Two different protein kinase activities are associated with the insulin receptor

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(Received 11 July 1983/Accepted 18 August 1983)

In intact rat hepatocytes insulin stimulates the phosphorylation of the β -subunit of its receptor exclusively on serine residues, which are also phosphorylated in the absence of insulin. In contrast, in partially purified insulin receptors derived from these same cells and in highly purified insulin receptors obtained by immunoprecipitation with anti-receptor antibodies, the receptor β -subunit is phosphorylated solely on tyrosine residues. For both cell-free systems, insulin's stimulatory action on receptor phosphorylation leads to an increase in phosphotyrosine. When partially purified receptors were used to phosphorylate two exogenous substrates, casein and histone, insulin was found to stimulate the phosphorylation of both tyrosine and serine. However, the basal and insulin-stimulated kinase activity of immunoprecipitated receptors was only tyrosine-specific. From these observations we propose that the insulin-receptor complex consists of two different insulin-stimulatable kinase activities: (1) a tyrosine-specific kinase, which is a constituent of the insulin-receptor structure and whose activation is likely to be the first post-binding event in insulin action; and (2) a serine-specific kinase, which is closely associated with the receptor in the cell membrane.

Insulin binds to its specific plasma membrane receptors in target cells and then initiates a complex array of biological responses (Kahn, 1976; Roth, 1979). A uniform picture of the insulin receptor has emerged from studies by a variety of methods (Yip *et al.*, 1980; Pilch & Czech, 1980; Jacobs *et al.*, 1980; Wisher *et al.*, 1980; Van Obberghen *et al.*, 1981; Hedo *et al.*, 1981). In brief, the native insulin receptor is a glycoprotein complex composed of two M_r -130 000 (α) and two M_r -95 000 (β) subunits linked together by disulphide bonds. The mechanism of the transduction of the binding event into cellular activation is poorly understood (Denton *et al.*, 1981). More recently, a promising advance has been made to contribute to our understanding of the transfer of information from the hormone receptor to the cell by the demonstration that insulin stimulates the phosphorylation of its own receptor, both in intact cells and in cell-free systems (Kasuga *et al.*, 1982a; Van Obberghen & Kowalski, 1982;

Petruzzelli *et al.*, 1982; Kasuga *et al.*, 1983; Van Obberghen *et al.*, 1983; Shia & Pilch, 1983). Further, we have identified an ATP-binding site on the β -subunit of the insulin receptor and found that insulin-stimulated receptor phosphorylation is retained in highly purified receptors (Van Obberghen *et al.*, 1983). From these studies, we proposed that the receptor β -subunit is a protein kinase capable of self-phosphorylation. This ligand-induced covalent receptor modification is likely to be an early, if not primary, event in insulin action.

In intact hepatoma cells the phosphorylation of the insulin receptor occurs on both serine and tyrosine residues (Kasuga *et al.*, 1982b), whereas in cell-free systems consisting of detergent-solubilized receptors obtained from placenta membranes (Shia & Pilch, 1983) or rat liver membranes only tyrosine is found to be phosphorylated (Kasuga *et al.*, 1982a). In the present study we have studied in detail the nature of the phosphoamino acids of insulin receptors phosphorylated in intact rat hepatocytes and in solubilized receptors derived from these cells. We have also analysed the protein kinase activities towards exogenous substrates of insulin receptors at different degrees of purity.

Abbreviations used: SDS, sodium dodecyl sulphate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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Materials and methods

Materials

Pig insulin (monocomponent) was purchased from Novo (Copenhagen, Denmark); collagenase (type II) and histone (H2B) were from Worthington; Nonidet P-40, ATP, *N*-acetyl-D-glucosamine, wheat-germ-agglutinin-agarose, bovine serum albumin (fraction V), bacitracin, casein, phosphoserine and phosphothreonine were from Sigma. [γ - 32 P]ATP (triethylammonium salt; aqueous solution; 5000 Ci/mmol) and [32 P] P_i (carrier-free, 5000 Ci/ml) were from The Radiochemical Centre (Amersham, Bucks., U.K.); *Staphylococcus aureus* cells (Pansorbin) were from Calbiochem-Behring. All reagents for SDS/polyacrylamide-gel electrophoresis were purchased from Bio-Rad. Serum from patient B₂, with autoantibodies to the insulin receptor, was generously given by C. R. Kahn (Joslin Research Laboratory, Boston, MA, U.S.A.) (Kahn *et al.*, 1976; Van Obberghen & Kahn, 1981); phosphotyrosine was kindly provided to us by Tony Hunter (Salk Institute, San Diego, CA, U.S.A.).

