proteins results in a number of downstream effects. The best characterized IRS proteins, IRS-1 and IRS-2, have different but overlapping functions. While IRS-1 has a predominant role in cell growth and insulin action in muscle and adipose tissue, the effects of IRS-2 are better defined in the β -cell and liver, in addition to its roles in brain growth, reproduction and food intake.

IRS-1-deficient mice because of compensatory hypersecretion of insulin [35]. Disruption of IRS-2, conversely, causes diabetes in mice and is ultimately fatal in young male mice and middle-aged female mice [40]. In contrast, mice deficient in IRS-3 or IRS-4 do not have a marked phenotype [44,47], although mice lacking IRS-4 exhibit small changes in growth, reproduction and glucose homeostasis compared with wild-type animals [47].

Euglycaemic-hyperinsulinaemic clamp studies in IRS-1- and IRS-2-deficient mice indicate a broader role for IRS-2 in liver, adipose tissue and muscle, whereas IRS-1 plays a more limited role in metabolic regulation that is focused

mainly in skeletal muscle [42]. Further studies indicate a central role for IRS-1 in the regulation of protein synthesis in muscle and of glucose transport in both muscle and adipose tissue [37,48]. IRS-2, on the other hand, appears to have numerous functions in peripheral insulin-sensitive tissues plus in β -cell survival/expansion and neuroendocrine regulation of reproduction and energy homeostasis [39–43]. Overall, however, studies have shown that there is not a simple separation of function between IRS-1 and IRS-2 in tissues but that it is the balance between these proteins that is important and the ability to compensate for IRS-1 deficiency in these mouse models depends ultimately on the level of IRS-2 [37,38].

Downstream effector molecules

While the IRS proteins are early components of the insulin signalling pathway, it is the subsequent specific recruitment of multiple downstream signalling proteins that ultimately generates the unique insulin responses in various cells and tissues. During insulin stimulation, tyrosine phosphorylation sites in the IRS proteins bind specifically to the Src-homology-2 (SH2) domains in various downstream signalling molecules, including phosphatidylinositol 3-kinase (PI 3-kinase), growth factor receptor binding protein 2 (Grb-2) and SH2-containing protein-tyrosine phosphatase-2 (SHP-2; Fig. 3). The outcome of insulin action in any cell depends on which of these effector molecules are expressed and recruited into the signalling complex and the pathways that are activated as a result [10]. In skeletal muscle and adipose tissue, insulin stimulation of the PI 3-kinase pathway enhances glucose utilization by regulating the expression or subcellular localization of glucose transporters, GLUT4 and GLUT1, and stimulates the storage of glucose as glycogen or fat [49–51]. In pancreatic β cells, the PI 3-kinase cascade probably promotes survival of β cells [41,52,53]. Moreover, insulin stimulation of PI 3-kinase

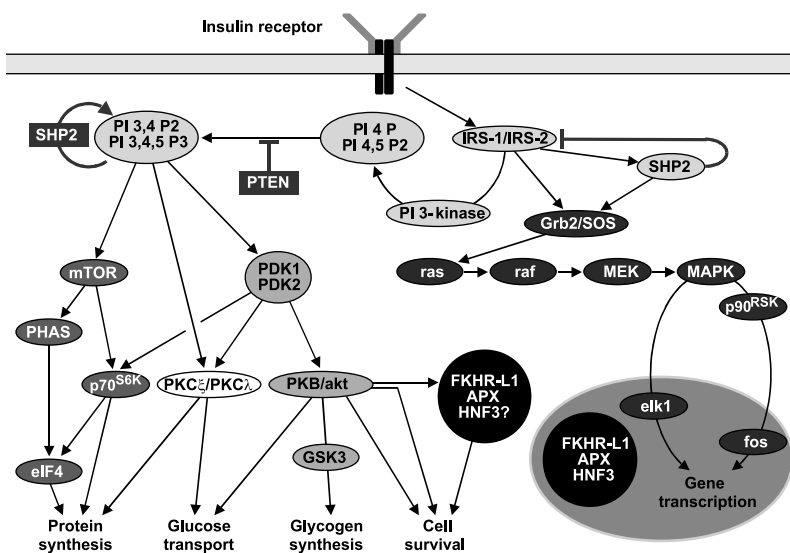


Figure 3 The insulin signalling cascade. When insulin binds to its receptor and activates the IRS proteins, this in turn triggers multiple downstream events that ultimately cause a unique insulin response, depending on the cells or tissues involved. Key molecules involved in this process are shown below, indicating some of the biological processes that are affected by changes in the insulin signalling cascade.

strongly activates total protein synthesis in most cell types that are regulated by the mammalian target of rapamycin (mTOR) pathway. Alternatively, activation of the adapter protein Grb-2 stimulates gene transcription through the mitogen-activated protein kinase (MAPK) cascade [54].

