Molecular Mechanisms of Insulin Resistance: Serine Phosphorylation of Insulin Receptor Substrate-1 and Increased Expression of p85α

The Two Sides of a Coin

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Initial attempts to unravel the molecular mechanism of insulin resistance have strongly suggested that a defect responsible for insulin resistance in the majority of patients lies at the postreceptor level of insulin signaling. Subsequent studies in insulin-resistant animal models and humans have consistently demonstrated a reduced strength of insulin signaling via the insulin receptor substrate (IRS)-1/phosphatidylinositol (PI) 3-kinase pathway, resulting in diminished glucose uptake and utilization in insulin target tissues. However, the nature of the triggering event(s) remains largely enigmatic. Two separate, but likely, complementary mechanisms have recently emerged as a potential explanation. First, it became apparent that serine phosphorylation of IRS proteins can reduce their ability to attract PI 3-kinase, thereby minimizing its activation. A number of serine kinases that phosphorylate serine residues of IRS-1 and weaken insulin signal transduction have been identified. Additionally, mitochondrial dysfunction has been suggested to trigger activation of several serine kinases, leading to a serine phosphorylation of IRS-1. Second, a distinct mechanism involving increased expression of p85α has also been found to play an important role in the pathogenesis of insulin resistance. Conceivably, a combination of both increased expression of p85α and increased serine phosphorylation of IRS-1 is needed to induce clinically apparent insulin resistance. *Diabetes* 55: 2392–2397, 2006

Even though insulin resistance has emerged as an enormous health care problem, trespassing the fields of obesity, diabetes, hypertension, and cardiovascular diseases (1,2), its molecular mechanism remains incompletely understood. Clinically, the term insulin resistance implies that higher-than-normal concentrations of insulin are required to maintain normoglycemia. On a cellular level, this term defines an inadequate strength of insulin signaling from the insulin receptor downstream to the final substrates of insulin action involved in multiple metabolic and mitogenic aspects of cellular function (3).

Insulin action is initiated by an interaction of insulin with its cell surface receptor (4). The insulin receptor is a heterotetrameric protein that consists of two extracellular α subunits and two transmembrane β subunits connected by disulfide bridges (5–7). Insulin binding to the extracellular α subunit induces conformational changes of the insulin receptor that activate the tyrosine kinase domain of the intracellular portion of the β subunit (8–11). Once the tyrosine kinase of insulin receptors is activated, it promotes autophosphorylation of the β subunit itself, where phosphorylation of three tyrosine residues (Tyr-1158, Tyr-1162, and Tyr-1163) is required for amplification of the kinase activity (12,13). Activation of the tyrosine kinase of the insulin receptor also leads to a rapid phosphorylation of the so-called "docking proteins," such as insulin receptor substrate (IRS)-1, -2, -3, and -4, and several Shc proteins (52-, 46-, and 64-kDa isoforms) (14,15) that, in turn, attract multiple intracellular signaling intermediates.

Initial attempts to unravel the molecular mechanism of insulin resistance have strongly suggested that a defect responsible for insulin resistance in the majority of patients lies at the postreceptor level of insulin signaling (16–18). Thus, numerous studies have demonstrated that the number and function (tyrosine kinase activity) of insulin receptors are either normal or only slightly reduced in patients and experimental animals with insulin resistance, insufficiently to account for a substantial reduction in insulin action.

The IRS and Shc proteins play an important regulatory role in the insulin signaling cascade, as in their phosphorylated form they become points of anchoring for intracellular proteins containing Src-homology-2 (SH-2) domains (rev. in 19). Whereas interaction of IRS and Shc proteins with the intracellular domain of the insulin receptor constitutes the first step in dispersing the directions of insulin signaling intracellularly, their ability to attract multiple signaling intermediates to their own phosphorylated domains further partitions insulin signaling downstream, thus accounting for the multitude of insulin's biological effects (20).

Most, if not all, of the metabolic and antiapoptotic effects of insulin are mediated by the signaling pathway

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IκB, inhibitor of κB; IKKβ, inhibitor of κB kinase β; IRS, insulin receptor substrate; JNK, c-Jun NH_2-terminal kinase; mTOR, molecular target of rapamycin; PI, phosphatidylinositol; PKC, protein kinase C; TNF, tumor necrosis factor.

