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Insulin receptors: Structure and function

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Introduction

More than fifty years after the discovery of insulin, its cellular mechanism of action still remains one of the major obstacles in cell biology. Recent progress in the molecular characterization of the insulin receptor itself due to concerted efforts in several laboratories have led to important discoveries. One of these, the kinase activity and autophosphorylation of the insulin receptor, will be reviewed and its putative role in insulin action discussed. Furthermore, conditions with cellular insulin resistance are coupled with decreased phosphorylation of the insulin receptor, giving a clue to a molecular defect in the disease states.

The molecular mechanism of insulin action

Regulation of cellular metabolism and growth by insulin is a result of a series of events initiated by the interaction of the hormone with specific cell surface receptors (fig. 1). In the past, insulin receptors on a large number of cell types have been characterized in detail by their structure and function[33, 38, 75]. This achievement is based on the development and application of a variety of biochemical methods including kinetic analysis for description of the receptor binding[16]; affinity labeling technique for identification of the receptor subunits[10]; and recently, recombinant DNA technology for the elucidation of receptor amino-acid sequences[13, 73]. In spite of this progress, the

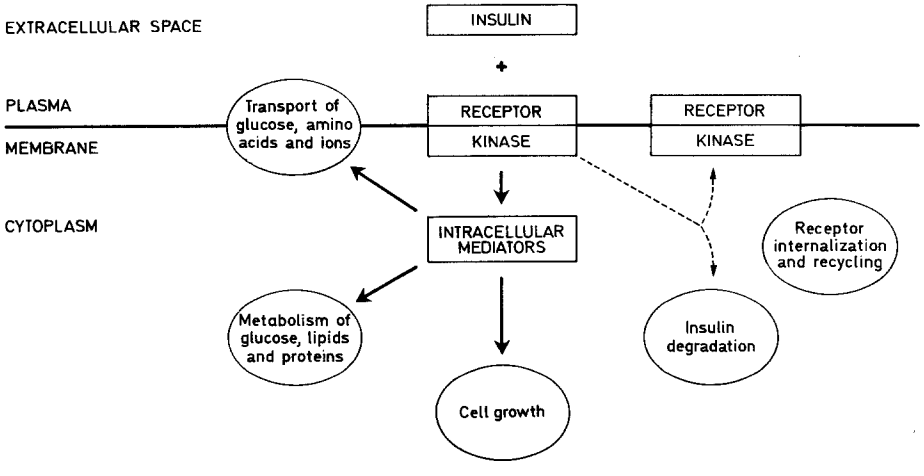

Figure 1. Cellular mechanism of insulin action. The receptor-kinase complex in the plasma membrane transmits the intracellular insulin signal to intracellular mediators e.g. phosphoproteins, which stimulate transport of glucose, amino acids and ions, metabolism of glucose, lipids and proteins and cell growth. The receptor-bound insulin is internalized and degraded whereas the receptor is recycled to the plasma membrane.

molecular mechanism of insulin action is still poorly comprehended as far as the events following the receptor binding and leading to the ultimate cellular responses are concerned. Many attempts to isolate a second messenger in insulin action have proved this to be difficult[10, 16, 33, 38, 75]. Recently, a promising discovery was made when it was demonstrated that the insulin receptor is an insulin-sensitive protein kinase[1, 24, 39, 42, 50, 60, 66, 78, 79]. This novel observation is of interest for our understanding of insulin-regulated processes, since it is now recognized that covalent phosphorylation-dephosphorylation of proteins is a mechanism whereby many cellular functions are regulated by hormones and neurotransmitters[7, 11]. Furthermore, protein kinases are also constituents of receptors for several polypeptide growth factors including epidermal growth factor (EGF)[8], platelet-derived growth factor (PDGF)[14], transforming growth factor (TGF-α)[57], and insulin-like growth factor-I (IGF-I)[35, 64], implying that receptor kinase activity may represent a general mechanism in transmembrane signaling of hormones and growth factors.