Glucose transporter knockout mice provide further information concerning the insulin signalling pathway downstream of the IRS proteins. The main insulin-responsive glucose transporter, GLUT4, is located primarily in muscle cells and adipocytes [55]. Perhaps surprisingly, complete disruption of GLUT4 in mice produces only mild glucose intolerance [56], although subsequent studies indicate severe insulin resistance and frank diabetes in some of the male animals [57,58]. Selective inactivation of GLUT4 in muscle or adipose tissue, however, has significant effects on glucose tolerance, leading to substantial reductions in insulin-stimulated glucose uptake in these tissues [59,60].

Regulation of β -cell function

β -cell function is regulated primarily by plasma glucose concentrations although numerous other signals are involved, including other sugars, amino acids, free fatty acids (FFA), hormones, growth factors and certain pharmacological agents (Fig. 4). Presented below is a brief overview of the many processes that together regulate β -cell function.

Regulation of insulin secretion

Glucose is a key regulator of insulin secretion. Regulation occurs via the process of stimulus–secretion coupling (Fig. 5), outlined below and reviewed in more detail elsewhere [61–63]. In brief, exposure to glucose increases the ATP : ADP ratio and triggers closure of ATP-sensitive K^+

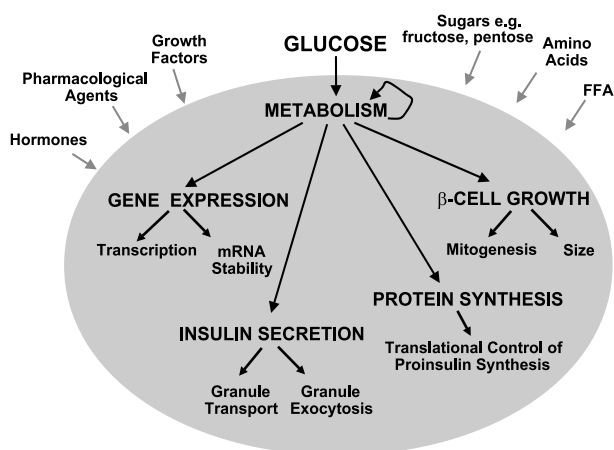


Figure 4 Regulators of β -cell function. Although β -cell function is primarily regulated by changes in plasma glucose concentrations, other factors may also have an effect. For example, other sugars, amino acids, free fatty acids, growth factors, hormones and pharmacological agents all influence β -cell function.

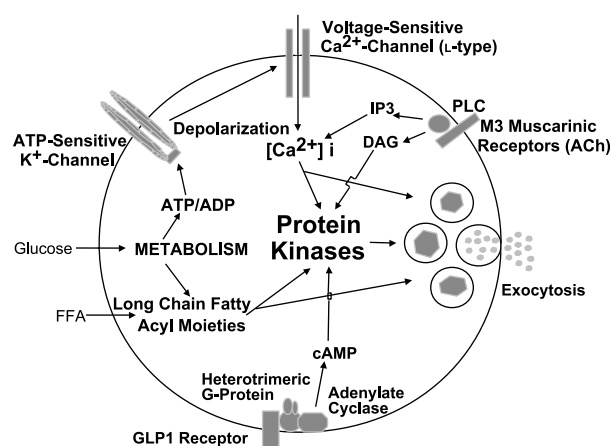


Figure 5 Stimulus–secretion coupling in the β cell. During stimulus–secretion coupling, rising plasma glucose concentrations result in an increased ratio of ATP to ADP and lead to closure of ATP-sensitive K^+ -channels, which in turn triggers membrane depolarization and opening of voltage-sensitive Ca^{2+} -channels. The influx of Ca^{2+} that follows causes protein kinase activation, resulting in exocytosis of insulin secretory particles. Insulin secretion is also triggered via other pathways and several other agents besides glucose influence stimulus–secretion coupling, for example, free fatty acids, glucagon-like polypeptide-1 (GLP-1) and cholinergic agents.

channels. This in turn causes membrane depolarization and stimulates the opening of voltage-dependent Ca^{2+} channels. The resultant Ca^{2+} influx leads to increased cytosolic Ca^{2+} concentrations and promotes exocytosis – an effect mediated through protein kinase C or through direct stimulation of secretory granules.

