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Review article

Insulin receptor : tyrosine kinase activity and insulin action

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Summary — The first step in insulin action consists in binding of the hormone to specific cell surface receptors. This receptor displays two functional domains : an extracellular α-subunit containing the majority or the totality of the hormone binding site and an intracellular β-subunit possessing insulin-stimulated tyrosine kinase activity.

A general consensus has been reached in favour of the idea that this receptor enzymic function is essential for generation of the metabolic and growth-promoting effects of insulin. Concerning the mechanism of transmembrane signalling, we like to think that interaction of insulin with the receptor α-subunit triggers a conformational change, which is propagated to the β-subunit and activates it. The active receptor kinase leads then to the phosphorylation of cellular protein substrates, which are likely to belong to two broad categories, those generating metabolic effects of insulin and those resulting in growth-promoting effects. The phosphorylated and active substrates then generate the final effects of insulin

insulin receptors — hormone signalling — tyrosine kinase

Résumé— Récepteur de l'insuline : activité tyrosine kinase et rôle dans le mécanisme d'action de l'hormone. *La première étape du mécanisme d'action de l'insuline implique la liaison de l'hormone à des récepteurs spécifiques exprimés au niveau de la membrane plasmique. Le récepteur présente deux domaines fonctionnels : un domaine extracellulaire liant l'insuline, contenu dans la sous-unité α; une activité tyrosine-kinase stimulée par l'insuline et contenue dans la partie intracellulaire de la sous-unité β. Un large consensus s'est dégagé sur le fait que cette activité enzymatique du récepteur est requise pour la génération des effets métaboliques et mitogéniques induits par l'insuline. En ce qui concerne le mécanisme de transmission du message hormonal, nous pensons que la liaison de l'hormone à la sous-unité α provoque une modification de conformation qui est répercutée au niveau de la sous-unité β et en active la fonction kinase. La kinase ainsi activée phosphoryle deux types de substrats cellulaires : ceux qui génèreront les effets métaboliques de l'insuline et ceux qui induiront ses effets sur la croissance cellulaire. Activées par la phosphorylation, ces protéines pourraient induire les différentes réponses biologiques de l'insuline.*

récepteur de l'insuline — activation cellulaire — tyrosine kinase

INTRODUCTION

Regulation of cellular metabolism and growth by insulin is the result of a series of events initiated by the interaction of the hormone with its cell surface receptors. The insulin receptor structure was elucidated through a variety of techniques (Van Obberghen, 1984) and, more recently, the aminoacid sequence of the human insulin receptor precursor has been unravelled by recombinant DNA technology (Ullrich *et al.*, 1985; Ebina *et al.*, 1985). Despite this progress, the molecular mechanism of insulin action is still not entirely understood with regard to the events following receptor binding and leading to the ultimate cellular responses. However, over the years, considerable evidence has been gathered indicating that reversible phosphorylation contributes to the mechanism of insulin action (Denton, 1986). In addition, a promising discovery was made by the demonstration that the insulin receptor is an insulin-sensitive protein kinase (Kasuga *et al.*, 1982a; Van Obberghen & Kowalski, 1982; Petruzzelli *et al.*, 1982). This observation is of particular interest for our understanding of insulin-regulated processes, since it is now recognized that phosphorylation-dephosphory-lation of proteins is a mechanism whereby many cellular functions are regulated by hormones and neurotransmitters. Furthermore, protein kinases are also constituents of receptors for several growth factors, implying that receptor kinase activity may represent a general mechanism in transmembrane signalling of hormones and growth factors.

INSULIN RECEPTOR PHOSPHORYLATION

In intact cells, insulin stimulates the phosphorylation of its receptor β subunit (Van Obberghen & Kowalski, 1982; Kasuga *et al.*, 1982a). In these experiments, cells were preincubated with [^{32}P]P_i to label cellular ATP, solubilized, and the glycoproteins purified on WGA-agarose. Immunoprecipitation by antibodies to insulin receptors followed by SDS/PAGE under reducing conditions and autoradiography revealed a labeled band (M_r 95 k), the phosphorylation of which was stimulated by insulin. Its identity with insulin receptor β subunit was established based on its appropriate electrophoretic mobility and on the fact that it was not precipitated with nonimmune serum. In intact cells, phosphoaminoacid analysis of the insulin receptor β subunit showed phosphorylation of serine, threonine, and tyrosine under basal conditions. Insulin induced a rapid, several-fold increase in ^{32}P incorporation on tyrosine, followed by a slower rise in labeling of phosphoserine (Kasuga *et al.*, 1982; Gazzano *et al.*, 1983; White *et al.*, 1985b; Ballotti *et al.*, 1987).