The insulin receptor kinase

The insulin receptor is an integral membrane glycoprotein ($M_r \sim 350{,}000$) composed of two α-subunits ($M_r \sim 130{,}000$) and two β-subunits ($M_r \sim 95{,}000$) linked by disulfide bonds[33, 38, 75] (fig. 2). Affinity labeling of the receptor using either photosensitive insulin analogues[34, 82], or cross-linking of insulin with bifunctional reagents[53, 68] have shown that the α-subunit is labeled predominantly by radioactive insulin, when compared to the β-subunit, the labeling of which is much weaker[47, 77], or even absent[82]. This suggests that the insulin binding site is located on the α-subunit of the receptor oligomer. In intact cells, insulin stimulates the phosphorylation of its receptor β-subunit. This was first demonstrated in rat hepatoma cells and human IM-9 lymphoblasts[39], and later in freshly isolated rat hepatocytes[78]. In these experiments, cells were preincubated with ^{32}P-ortho-phosphate to label cellular ATP, solubilized in detergent, and the glycoproteins purified on wheat-germ-agglutinin-agarose. Immunoprecipitation of phosphorylated proteins by antibodies to insulin receptor followed by sodium-dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis under reducing conditions and autoradiography revealed a labeled band ($M_r \sim 95{,}000$), the phosphorylation of which was stimulated by insulin. Its identity with the insulin receptor β-subunit was established for the following reasons. First, non-immune serum did not precipitate a band with a similar electrophoretic mobility. Second, the molecular size was identical with that determined previously, using biosynthetic and affinity labeling methods[28, 47, 53, 68, 77].

Subsequently, the phosphorylation of the β-subunit of the insulin receptor was demonstrated in cell-free systems using [γ-^{32}P] ATP in solubilized and partially purified receptor preparations from rat liver (fig. 3) and human placenta[1, 24, 42, 50, 60, 66, 78, 79]. Phosphoaminoacid analysis of the phosphorylated β-subunit of partially purified receptors showed phosphoserine, phosphothreonine and phosphotyrosine under basal conditions. Insulin induced a several-fold increase in ^{32}P-incorporation in tyrosine, and had in addition a smaller, but consistent stimulating effect on the labeling of phosphoserine[19, 43].

The insulin receptor exhibits insulin-dependent tyrosine-kinase activity. This was demonstrated in cell-free systems with detergent-solubilized and highly purified receptors obtained from various tissues. The purification scheme was based on sequential affinity chromatography on wheat-germ-agglutinin- and insulin-aga-

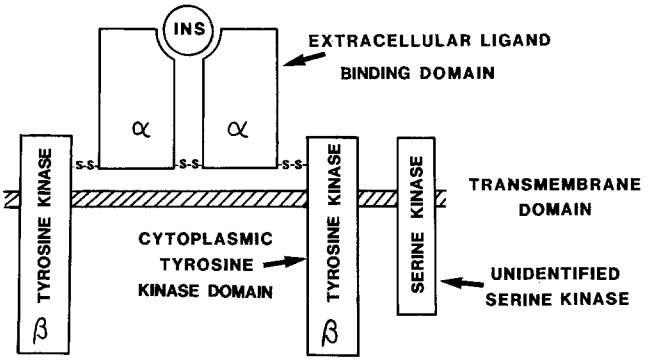

Figure 2. Schematic model of the insulin receptor kinase complex.