Although glucose provides the primary stimulus, several other molecules such as FFA, amino acids and keto acids also influence stimulus–secretion coupling [64,65]. In addition, a number of hormones and neuromodulators stimulate insulin secretion, including glucagon-like polypeptide-1 (GLP-1) that increases cAMP levels and activates protein kinase C through specific G-protein-coupled receptors [66]. Another pathway operates via binding of cholinergic agents to muscarinic receptors, which stimulates the production of inositol triphosphate (IP_3) and diacylglycerol (DAG) and thus increases intracellular calcium concentrations and promotes protein kinase C activity [67–69]. The exact mechanism by which Ca^{2+} and its associated protein kinases induce transport of secretory granules to the plasma membrane and subsequently stimulate granule exocytosis is currently unclear.

Regulation of insulin production

Production of proinsulin, the precursor molecule of insulin, is regulated by glucose at both the transcriptional and post-transcriptional levels [70]. Such multifaceted gene regulation allows rapid replenishment of intracellular hormone stores during periods of high insulin secretion [71–73]. The stimulatory effects of glucose are selective for insulin, as

proinsulin biosynthesis can be stimulated up to 30-fold at the translational level, while general protein synthesis increases only twofold [72,74–76].

Numerous reports demonstrate the importance of untranslated regions of certain mRNAs in the specific regulation of that protein's synthesis. Recently, it has been indicated that a secondary 'stem loop' structure in the 5'-untranslated region and a short primary sequence in the 3'-untranslated region of preproinsulin mRNA confers a specific regulation of preproinsulin translation in response to glucose [76]. A glucose-induced rise in preproinsulin mRNA translation is paralleled by specific increases in the biosynthesis of proinsulin-converting enzymes PC2 and PC3 [77,78]. Increased synthesis of these proteins has also been attributed to changes at the level of mRNA translation [72,73,77–79], although whether this occurs through the same mechanism as for proinsulin gene expression remains to be confirmed [76]. Nonetheless, increased biosynthesis of PC2 and PC3 in parallel to their proinsulin substrate provides a means whereby proinsulin to insulin conversion in the β -cell adapts to changes in glucose homeostasis.

Under normal circumstances, glucose stimulates both proinsulin biosynthesis and insulin secretion in a co-ordinated manner. Other nutrients may also impact on these processes, for example, elevated concentrations of FFA promote increased basal insulin secretion in rats [80]. However, since elevated FFA do not stimulate proinsulin synthesis to a corresponding extent in these animals, the net result is a decrease in β -cell insulin content [80]. These data are supported by findings in isolated β cells [81]. This suggests a mechanism by which chronically elevated FFA may contribute to β -cell dysfunction in the pathogenesis of type 2 diabetes, i.e. by increasing basal insulin secretion without inducing a compensatory increase in proinsulin biosynthesis [80]. Further insight comes from a glucose-infusion rat model of type 2 diabetes [82]. In hyperglycaemic animals, chronically elevated glucose levels induce a substantial increase in proinsulin biosynthesis leading to hyperproinsulinaemia [83]. This appears to be due to premature secretion of proinsulin rather than to inefficient processing of proinsulin to insulin as a result of an acquired deficiency in PC2 and PC3 enzyme activities [83]. For a further discussion of the characteristic elevation in proinsulin levels in individuals with type 2 diabetes, see accompanying article in this supplement by Bergman *et al.* [84].

Regulation of β -cell mass

The net rate of β -cell turnover is an important factor in regulating glucose homeostasis. Since the β -cell mass exists in a dynamic state, compensatory changes, for example to correct for changes in insulin sensitivity, ensure maintenance of euglycaemia in healthy individuals. A number of factors affect β -cell mass, for example, changes in the rate of replication, neogenesis and cell death (both necrosis and apoptosis), as well as β -cell volume [85]. Although adult β cells are generally well-differentiated, with only around 0.5% of cells undergoing mitosis at any one time, β -cell proliferation