Subsequently, insulin-stimulated phosphorylation of the insulin receptor β subunit was demonstrated in cell-free systems using [γ-^{32}P]ATP and purified receptors (Van Obberghen & Kowalski, 1982; Kasuga *et al.*, 1982c; Van Obberghen *et al.*, 1983; Shia & Pilch, 1983; Petruzzelli *et al.*, 1982; 1984).

These purified receptor preparations exhibited insulin-stimulated protein kinase activity which catalysed phosphorylation of

both the β subunit and exogenous substrates. With a highly purified receptor, the phosphorylation occurred exclusively on tyrosine residues under basal conditions, and insulin stimulatory action was accounted for by a several-fold increase in phosphotyrosine. Thus, the tyrosine kinase appeared as a constituent of the insulin receptor. Further, the β subunit contains an ATP-binding site as demonstrated by covalent affinity labeling (Van Obberghen *et al.*, 1983; Shia & Pilch, 1983; Roth & Cassell, 1983). The simultaneous presence of phosphorylation sites and an ATP binding site on the receptor β subunit indicates that the insulin receptor acts as its own tyrosine kinase. Further proof is the demonstration that the insulin-binding activity and insulin-dependent tyrosine-kinase activity copurified to homogeneity at a constant stoichiometric ratio (Petruzzelli *et al.*, 1984). In addition, insulin binds to and promotes phosphorylation of the insulin receptor precursor (M_r 210 Kd) (Rees-Jones *et al.*, 1983).

BIOCHEMISTRY OF THE INSULIN RECEPTOR KINASE

Following the identification of the insulin receptor protein kinase activity, its biochemical properties have been investigated in detail. For original references, see Gammeltoft & Van Obberghen (1986).

ROLE OF INSULIN RECEPTOR TYROSINE KINASE IN HORMONE ACTION

Characteristics of insulin receptor tyrosine kinase

Since the discovery that the insulin receptor is an insulin-dependent protein tyrosine kinase, it was anticipated that this receptor enzymic function was involved in insulin action (Gazzano *et al.*, 1983). For the validity of this contention, the following five criteria were expected to be fulfilled. First, the insulin dose-response relationship of the kinase should be within the physiological range and correlate with that of receptor binding. Several authors found that the kinase activation was half-maximal at an insulin concentration of 2-5 nM (ED_{50}), which corresponded to the apparent K_d of the receptor-insulin complex of the same solubilized receptor preparations (Kasuga *et al.*, 1982b; Shia & Pilch, 1983; Petruzzelli *et al.*, 1984; Sadoul *et al.*, 1985). In contrast, a dissociation between dose-response curves of insulin binding and kinase activation was observed with soluble receptors from rat liver and human erythrocytes (Grigorescu *et al.*, 1983). In this instance, the apparent K_d exceeded the ED_{50} by a factor of 3-10, suggesting that the phenomenon of "spare receptors", observed for other insulin actions, was also applicable for kinase activation. It is not clear whether these findings are explained

by differences in tissues, purification procedures, or assay methods. In conclusion, in most instances, the receptor kinase is activated by insulin concentrations within a physiological range corresponding to receptor binding.