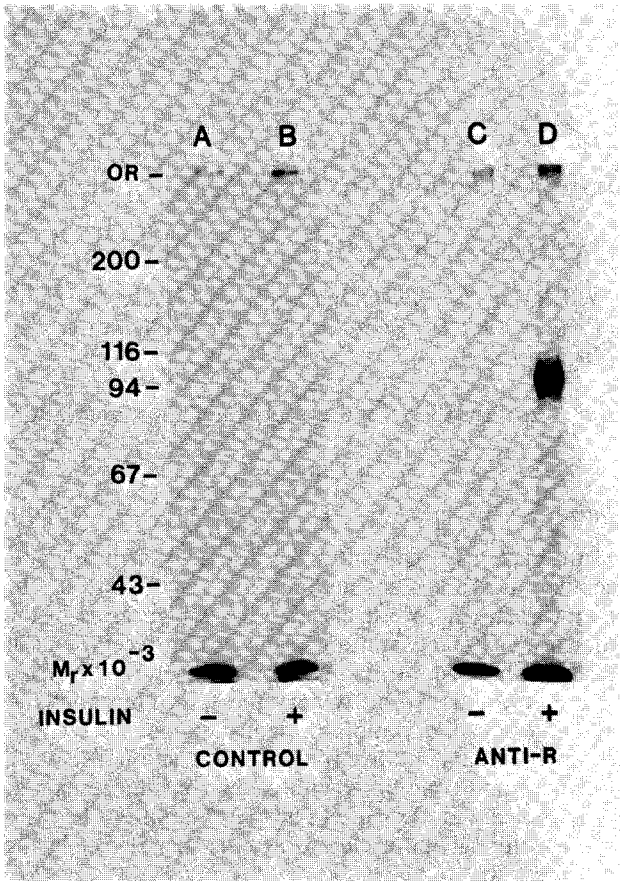

Figure 3. Phosphorylation of insulin receptors. Partially purified receptors from rat liver were incubated 30 min at 20 °C in the absence (A and C) or presence of insulin (10^{-7} mol/l) (B and D) and phosphorylated with [γ-^{32}P]ATP. The phosphoproteins were immunoprecipitated with anti-insulin receptor antiserum (C and D) or normal serum (A and B) and analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis followed by autoradiography[78].

rose[15,34,40,41,48,51]. Alternatively, the lectin-purified receptors were immunoprecipitated with antibodies to insulin receptor, obtained from patients with severe insulin-resistance and Acanthosis nigricans[27,42,43,79], or monoclonal IgG directed against insulin receptors[45,59,63]. These purified receptor preparations exhibited insulin-stimulated protein kinase activity which catalyzed phosphorylation of both the β-subunit and exogenous substrates like casein, histones and synthetic tyrosine-containing peptides[1,19,41,43,50,51,60,66,79]. In contrast to the partially purified receptor, the phosphorylation occurred exclusively on tyrosine residues in highly purified receptors under basal conditions, and the insulin stimulatory action was accounted for by a several-fold increase in phosphotyrosine[1,19,43,50,60,66]. Thus, the tyrosine kinase is a constituent of the insulin receptor, whereas the serine kinase is non-covalently associated with the receptor (fig. 2). In addition to being the principal substrate for autophosphorylation, the β-subunit has an ATP-binding site as demonstrated by covalent affinity labeling with oxidized [α-^{32}P]ATP[79] or photoreactive azido [α-^{32}P]ATP[66]. The simultaneous presence of a phosphorylation site and an ATP-binding site on the β-subunit suggests that the insulin receptor acts as its own tyrosine kinase. Further proof of the identity of the insulin receptor kinase seems to be the demonstration that the insulin-binding activity and the insulin-dependent tyrosine-kinase activity co-purified at a constant stoichiometric ratio to homogeneity[40,41,48,51,60]. Thus, the functional cell-surface insulin receptor is composed of two functional domains, one with binding activity and another with tyrosine-specific protein kinase activity. In addition, insulin binds to and promotes phosphorylation of the insulin receptor precursor, a monomeric protein of $M_r \sim 210{,}000$[3,54].

Biochemical properties of the insulin receptor kinase

Following the identification of the protein kinase activity of the insulin receptor β-subunit, its biochemical properties were investigated (table 1). These included temperature dependence, metal ion requirements, nucleotide and substrate specificity, and kinetic parameters of the phosphorylation reaction. In the absence of insulin phosphorylation occurred slowly, but addition of insulin (100 nM) rapidly stimulated the incorporation of ^{32}P from [γ-^{32}P]ATP into the β-subunit of the receptor. Within 30 s at 22 °C, autophosphorylation of the insulin-stimulated receptor reached 50% of maximum and a steady state value was reached after about 10 min[81]. Even at 4 °C, the phosphorylation was rapid; the ^{32}P-content of the receptor reached half-maximal level by 5 min and maximum after about 20 min[88].