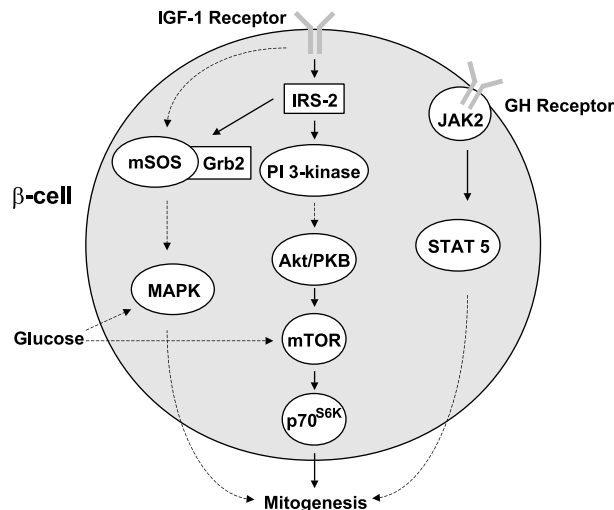


Figure 6 IGF-1 and GH signal transduction pathways in the β cell. An overview of key events occurring in the IGF-1 and GH signalling pathways that lead to stimulation of β -cell mitogenesis. Note that increased glucose metabolism can lead to activation of elements in the signal transduction pathway downstream of IRS-2. A solid arrow indicates a single step while a broken arrow is indicative of several steps. For a more detailed review, see ref. [93].

can be increased by nutrients such as glucose and amino acids [86,87]. Normal cell growth is influenced by glucose on a number of levels, including mitogenesis, DNA synthesis and cell proliferation, while the regulation of β -cell size is also considered glucose-dependent. Recently, Akt/PKB and p70^{S6K} transgenic mouse models have indicated that β -cell size can be controlled via Akt/PKB, mTOR and p70^{S6K} signal transduction pathways, downstream of IRS-2/PI 3-kinase activation [52,53,88].

The role of insulin signalling molecules such as the IRS proteins in the regulation of β -cell mitogenesis is of particular interest (Fig. 6). For example, IGF-1 stimulates β -cell proliferation, giving up to a 50-fold stimulation of mitogenesis in pancreatic β -cell line models [89]. The increased β -cell proliferation is glucose-dependent, but also requires the recruitment of PI 3-kinase and Grb2 to IRS-2, resulting in the activation of MAPK and p70^{S6K}, and, ultimately, increased β -cell proliferation [89]. These findings support the important part that IRS-2 plays in regulating β -cell mass as found in studies of IRS-2 knockout mice [40,41]. In addition to the effects of IGF-1, growth hormone (GH) is also important in potentiating glucose-dependent β -cell mitogenesis, although this occurs via a JAK2/STAT5 mitogenic signalling pathway independent of IRS [90].

Recent experiments have helped to elucidate the nature of the glucose-dependency of IGF-1-induced β -cell proliferation. Glucose can independently cause MAPK activation in a Ca²⁺-dependent manner that requires glucose metabolism [89,91]. Although glucose can modestly increase PI 3-kinase activity [92,93] this surprisingly does not result in a glucose-induced activation of Akt/PKB in β cells [94]. In addition, Akt/PKB can be activated by IGF-1 independently of glucose in the β cell. However, glucose does act

downstream of Akt/PKB by independently activating the mTOR/p70^{S6K} pathway [94]. There is a fine balance between the MAPK and PI 3-kinase signalling pathways in the β cell that highlights the complex nutrient and growth factor regulation of mitogenesis (discussed in detail in Dickson *et al.* [94]). By gaining further understanding of the mechanism by which β -cell proliferation and survival is stimulated, we may get one step closer to learning how we can preserve, or even enhance, β -cell mass in subjects with type 2 diabetes as a means to compensate for insulin resistance.

Abnormal insulin signalling: insulin resistance and β -cell dysfunction

We have outlined above the critical parts played by signalling molecules in regulating insulin action and secretion under normal circumstances. What happens when signalling pathways are disrupted, for example, in type 2 diabetes? In this case, abnormal insulin signalling in muscle, adipose tissue, liver and pancreas leads to insulin resistance and β -cell dysfunction.

Data from knockout mouse models indicate that deficiencies in a number of key signalling proteins, such as IRS-1 and IRS-2, lead to insulin resistance in peripheral tissues [35,36,40]. This is reinforced by additional evidence implicating decreased insulin signalling in the development of peripheral insulin resistance and supporting a role for elevated FFA concentrations and/or accumulation of intracellular lipid in reducing insulin sensitivity, particularly in skeletal muscle [95]. More specifically, increased circulating FFA levels have been linked with multiple abnormalities in the insulin-signalling pathway, including decreased IRS-1 tyrosine phosphorylation, reduced PI 3-kinase activity and increased activation of PKC [96,97]. The ultimate consequence is a decrease in GLUT4 translocation, leading to significant reductions in muscle glucose transport [96]. Preliminary data indicate that thiazolidinediones may, at least in part, exert their insulin sensitizing effects by reducing elevated plasma FFA and intracellular lipid concentrations, thus restoring insulin signalling in skeletal muscle [98,99]. For example, there is evidence that rosiglitazone modifies muscle PKC θ and PKC ϵ activity in high-fat-fed rats [100], which may provide a potential mechanism to explain these effects. More details on the role of elevated FFA in the development of insulin resistance are given in another article in this supplement by Boden and Shulman [101].