Preparation of partially purified insulin receptors

Hepatocytes were isolated from male Wistar rats (150–200 g) as described by Le Cam & Freychet (1977). Immediately after isolation, hepatocytes were washed twice with cold buffer containing Hepes (50 mM; pH 7.4), NaCl (150 mM), bacitracin (1 mM), aprotinin (1000 trypsin-inhibitor units/ml), and soya-bean trypsin inhibitor (10000 trypsin-inhibitor units/ml). Cells were collected by centrifugation, resuspended in the washing buffer containing Nonidet P-40 (0.5%) and deoxycholate (0.5%), and homogenized in a Dounce homogenizer (140 strokes). Thereafter, the broken cells were solubilized for 90 min at 4°C by continuous stirring. This preparation was centrifuged at 100000 g for 90 min at 4°C, and the insoluble fraction was discarded. The supernatant was applied to a wheat-germ-agglutinin-agarose column, which was extensively washed at 4°C; bound glycoproteins were desorbed with *N*-acetylglucosamine (0.3 M). This chromatography allows a 20-fold purification with nearly 100% recovery of the insulin receptor, as determined by 125 I-labelled-insulin binding. The two fractions, which are known to contain insulin receptors (Van Obberghen *et al.*, 1981), were pooled.

Immunoprecipitation of insulin receptors

Insulin receptors were specifically immunoprecipitated as previously described with antiserum containing autoantibodies to the insulin receptor (Van Obberghen *et al.*, 1981; Kasuga *et al.*, 1981). In brief, solubilized insulin receptors (300 μ l) were incubated for 6 h at 4°C with anti-receptor anti-

serum at a 1:800 dilution, corresponding to an immunoglobulin G concentration of 25 μ g/ml. Immunoprecipitation with protein A was performed by addition of *Staphylococcus aureus* cells (Pansorbin). After 2 h at 4°C, the immunoprecipitates were collected by centrifugation (5 min at 15000 g at 4°C), and the pellets were washed three times in 50 mM-Hepes/50 mM-NaCl, pH 7.6.

Phosphorylation of insulin receptors in intact hepatocytes

The technique used is identical with that we used previously, except that the cells were incubated for 60 min at 37°C with [32 P] P_i followed by 15 min without or with insulin (0.1 μ M) (Van Obberghen & Kowalski, 1982).

Phosphorylation of partially purified and immunoprecipitated insulin receptors

Partially purified or immunoprecipitated insulin receptors were incubated in a reaction mixture containing Hepes (20 mM; pH 7.6), MnCl₂ (4 mM), MgCl₂ (8 mM), and, if present, insulin (0.1 μ M). To allow for insulin binding, the tubes were incubated for 2 h at 20°C. Thereafter, the phosphorylation reaction was initiated by the addition of [γ - 32 P]ATP (20 μ M). The reaction tubes were incubated for 15 min at 20°C. The reaction was terminated by addition of ice-cold stopping solution containing NaF and EDTA (final concns. 100 mM and 10 mM respectively). The partially purified phosphorylated insulin receptors were then either immediately analysed by gel electrophoresis or first immunoprecipitated as described above, collected by centrifugation, washed three times with ice-cold stopping solution, and then subjected to gel electrophoresis.

Phosphorylation of exogenous substrates

Histone H2B (0.50 mg/ml) or casein (0.50 mg/ml) was incubated for 10 min at 20°C with either partially purified insulin receptors or immunoprecipitated insulin receptors which had been incubated first for 2 h in the absence or presence of insulin (0.1 μ M). Phosphorylation was initiated by the addition of [γ - 32 P]ATP, MnCl₂ and MgCl₂ at final concentrations of 20 μ M, 4 mM and 8 mM respectively. After 15 min at 20°C the reaction was stopped and the substrates were analysed by SDS/polyacrylamide-gel electrophoresis.

Polyacrylamide-gel electrophoresis and autoradiography

Samples of the phosphorylated partially purified or immunoprecipitated insulin receptors, or of the phosphorylated substrates, were boiled for 5 min in a solution containing 3% (w/v) SDS, 10% (v/v) glycerol, 10 mM-sodium phosphate, 2% (v/v) 2-mercaptoethanol and 0.01% Bromophenol Blue. The

samples were analysed by one-dimensional SDS/polyacrylamide-gel electrophoresis as described by Laemmli (1970), with a 5–15% linear gradient of acrylamide as the resolving gel. The M_r values of the standards used were: myosin, 200 000; β -galactosidase, 116 000; phosphorylase *b*, 94 000; bovine serum albumin, 67 000; ovalbumin, 43 000; carbonic anhydrase, 30 000; soya-bean trypsin inhibitor, 20 000; lysozyme, 14 400. The gels were stained, dried and autoradiographed by exposing them to Kodak X-Omat film as described by Van Obberghen *et al.* (1981). The autoradiograms were scanned in a micro-densitometer (Gelman), or the bands were cut out, and the radioactivity was eluted and counted for quantitative analysis.

Analysis of phosphoamino acids

We used the technique originally described by Hunter & Sefton (1980). The region of the polyacrylamide gel of interest (i.e. ^{32}P -labelled insulin receptor or substrates) was excised and the radioactive material eluted by incubating the pieces of gel at 37°C in SDS solution. The eluted material was precipitated with trichloroacetic acid after addition of $50\mu\text{g}$ of γ -globulin carrier and then hydrolysed with 6M-HCl at 110°C for 1 h. Phosphoamino acids were separated on thin-layer cellulose plates by one-dimensional electrophoresis at pH 3.5 toward the anode and located by autoradiography. Unlabelled phosphoamino acid markers (phosphoserine, phosphotyrosine and phosphothreonine) were added to each sample ($1\mu\text{g}$) and located on the plates by spraying with a ninhydrin solution and heating.