As with the EGF-stimulated phosphorylation of the EGF-receptor[6,52], Mn^{2+} was the most potent cation in augmenting the insulin-stimulated phosphorylation of the insulin receptor[1,48,51,52,81,88]. The effect of Mn^{2+} was maximal at concentrations above 2 mM and constant up to 10 mM[52], but showed a complex relationship with the ATP-concentration (see below). Mg^{2+} was also effective, but concentrations above 15 mM were required for a maximal effect. However, the insulin-stimulated kinase showed greater activity in the presence of a combination of 2 mM Mn^{2+} and 12 mM Mg^{2+} than when either metal ion was used alone[43]. Ca^{2+} as well as Zn^{2+} and Cr^{2+} were totally ineffective, whereas Co^{2+} (2 mM) had some effect[1,88]. This ion dependency is characteristic of tyrosine kinases compared to serine kinases and threonine kinases[8]. In the cell-free system, the source of phosphate used to phosphorylate the β-subunit was identified as ATP[42], and the K_m value for ATP of the insulin-stimulated receptor kinase was determined as 30–150 μM[48,51,52,81]. No other nucleotide triphosphate (GTP, CTP, TTP or UTP) competed with [γ-^{32}P]ATP in the receptor phosphorylation assay, whereas addition of ATP and ADP, but not AMP, gave significant inhibition of ^{32}P incorporation[48,52,81]. Cyclic AMP had no effect on the phosphorylation of the receptor[39,48,50]. Thus, the insulin receptor kinase showed specificity for adenosine di- and tri-nucleotides. As mentioned above, Mn^{2+} and ATP showed a complex relationship in their activation of the kinase. Kinetic data showed that Mn^{2+} acted predominantly by decreasing the K_m for ATP presumably through binding to a specific regulatory site on the kinase rather than chelating with ATP. On the other hand, increasing ATP concentration decreased the K_m for Mn^{2+}, showing that a high substrate concentration can activate the kinase even when the metal activator concentration is low[81].

The substrate specificity of the insulin receptor kinase was assessed using both naturally occurring proteins and synthetic peptides including histones, casein, tubulin, troponin, angiotensin II, angiotensin II inhibitor, β-lipotropin, pp60*src* (a gene product of the Rous sarcoma virus), anti-pp60*src* IgG, and several synthetic peptide fragments containing a tyrosine residue[19,41,48,52,70]. In the proteins, phosphoaminoacid analysis showed only phosphorylation on tyrosine residues. Among the synthetic peptides, even a dipeptide, Tyr-Arg was a substrate although with very high K_m[70]. The K_m values varied significantly among the substrates from 1 μM to a value > 80 mM, but insulin acted by stimulating the V_{max} with no alteration of K_m[41,48,52,70]. The substrate specificity of the insulin receptor kinase was similar, but not identical with that of the EGF receptor[46,52] and the pp60*src* kinases[30], suggesting that they are members of a superfamily of tyrosine

Table 1. Major features of the insulin receptor kinase*

1. Intrinsic to the receptor
   - ATP-binding site on the receptor β-subunit
   - Phosphorylation of highly purified receptors
   - Co-purification of insulin binding activity and insulin-stimulated kinase activity
   - Present when receptors present
2. Regulators
   - Insulin
   - ATP (phosphate donor)
   - Mn^{2+}, Mg^{2+}
3. Substrates
   - Receptors: autophosphorylation
   - Substrates: exogenous and endogenous
4. Phosphoamino acids in receptor
   - Intact cells: tyrosine and serine
   - Cell-free systems: predominantly tyrosine
5. Multiple sites phosphorylated on receptor β-subunit

*The features listed were compiled from data in refs. 1, 41–43, 48, 50–52, 58, 60, 66, 70, 79, 81, 83, 84, 88.

kinases which has diverged from a common evolutionary origin.