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TABLE 1
Causes of IRS-1 serine phosphorylation

Causes
mTOR (76–79)
p70S6 kinase
Amino acids
Hyperinsulinemia
TSC1–2 depletion
Nutrition
JNK (55–58,80)
Stress
Hyperlipidemia
Inflammation
IKK (59–63)
Inflammation
TNFα (64–68)
Obesity
Inflammation
Mitochondrial dysfunction (69–71)
PKCθ (58,72–75)
Hyperglycemia
Diacylglycerol
Inflammation

involving IRS proteins, phosphorylation, and activation of phosphatidylinositol (PI) 3-kinase, Akt (also known as protein kinase B), molecular target of rapamycin (mTOR), and p70 S6 kinase (21–24). Activation of PI 3-kinase, Akt, and atypical protein kinase C (PKC) via the phosphoinositide-dependent protein kinase (25) appears to be critical in the mechanism of insulin action on GLUT-4 translocation and glucose transport. In contrast, nonmetabolic, proliferative, and mitogenic effects of insulin are mediated largely via the activation of Ras (mostly through Shc and, to a lesser degree, through IRS proteins), Raf, and mitogen-activated protein kinases Erk 1 and Erk 2 (26–30).

Subsequent studies (31–33) in insulin-resistant animal models and humans have consistently demonstrated a reduced strength of insulin signaling via the IRS-1/PI 3-kinase pathway, resulting in diminished glucose uptake and utilization in insulin target tissues. However, the nature of the culprit that initiates and sustains impaired insulin signal transduction along the IRS-1/PI 3-kinase pathway is still largely enigmatic. Two separate, but likely, complementary mechanisms have recently emerged as a potential explanation for the reduced strength of the IRS-1/PI 3-kinase signaling pathway.

SERINE PHOSPHORYLATION OF IRS-1

First, it became apparent that serine phosphorylation of IRS proteins can reduce the ability of IRS proteins to attract PI 3-kinase, thereby minimizing its activation (34–40), and can also lead to an accelerated degradation of IRS-1 protein (41). Thus, in contrast to a signal promoting tyrosine phosphorylation, excessive serine phosphorylation of IRS proteins could become detrimental for normal conductance of the metabolic insulin signaling downstream, causing insulin resistance. Serine phosphorylation of IRS proteins can occur in response to a number of intracellular serine kinases (Table 1).

A cellular nutrient sensor, mTOR, has been identified as a critical element integrating cellular metabolism with growth factor signaling (42–45). In response to insulin and amino acids, mTOR, which is a serine/threonine kinase, phosphorylates and modulates activities of p70 S6 kinase