Second, the receptor kinase should be capable of phosphorylating cellular substrates other than the receptor itself, in order to propagate the insulin response. The insulin receptor kinase can phosphorylate a number of substrates on tyrosine *in vitro*, although none of the proteins tested are proven to be physiologically relevant substrates. The first two «putative» substrates described were a 110-120 kDa protein and a 185 kDa protein. In 1985, two laboratories independently identified in purified glycoproteins from rat liver and rabbit brown adipose tissue, a cellular protein «substrate» of M_r 110 K for the insulin receptor kinase (Sadoul *et al.*, 1985; Rees-Jones & Taylor, 1985). This glycoprotein appears as a monomeric structure and is not part of the insulin receptor itself. Phosphorylation of the M_r 110 K protein and of the receptor β subunit were stimulated by insulin in a remarkably similar dose-dependent fashion ($ED_{50} \approx 1$ nM). In addition, kinetic studies suggested that phosphorylation of the M_r 110 K protein occurred after activation and phosphorylation of the insulin receptor kinase. The nature and function of this endogenous substrate is as yet unknown. In the same period, a different putative substrate for the insulin receptor kinase was identified in a hepatoma cell line, Fao (White *et al.*, 1985a). This M_r 185 kDa substrate does not contain carbohydrate moieties and appears to be monomeric. Since the reports on these two «putative» substrates, a number of other phosphoproteins have been described, including an M_r 15 kDa that may play a role in insulin-mediated glucose transport (Bernier *et al.*, 1987). However, at present, it remains to be seen whether any of these proteins have a physiological significance. One is forced to admit that, despite intensive efforts, extremely little is known about «putative» substrates. This is likely due to the fact that they are rare and labile. In addition, as expected from the large array of biological responses induced by insulin, a whole series of non-abundant regulatory proteins are likely to exist to account for the metabolic and growth-promoting effects of insulin.

The third criterion is 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. Lectin-purified receptor preparations were found to contain phosphatase activity which slowly reduced the ^{32}P content of phosphorylated receptor and was insulin-independent (Kowalski *et al.*, 1983). Exposure of phosphorylated insulin receptor to alkaline phosphatase resulted in the removal of about 50% of the β subunit phosphotyrosine and about 65% reduction in kinase activity (Yu & Czech, 1984). Thus, the insulin receptor kinase can be de-activated through dephosphorylation of tyrosine residues.

The fourth criterion concerns the specificity of insulin effect on its receptor kinase. Several insulin analogues stimulated receptor phosphorylation with potencies relative to porcine insulin and identical with their relative binding affinities and with potencies in other assay systems (Kasuga *et al.*, 1982a; Grigorescu *et al.*, 1983). Finally, polyclonal antisera to insulin receptor, which exert insulin-like effects in several cell types, were also able to stimulated the receptor tyrosine kinase (Gammeltoft & Van Obberghen, 1986; Gherzi *et al.*, 1987). In conclusion, the insulin effect on receptor phosphorylation has the affinity

and specificity of a typical insulin receptor mediated event.

Combined, the kinase activity of the insulin receptor seems to be a fundamental receptor property, since whenever insulin receptors are present, insulin-stimulated autophosphorylation occurs (Gammeltoft *et al.*, 1984, 1985; Gazzano *et al.*, 1985; Grigorescu *et al.*, 1983; Kasuga *et al.*, 1982a, c; Petruzzelli *et al.*, 1984; Shia & Pilch, 1983; Tanti *et al.*, 1986; Van Obberghen & Kowalski, 1982). An important feature of the insulin receptor tyrosine kinase is that receptor autophosphorylation on one or more tyrosyl residues activates the receptor kinase towards exogenous substrates without affecting the insulin-binding caracteristics (Rosen *et al.*, 1983; Yu & Czech, 1984).

Insulin receptor tyrosine kinase and hormone action

A general consensus has been reached for a role of insulin receptor kinase in hormone action. The first series of suggestive observations was provided by studies, showing that alterations in insulin action are associated with parallel alterations in insulin receptor tyrosine kinase activity. Thus, the receptor kinase is impaired in various insulin-resistant states including the syndrome of extreme insulin resistance type A (Grunberger *et al.*, 1984), melanoma cell cultures (Häring *et al.*, 1984), gold-thioglucose obese mice (Le Marchand-Brustel *et al.*, 1985), and streptozotocin diabetic rats (Kadowaki *et al.*, 1984). Conversely, insulin receptor kinase is hyperactive in insulin hyperresponsive adipocytes of young obese Zucker rats (Debant *et al.*, 1987). Further, insulinomimetic agents (vanadate, lectins, trypsin) increased receptor autophosphorylation (Tamura *et al.*, 1983; Roth *et al.*, 1983). Introduction into mammalian cells of a monoclonal antibody which inhibits insulin receptor kinase, blocks the rapid effects of insulin (Morgan & Roth, 1987). Conversely, microinjection of antiphosphotyrosine antibodies, which stimulate the insulin receptor kinase, induces enhanced insulin-evoked glucose transport and aminoacid uptake (Ballotti *et al.*, 1989).