It seems that the insulin receptor β-subunit is the best substrate for its own kinase. This conclusion is based on the observation that the V_{max} for autophosphorylation of the insulin receptor kinase was increased nearly 20-fold by insulin[81], whereas the V_{max} values for other substrates were only increased 2–5-fold[41, 48, 52, 70]. Alternatively, it has been suggested that the kinase activity associated with the insulin receptor is increased by tyrosine phosphorylation of the receptor β-subunit[58, 83, 84]. Phosphorylation on tyrosine residues induced by insulin leads to increased kinase activity, whereas dephosphorylation of the tyrosine residues by alkaline phosphatase is accompanied by a marked inhibition[83]. Thus, the autophosphorylation on tyrosine residues may play a key role in regulating the insulin receptor kinase. The additional phosphorylation of serine and threonine residues on the receptor by non-covalently associated kinases may also exert a regulatory role on insulin receptor kinase and binding activity[10].

Role of receptor phosphorylation in insulin action

The biological relevance of insulin receptor phosphorylation is not clear. It is possible that it plays a role in cellular processes such as receptor affinity regulation, hormone and receptor internalization and signal transmission. These phenomena are well-characterized[10, 16, 33, 38, 75], but their molecular mechanism is almost completely unknown. Most likely, receptor regulation, internalization and transmembrane signaling are integrated events in insulin action, and receptor autophosphorylation per se is involved in transmission of the insulin message to cellular enzymes and transport carriers. At present, it is tempting to suggest that the covalent receptor modification is an early step in insulin action and that the increased kinase activity of the insulin receptor evoked by hormone binding would lead to phosphorylation-dephosphorylation of other cellular proteins, and through the generation of a cascade of reactions this would result in the final effects of insulin. Five requirements should be fulfilled by the insulin-induced receptor kinase activation and autophosphorylation before one can say with certainty that they are involved in physiological insulin action.

First, the insulin dose-response relationship of the kinase should be within the physiological range and correlate with that of the binding to the receptor. Several authors found that the kinase activation was half-maximal at an insulin concentration of 2–5 nM ($\sim ED_{50}$), which corresponded to the apparent K_d of the receptor-insulin complex as determined with the same preparations of solubilized receptor of varying purity obtained from human placenta[1, 43, 48, 51, 52, 58, 65, 66]. In contrast, a dissociation between the dose-response curves of insulin binding and kinase activation was observed with soluble receptors from rat liver and human erythrocytes[21, 88]. In these studies, the apparent K_d exceeded the ED_{50} by a factor of 3–10, which suggested that the phenomenon of 'spare receptors' observed for other insulin actions[16] is also applicable for kinase activation. It is not clear whether the different findings are the result of differences in the tissues, purification procedures, or assay methods used. In conclusion, the receptor kinase is activated by insulin concentrations within a physiological range corresponding to the receptor binding.

Secondly, the receptor kinase should be capable of phosphorylating cellular substrate other than the receptor itself, in order to propagate the insulin response. As discussed in detail above, the insulin receptor kinase is capable of phosphorylating a number of substrates on tyrosine residues, in vitro, although none of the proteins tested are physiological substrates[19, 41, 51, 52, 70]. Recently, two laboratories, independently, identified a cellular protein substrate of $M_r \sim 110{,}000$–$120{,}000$ for the insulin receptor kinase in wheat-germ-agglutinin purified glycoproteins from rat liver and rabbit brown adipose tissue[56, 65]. The naturally occurring glycoprotein appears as a monomeric structure, and it is not part of the insulin receptor itself, because it was not immunoprecipitated by highly specific antibodies to insulin receptor. Phosphorylation of the $M_r \sim 110{,}000$ protein and autophosphorylation of the receptor β-subunit ($M_r \sim 95{,}000$) were stimulated by insulin in a remarkably similar dose-dependent fashion with an ED_{50} of 1 nM. Further kinetic studies suggested that the phosphorylation of the $M_r \sim 110{,}000$ protein occurred after autophosphorylation of the insulin receptor kinase[65]. The nature and function of this endogenous substrate is as yet unknown; nor can we answer the intriguing question whether it displays kinase or phosphatase activity.