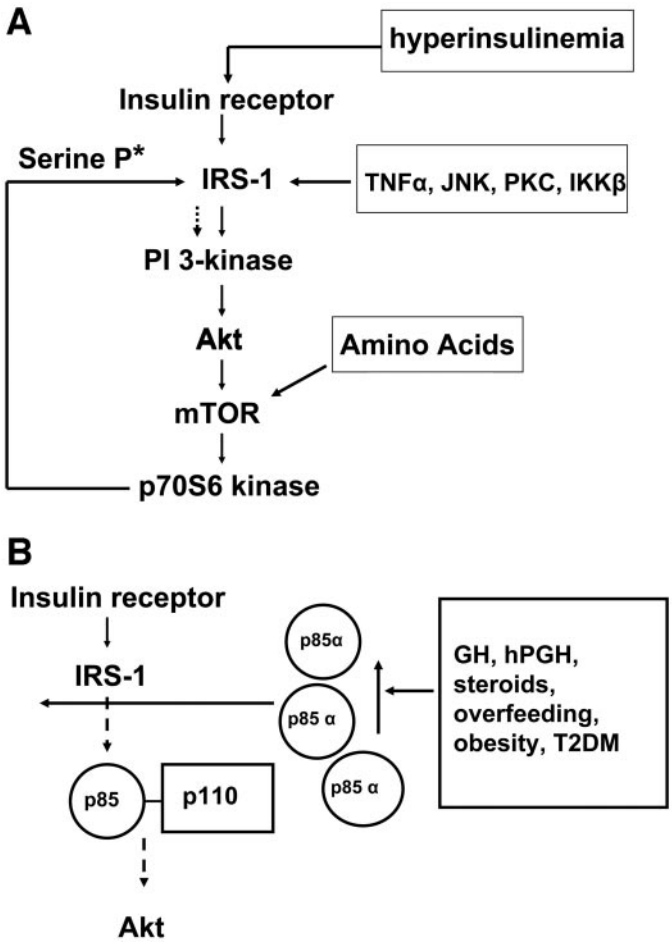

FIG. 1. Inhibition of the metabolic insulin signaling. IRS-1 is phosphorylated by the tyrosine kinase of the insulin receptor in response to insulin binding. Protein/lipid kinase, PI 3-kinase, binds to the specific MYMX motifs of IRS-1, containing phosphorylated tyrosine residues. PI 3-kinase is then activated and initiates a downstream cascade of events leading to the phosphorylation and activation of Akt, mTOR, and p70S6 kinase. Activation of Akt appears to be important for glucose transport, while activation of mTOR and p70S6 kinase participates in the process of protein synthesis. *A*: Hyperactivation of mTOR by amino acids, Akt, or hyperinsulinemia results in serine phosphorylation of IRS-1 by p70S6 kinase, with a subsequent decrease in the strength of the IRS-1/PI 3-kinase signaling. In addition, serine phosphorylation of IRS-1 can be promoted by JNK, PKC, IKKβ, and TNFα. *B*: Increased expression of p85α monomer competes with and displaces the p85-p110 heterodimer from the IRS-1 binding sites. The resultant decrease in association of p110 with IRS-1 diminishes PI 3-kinase activity and the downstream effects of this kinase. Steroids, growth hormone (GH), human placental growth hormone (hPGH), short-term overfeeding, obesity, and type 2 diabetes (T2DM) have been shown to increase p85α expression (see text for details and references).

(S6K1 kinase) and an inhibitor of translational initiation, eIF-4E binding protein (46–48). While insulin activates mTOR and S6K1 kinase via the IRS-1/PI 3-kinase/Akt pathway (49,50), amino acids seem to exert their effect through a direct influence of mTOR (44,51,52). In any event, activation of mTOR and S6K1 kinase causes serine phosphorylation of IRS-1, with a subsequent decline in the IRS-1–associated PI 3-kinase activity (Fig. 1*A*). In contrast to wild-type littermates, transgenic mice lacking S6K1 kinase (S6K1-deficient mice) displayed a strong resistance to age- and diet-induced obesity and insulin resistance (37). Moreover, because wild-type mice on a high-fat diet demonstrated significantly elevated S6K1 kinase activity and serine phosphorylation of IRS-1, it has been suggested that under conditions of nutrient saturation, S6K1 kinase

may negatively regulate insulin signaling and sensitivity (37,53,54).

Because insulin resistance can be induced by mechanisms other than nutritional excess, serine phosphorylation of IRS-1 has been examined under various circumstances. It appears that in addition to the mTOR-S6K1–dependent mechanism, various serine kinases, such as c-Jun NH_2-terminal kinase (JNK), stress-activated protein kinases, tumor necrosis factor (TNF)α, and PKC, among others, can promote serine phosphorylation of IRS-1 (Table 1 and Fig. 1*A*).

Activation of JNK by free fatty acids, stress, and inflammation (55–58) has been shown to increase serine phosphorylation of IRS-1 with a resulting decline in the strength of insulin signaling along the metabolic pathway. Blocking JNK activation rescued the cellular and molecular defects induced by free fatty acids (56). Furthermore, JNK-1 knockout mice were found to be resistant to diet-induced obesity and insulin resistance (55). Similarly, activation of the proinflammatory kinase that phosphorylates the inhibitor of nuclear factor-κB (IKKβ) has been shown to induce insulin resistance (59–61). In an unstimulated state, nuclear factor-κB dimers are restrained in the cytoplasm in association with inhibitory protein inhibitor of κBs (IκB). In response to proinflammatory stimuli, such as TNFα, IKKβ is activated and phosphorylates two serine residues of the IκB. Phosphorylated IκB is rapidly degraded by proteasomes, releasing nuclear factor-κB for translocation to the nucleus where it activates transcription of target genes. Inhibition of IKKβ with salicylates has been shown to prevent and reverse diet- and obesity-induced insulin resistance (62,63).

TNFα, an agent responsible for cachexia, has been shown to be increased in adipose tissue of obese, insulin-resistant humans and animals. Because removal of TNFα appeared to reverse insulin resistance in animal models, it has been suggested that TNFα plays an important role in the pathogenesis of insulin resistance in obesity (64–66). Furthermore, mice lacking TNFα function were protected from obesity-induced insulin resistance (67). More recently, TNFα has been shown to block insulin signaling by promoting serine phosphorylation of IRS-1 (68), with a resultant decline in IRS-1–associated PI 3-kinase activity.

Recently, a hypothesis that mitochondrial dysfunction or reduced mitochondrial content accompanied by a decreased mitochondrial fatty acid oxidation and accumulation of fatty acid acyl CoA and diacylglycerol can cause insulin resistance has gained substantial experimental support (69–71). The mechanism of insulin resistance in these cases has been suggested to involve activation of a novel PKC that either by itself or via IKKβ or JNK-1 could lead to increased serine phosphorylation of IRS-1.

The proinflammatory novel PKCθ has been found to cause serine phosphorylation of IRS-1 (72,73), while PKCθ knockout mice have been shown to be protected from fat-induced insulin resistance (74). Increased activity of PKCθ, along with increased activity of JNK, has also been found in skeletal muscle of obese and type 2 diabetic subjects (58,75), supporting a potential role of these serine kinases in the pathogenesis of insulin resistance.