Although the concept of 'insulin resistance' in the β cell is relatively new, evidence from IRS-2, Akt/PKB and p70^{S6K} knockout mice for example suggest that defective IGF-1 signalling in the endocrine pancreas contributes to β -cell dysfunction in type 2 diabetes [24,40,41,52,53,88]. While there is a mounting body of evidence indicating that elevated fat significantly inhibits insulin signalling in skeletal muscle, it is not yet known if accumulation of fat in β cells reduces insulin signalling in the pancreas. However, elevated plasma FFA concentrations, as commonly seen in

individuals with type 2 diabetes, appear to have a lipotoxic effect on the pancreas and there is evidence from rodent models that increased FFA contribute to β -cell dysfunction by increasing β -cell apoptosis [102,103]. Furthermore, *in vitro* data indicate an inhibitory effect of long-chain FFA on glucose- and IGF-1-induced DNA synthesis, leading to alterations in the activity of several protein kinases (especially inhibition of Akt/PKB activation) involved in the insulin/IGF-1 signalling pathway [91]. A hypothesis has recently been put forward suggesting that intracellular fat accumulation in β cells during obesity may lead to inhibition of β -cell mass expansion and thus failure to compensate for peripheral insulin resistance, which in turn leads to type 2 diabetes [93]. In addition to the effects of fat on β -cell insulin/IGF-1 signalling, there is evidence that high glucose concentrations are also toxic to β cells (glucotoxicity) and induce apoptosis [104]. At elevated glucose concentrations, glucose-dependent IGF-1-induced β -cell proliferative pathways are also reduced, again indicating the adverse effects of hyperglycaemia on β -cell function [89,90]. Preliminary data from Zucker Diabetic Fatty (ZDF) rats treated with rosiglitazone indicate that the thiazolidinediones, which have been shown to reduce plasma FFA and glucose levels, may also prevent the characteristic decline in β -cell mass seen in untreated animals [105,106].

While defective insulin signalling appears to be important in peripheral insulin resistance, it is becoming clearer that defective IGF-1 signalling also contributes to β -cell dysfunction. This raises the possibility of a common signalling molecule linking insulin/IGF-1 action and insulin secretory deficiencies that is disrupted in type 2 diabetes. Although IRS-1 has been put forward as providing a novel functional link between the insulin signalling and insulin secretion pathways [107], there is more evidence supporting IRS-2 as the key molecule since mice deficient in this protein develop both insulin resistance and β -cell dysfunction [39–42]. Unlike IRS-1 knockout mice, which are hyperinsulinaemic owing to higher-than-normal β -cell mass that allows them to compensate for insulin resistance, IRS-2 knockouts have a characteristic >50% reduction in β -cell mass [108]. Further clarification of the specific role of the IGF-1 \rightarrow IRS-2 signalling pathway will help to elucidate whether it plays a central part in regulating both insulin action and secretion and, in particular, the development of a β -cell-specific IRS-2 knockout may provide further insight. Research is also underway to investigate whether the thiazolidinediones, which have been shown to improve β -cell function as well as insulin sensitivity, have an impact on insulin signalling in β cells as well as peripheral tissues [109].

Conclusion

Since the IRS-2 branch of the insulin/IGF-1 signalling pathway is such a fundamental process in both insulin action and β -cell function, abnormalities might contribute to both the insulin resistance and β -cell dysfunction seen in type 2 diabetes.

If this is the case, what is the likelihood that a single molecular defect can give rise to both insulin resistance and β -cell dysfunction? Although we have discussed evidence supporting the central role of IRS-2 in linking these two abnormalities, it is likely that other factors, especially those that counter-regulate the IGF-1 \rightarrow IRS-2 pathway, might be involved. While this remains a subject for debate, it is clear that any molecules linking peripheral insulin action and β -cell secretory function are of interest as potential targets for therapeutic intervention because such an intervention might delay disease progression.

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