In intact cells, a rapid insulin-stimulated phosphorylation of its receptor on tyrosine residues is followed by a slower serine phosphorylation[43]. Furthermore, in a cell-free system of partially purified receptor, some laboratories have reported that insulin stimulates phosphorylation of both tyrosine and serine residues of its receptor[43, 83, 85], as well as on exogenous substrates[19]. The serine kinase is non-covalently associated with the receptor, and is removed during further purification, because the highly purified receptor displayed only tyrosine kinase activity[19, 41, 42, 48, 51]. The relationship between the two protein kinase activities associated with the receptor and their cellular role remains to be established. Two possibilities exist (fig. 4), one in which both kinases serve separate cellular functions, and another one with sequential activation of the kinases[76].

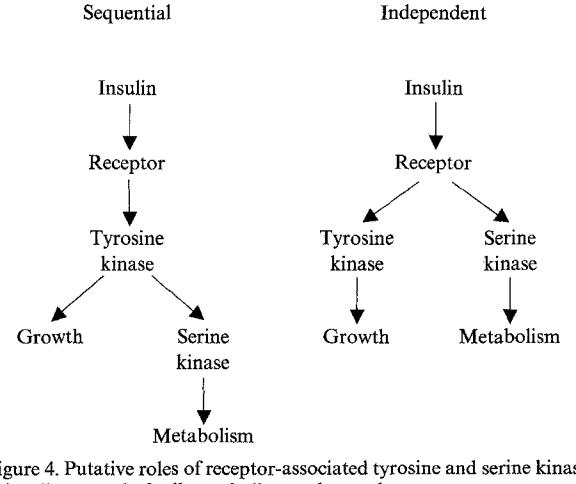

Figure 4. Putative roles of receptor-associated tyrosine and serine kinases in insulin control of cell metabolism and growth.

The first model implies that the tyrosine-specific enzyme activity is involved in insulin's growth-promoting action in a similar way to the tyrosine phosphorylations mediating the cellular responses to growth factors such as EGF[8], PDGF[14] and TGF-α[57] and several cellular and retroviral oncogene proteins[2,29,31]. In contrast, the serine kinase would play a role in insulin's metabolic actions. All kinases involved in the control of intermediary metabolism are indeed serine or threonine specific[7,11], and phosphoserine and phosphotreonine constitute about 99.97% of all phosphorylated amino acids; phosphotyrosine accounts for the remaining 0.03%[32]. In the second model, the two kinases are activated sequentially. Insulin binding to its receptor leads to activation of the constituent tyrosine kinase, which then induces activation of the receptor-associated serine kinase and this last one accounts for the generation of cellular responses to insulin. Future work should be directed towards the identification of the serine kinase.

The third criterion is that of reversibility of insulin receptor phosphorylation. To exert a regulatory function, the phosphorylated and activated receptor kinase should return to basal activity through a dephosphorylation reaction. Solubilized, lectin-purified receptors from rat liver membranes contained phosphatase activity, which slowly reduced the ^{32}P-content of the phosphorylated receptor, and which was insulin-independent[25,44,86]. The physiological significance of this reaction is difficult to ascertain, in particular because a 2-h incubation at 22 °C was required for complete dephosphorylation of the receptor[44]. A different approach was taken by incubation of the phosphorylated insulin receptor with alkaline phosphatase, which resulted in removal of about 50% of the phosphotyrosine in the β-subunit, and about 65% reduction in kinase activity, suggesting that dephosphorylation is accompanied by deactivation of the receptor kinase[83,86]. These observations demonstrate that the insulin receptor kinase can be deactivated through dephosphorylation of phosphotyrosine residues, although the physiological mechanism remains to be elucidated.