INCREASED EXPRESSION OF P85A

A second molecular mechanism that can potentially lead to insulin resistance is a disruption in the balance between the amounts of the PI 3-kinase subunits (81). PI 3-kinase belongs to the class 1a 3-kinases (82), which exist as heterodimers, consisting of a regulatory subunit (p85), which is tightly associated with a catalytic subunit, p110. The regulatory subunit, p85, is encoded by at least three genes that generate highly homologous products. Two isoforms are termed p85α (PIK3R1) and p85β (products of the two genes). Three splice variants of p85α have been reported, including p85α itself, p55α, and p50α. The third gene product is p55γ. p85α, however, appears to be the most abundant isoform (82).

TABLE 2
Causes of an imbalance between PI 3-kinase subunits

Causes
Steroids (89)
Growth hormone (93)
Human placental growth hormone (87,93)
Short-term overfeeding (88)
Obesity and diabetes (58)

Normally, the regulatory subunit exists in stoichiometric excess to the catalytic one, resulting in a pool of free p85 monomers not associated with the p110 catalytic subunit. Thus, there exists a balance between the free p85 monomer and the p85-p110 heterodimer, with the latter being responsible for the PI 3-kinase activity. Increases or decreases in expression of p85 shift this balance in favor of either free p85 or p85-p110 complexes (83–86). Because the p85 monomer and the p85-p110 heterodimer compete for the same binding sites on the tyrosine-phosphorylated IRS proteins, an imbalance could cause either increased or decreased PI 3-kinase activity (Fig. 1*B*). This possibility has been recently supported by studies in insulin-resistant states induced by human placental growth hormone (87), obesity, and type 2 diabetes (58) and by short-term overfeeding of lean nondiabetic women (88).

One of the first indications that an imbalance between the abundance of p85 and p110 can alter PI 3-kinase activity came from experiments with L-6 cultured skeletal muscle cells treated with dexamethazone (89). This treatment significantly reduced PI 3-kinase activity, despite an almost fourfold increase in expression of p85α (no change in p85β) and only a minimal increase in p110. The authors concluded that p85α competes with the p85-p110 heterodimer, thus, reducing PI 3-kinase activity (Table 2).

Subsequently, animals with a targeted disruption of *p85α* ($p85^{+/-}$ heterozygous mice) have been found to have a higher ratio of p85-p110 dimer to free p85 and to be more sensitive to insulin (80,81,89–91). To determine this ratio, the authors immunodepleted p110 and blotted both the immunoprecipitates and the supernatant with p85 antibody. The amounts of p85 in the p110 immunoprecipitates denote p85 bound to p110, while the amount of p85 in the supernatant represents free (excess) p85. The greater the ratio of bound to free, the greater the insulin sensitivity the mice display. The same group of authors then overexpressed p85α in cultured cells. This overexpression significantly inhibited the PI 3-kinase activity (85,86,92). Overexpression of p50α or p55α did not inhibit PI 3-kinase activity to the same extent. These experimental results were consistent with the competition hypothesis.

Recently, Barbour and colleagues (87,93) demonstrated that insulin resistance of pregnancy is likely due to increased expression of skeletal muscle p85 in response to increasing concentrations of human placental growth hor-

mone. Furthermore, women remaining insulin resistant postpartum have been found to display higher levels of p85 in the muscle (94). Thus, results reported in the literature support the hypothesis that the p85 monomer completes with a p85-p110 dimer and that the removal of the excess of p85 improves insulin sensitivity by allowing the remaining isoforms to bring p110 to its site of action.

Finally, in a small study of eight healthy lean women without a family history of diabetes, Cornier et al. (88) were able to show that 3 days of overfeeding (50% above usual caloric intake) led to a significant increase in expression of p85α, ratio of p85α to p110, and a decline in insulin sensitivity. Within this experimental time frame, overfeeding did not cause any change in serine phosphorylation of either IRS-1 or S6K1 (88), suggesting that increased expression of p85α may be an early molecular step in the pathogenesis of the nutritionally induced insulin resistance.

SUMMARY

There have been substantial strides made in our understanding of the genesis of insulin resistance. A number of serine kinases that could phosphorylate serine residues of IRS-1 and thereby diminish insulin signal transduction have been identified. Potential triggering mechanisms such as mitochondrial dysfunction have also been proposed and supported by experimental and observational data. On the other hand, an additional and possibly complementary mechanism involving increased expression of p85α has also been found to play an important role in the pathogenesis of insulin resistance under certain circumstances. Conceivably, a combination of both increased expression of p85α and increased serine phosphorylation of IRS-1 is needed to induce clinically apparent insulin resistance. Further studies are needed in order to evaluate this hypothesis.

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