The most convincing and elegant evidence for the idea that insulin action depends on receptor protein tyrosine kinase activity, comes from mutagenesis experiments involving the receptor. Thus, insulin receptors mutated on the ATP binding site, lack protein tyrosine kinase activity and fail to mediate insulin post-receptor effects, including glucose transport, glycogen synthesis, S6 kinase activity and thymidine uptake (Chou *et al.*, 1987).

SERYL (AND THREONYL) PHOSPHORYLATION OF INSULIN RECEPTORS

In intact cells, the rapid insulin-stimulated phosphorylation of its receptor on tyrosine is followed by a slower serine phosphorylation (Kasuga *et al.*, 1982c; Gazzano *et al.*, 1983; White *et al.*, 1985b; Ballotti *et al.*, 1987; Pang *et al.*, 1985). In addition, with partially purified insulin receptor, insulin-stimulated phosphorylation of both tyrosine and serine on its receptor (Kasuga *et al.*, 1982; Zick *et al.*, 1983), as well as on exogenous substrates (Gazzano *et al.*, 1983; Ballotti *et al.*, 1986) was shown. The serine kinase activity appears to be non-covalently associated with the receptor and is removed during further purification, because highly purified receptor solely displayed tyrosine kinase activity. The relationship between the two protein kinase

activities associated with the receptor, and their cellular role, remain to be established. At least two possibilities exist, one in which the two kinase activities serve separate cellular functions, and another with sequential kinase activation (Gazzano *et al.*, 1983). According to the first model, the tyrosine kinase would be involved in insulin's growth-promoting action similar to tyrosine phosphorylations, mediating cellular responses to growth factors and to cellular and retroviral oncogene proteins (Hunter & Cooper, 1985). In contrast, the serine kinase activities would play a role in insulin's metabolic actions. All kinases involved in the control of intermediary metabolism are indeed serine- or threonine-specific. In the second model, the two types of kinases are activated sequentially. Insulin binding to receptor leads to activation of the constituent tyrosine kinase, which induces activation of the receptor-associated serine kinase(s), accounting for the generation of cellular responses to insulin.

In contrast to insulin receptor autophosphorylation on tyrosine residues, phosphorylation of insulin receptor β subunit on serine and threonine residues results in a decrease in receptor tyrosine kinase activity. This has been observed in the following situations :

— intact cells treated either with phorbol esters, thought to act through protein kinase C (Jacobs *et al.*, 1983; Häring *et al.*, 1986), or with agents leading to an increase in cellular cAMP (Stadtmauer & Rosen, 1986);

— purified insulin receptors exposed to cAMP-dependent protein kinase (Roth & Beaudoin, 1987) or protein kinase C (Bollag *et al.*, 1986).

The role of serine and threonine phosphorylation of the insulin receptor remains a matter of speculation. It is tempting to suggest that at least part of the antagonistic action of some hormones, which oppose insulin's effects and act through cAMP dependent protein kinase or protein kinase C, is mediated by seryl and threonyl β subunit phosphorylation with a concomitant decrease in receptor tyrosine kinase activity. Furthermore, like to think that seryl and threonyl insulin receptor phosphorylation might also be the endpoint of a negative feed-back loop in the regulation of insulin action. According to this idea, the activated insulin receptor tyrosine kinase would phosphorylate and activate a series of functional substrates, one of which would be a serine/threonine kinase. This kinase would phosphorylate the insulin receptor on serine/threonine residues leading to reduced tyrosine kinase activity and, consequently, to a decreased insulin signal.

SIGNAL TRANSDUCTION

A large body of evidence establishes insulin receptor tyrosine kinase as fundamental property of the receptor and indicates that this receptor enzymic function is essential for generation of the metabolic and growth-promoting effects of insulin. The insulin receptor displays two functional domains, an extracellular insulin binding α subunit and an insulin-responsive protein kinase contained within the intracellular domain of the β subunit. At present, it has not been established how hormone recognition at the cell surface transmits a signal to the cytoplasmic receptor domain through a unique transmembrane stretch. The simplest mechanism would be that interaction of insulin with the receptor α subunit trigger a conformational change which is propagated at the level of the contact region between α–β subunits, resulting in ac-

tivation of the receptor kinase. How the activated receptor kinase transduces the hormone signal is not known at present. We favour the idea that the insulin-stimulated receptor kinase leads to phosphorylation of cellular protein substrates, which are likely to belong to two broad categories those generating metabolic effects of insulin and those resulting in growth-promoting effects.

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