The fourth criterion is the specificity of the insulin effect on its receptor kinase. Several insulin analogues including porcine proinsulin, desoctapeptide insulin, desalanine-desasparagine insulin, guinea pig insulin, insulin-like growth factor II and covalently linked insulin dimers stimulated receptor phosphorylation with potencies relative to porcine insulin which were identical to their relative binding affinities and potencies in other assay systems[21,42,50,52,62,88]. Furthermore, polyclonal antisera directed against the insulin receptor, which show insulin-like effects in several cell-types, were also able to stimulate the tyrosine-specific kinase associated with the receptor[50,85]. However, some antibodies were inactive, although they showed both insulin-like effects in intact cells and interaction with receptors in cell-free preparations[69,87]. The reason for this discrepancy is not clear, but a possible explanation is that activation of the receptor-associated tyrosine kinase mediates the growth activity of insulin and not the metabolic actions (fig. 4). Finally, other hormones which do not bind to the insulin receptor, including EGF, which activates its own receptor kinase, had no effect on insulin receptor kinase activity[53,82]. In conclusion, the insulin effect on phosphorylation of its own receptor has the affinity and specificity of a typical insulin receptor mediated event.

Finally, the kinase activity is present whenever insulin receptors are present. So far, receptors in all cell-types investigated have shown insulin-stimulated phosphorylation of the β-subunit. These include liver[42,78,79], adipose tissue[24,80], skeletal muscle[5,26,46], placenta[50,60,66], lymphocytes[22], erythrocytes[21], fibroblasts[76], brain cortex[17,55] and various tumor cell lines like IM-9 lymphoblasts[39], 3T3-L1 adipocytes[50], hepatoma[39,43,87] and insulinoma cells[18]. Thus, the insulin-sensitive kinase is a general feature of the insulin receptor.

Additional evidence for a role of insulin receptor kinase in insulin action was obtained from two kinds of observations (table 2). First, the receptor kinase activity is impaired in cells from various insulin-resistant states including the syndrome of insulin resistance and Acanthosis nigricans, type A[20-22], from melanoma cell cultures[27], from goldthioglucose obese mice[46], and from streptozotocin-diabetic rats[37]. Second, insulinomimetic agents like vanadate, concanavalin A, wheat-germ-agglutinin, and trypsin, which act via the insulin receptor, increased the receptor autophosphorylation[44,61,71,72].

In conclusion, five criteria are fulfilled which establish the kinase activity as a fundamental property of the insulin receptor and strongly suggest an important role in insulin action. Data from several laboratories suggest that receptor phosphorylations are involved in insulin receptor autoregulation and in the transmission of the insulin signal. At present there is no information on a role in insulin receptor internalization. It has been proposed that tyrosine phosphorylation of the β-subunit regulates its kinase activity, whereas receptor phosphorylation on serine and threonine residues could play a role in modulation of the binding affinity of the α-subunit as well as kinase activity of the β-subunit. Furthermore, phosphorylation of an endogenous substrate on tyrosine and serine might represent a secondary event leading to insulin actions on cellular metabolism and growth.

Structure-function relationship of the insulin receptor kinase

Several authors have attempted to purify the insulin receptor for structural analysis. The protocols used were mainly based on affinity chromatography using agarose conjugated with lectins such as concanavalin or wheat-germ-agglutinin, followed by insulin-agarose[9,15,36,41,48,51,63,67,68]. At least three laboratories succeeded

Table 2. Evidence for a role of insulin receptor kinase in insulin action*

Impaired insulin receptor kinase activity in insulin-resistant states
1. Syndrome of insulin resistance and Acanthosis nigricans type A
2. Cultured melanoma cells
3. Mice rendered obese by goldthioglucose
4. Streptozotocin-diabetic rats
Increased insulin receptor phosphorylation induced by insulinomimetic agents
1. Vanadate
2. Trypsin
3. Concanavalin A
4. Wheat-germ-agglutinin

*Data from refs. 22, 23, 27, 37, 44, 46, 61, 71, 72.

in purifying the receptor from placental membranes[15, 51, 63]. The pure insulin receptor has a binding capacity of 1.1–1.5 mol insulin per mol of receptor (M_r 300,000)[15, 51], and protein kinase activity with a V_{max} of 80 mmol/min/mg (using angiotensin as substrate)[51].

Recently, the amino acid sequence of the human insulin receptor precursor was deduced from human placental complementary DNA (cDNA) clones[9, 73]. This achievement was based on amino-terminal sequences obtained for both α- and β-subunits of the purified receptor, which were used for the design of single long synthetic DNA probes and hybridization screening of a DNA library to identify human insulin receptor cDNA clones. Nucleotide sequence analysis of cDNA positive clones which hybridized with both α- and β-subunit DNA probes revealed a sequence of 5181 base-pairs which coded for 1382 amino acids, including a 27-residue signal peptide. This is the amino acid sequence of the insulin receptor single chain precursor, composed of a N-terminal α-subunit (735 residues) followed by a β-subunit (620 residues) and an intervening peptide composed of 4 basic amino acids (Arg-Lys-Arg-Arg), which probably represents the cleavage site for the receptor precursor processing enzyme[9, 73]. The α-chain is largely hydrophilic, with a few short hydrophobic stretch and contains sequences for asparagine N-linked glycosylation and an unusually large number of 37 cysteine residues. The β-chain contains a sequence of 23–26 hydrophobic amino acids which probably represents a single transmembrane region dividing the β-subunit into a shorter extracellular portion, which links the α-subunit through disulfide bridges, and a longer cytoplasmic part (fig. 2).

The cytoplasmic part of the insulin receptor β-subunit shows some homology with other tyrosine-specific kinases like the *src* oncogene kinases[29, 31] and the EGF-receptor kinase[12, 74]. The similarities in sequence include the ATP-binding site and the residues essential for kinase activity as well as tyrosine residues which can be phosphorylated, demonstrating that the insulin receptor is a member of the *src* family of tyrosine kinases. No cellular proto-oncogene has yet been identified which is identical with the insulin receptor β-subunit as is the case for the EGF receptor and *v-erb B* oncogene product[73, 74], although one region of the insulin receptor β-subunit (51 residues) is practically identical with a portion of the *v-ros* transforming protein[49]. It is possible that the insulin receptor is the cellular homologue of the *v-ros* transforming protein, which has a $M_r \sim 68{,}000$, tyrosine kinase activity and a hydrophobic transmembrane region at the N-terminus[49].

In conclusion, the amino acid sequence of the insulin receptor gives evidence that the β-subunit is a tyrosine kinase. Future studies will define the phosphorylation site at tyrosine and serine residues which might help in understanding the functional role of receptor phosphorylations.

Summary and conclusions

The recent characterization of the human insulin receptor structure and its intrinsic tyrosine kinase activity represent major advances in our understanding of the mechanism of insulin action. It is reasonable to think that the insulin-induced autophosphorylation and activation of its receptor kinase represent an important event in the action of insulin on cell metabolism and growth. The fundamental research reviewed may be followed by the discovery of molecular receptor defects in clinical syndromes of insulin resistance.

Note added in proof:
Two papers have recently described that in intact cells, insulin induces a rapid several-fold increase in ^{32}P-incorporation in tyrosine of its receptor β-subunit followed by a slower rise in labeling of phosphoserine (Pang, D. T., Sharma, B. R., Schafer, J. A., White, M. F., and Kahn, C. R., Predominance of tyrosine phosphorylation of insulin receptors during the initial response of intact cells to insulin. J. biol. Chem. *260* (1985) 7131–7136; White, M. F., Takayama, S., and Kahn, C. R., Differences in sites of phosphorylation of the insulin receptor in vivo and in vitro. J. biol. Chem. *260* (1985) 9470–9478).

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Internalization of polypeptide hormones and receptor recycling

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Introduction

The insulin receptor is an integral plasma membrane glycoprotein of most cells. It consists of 2 subunits linked by disulfide bonds; the alpha and beta subunits of the receptor are synthesized by way of a single chain proreceptor which is cleaved and further processed, by the addition of complex carbohydrates, prior to insertion into the plasma membrane[49]. Recently a cDNA encoding the proreceptor has been cloned and the protein sequence