Results

Phosphoamino acid analysis of insulin receptors phosphorylated in intact hepatocytes

As we previously reported (Van Obberghen & Kowalski, 1982), insulin stimulates the phosphorylation of its own receptor β -subunit (M_r 95 000) (results not shown). To identify the amino acid(s) phosphorylated in the insulin receptor, the region comprising the M_r -95 000 ^{32}P -labelled peptide was excised from the gel. The ^{32}P -labelled peptide was eluted, precipitated, subjected to acid hydrolysis and finally to thin-layer electrophoresis. In the absence of insulin, two labelled spots were found, corresponding to phosphoserine and phosphothreonine. The stimulating effect of insulin on its own receptor phosphorylation is solely due to an increase in the amount of phosphoserine (Fig. 1).

Phosphoamino acid analysis of insulin receptors phosphorylated in a cell-free system

Partially purified insulin receptors were first incubated without or with insulin, then phosphoryl-

Fig. 1. Analysis of phosphoamino acids in the β -subunit of insulin receptors phosphorylated in intact hepatocytes

Freshly isolated rat hepatocytes were incubated in medium containing [^{32}P]P_i for 60 min at 37°C , and thereafter without (a) or with insulin ($0.1\mu\text{M}$) (b) for 15 min. Insulin receptors were solubilized, partially purified and subjected to immunoprecipitation with autoantibodies to the insulin receptor. The immune precipitates were analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions, followed by autoradiography. The labelled bands corresponding to the receptor β -subunit were excised from the gel, and the radioactivity was eluted and treated for phosphoamino acid analysis as described in the Materials and methods section. The phosphoamino acids were separated by thin-layer electrophoresis. An autoradiograph of the plate is shown.

ated, and finally immunoprecipitated with anti-receptor antibodies. As we previously demonstrated in the same system (Van Obberghen & Kowalski, 1982),

the β -subunit (M_r 95 000) of the insulin receptor is phosphorylated, and insulin induced a 3–5-fold increase in ^{32}P incorporation in the receptor β -subunit (results not shown). Amino acid analysis of the β -subunit of the insulin receptor labelled in the absence of insulin revealed a major labelled spot corresponding to phosphotyrosine. The stimulating effect of insulin on its own receptor phosphorylation is solely due to an increase in the amount of phosphotyrosine (Fig. 2, lanes *a* and *b*).

Similar results were observed when the insulin receptor was first immunoprecipitated and then phosphorylated. Analogous to our previously published data (Van Obberghen *et al.*, 1983), we found not only that the immunoprecipitated receptor β -

Fig. 2. Analysis of phosphoamino acids in the β -subunit of insulin receptor phosphorylated in a cell-free system

Partially purified insulin receptors (*a*, *b*) or immunoprecipitated insulin receptors (*c*, *d*) were first incubated without (*a*, *c*) or with insulin (*b*, *d*). Thereafter, both receptor preparations were phosphorylated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. The reaction was stopped and the phosphorylated solubilized receptors (*a*, *b*) were immunoprecipitated as described in the Materials and methods section. The immune precipitates were analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions followed by autoradiography. The labelled bands corresponding to the receptor β -subunit were excised from the gel, and the radioactivity was eluted and treated for phosphoamino acids analysis as described in the Materials and methods section. The phosphoamino acids were separated by thin-layer electrophoresis; an autoradiograph of the plate is shown.

subunit is a substrate for phosphorylation reactions, but, more important, that insulin increases several-fold the ^{32}P labelling of its own receptor subunit (results not shown). The analysis of the phosphoamino acids present in the receptor β -subunit is shown in Fig. 2 (lanes *c* and *d*). In the absence of insulin the β -subunit contains mainly $[\text{P-Tyr}]$ phosphotyrosine, and trivial amounts of $[\text{P-Ser}]$ phosphoserine and $[\text{P-Thr}]$ phosphothreonine. Again, the insulin-induced stimulation of receptor phosphorylation is entirely accounted for by an increase in $[\text{P-Tyr}]$ phosphotyrosine.

Phosphorylation of exogenous protein substrates by insulin receptors

We first investigated whether partially purified insulin receptors were able to catalyse the phosphorylation of histone and casein. Both protein substrates were phosphorylated by partially purified insulin receptors (Fig. 3, lanes *c* and *e*). More important, the addition of insulin enhanced the insulin-receptor kinase activity, resulting in a marked increase in ^{32}P incorporation in histone and casein (Fig. 3, lanes *d* and *f*). Thus determination of

Fig. 3. Insulin-receptor-mediated phosphorylation of exogenous substrates

Partially purified insulin receptors were incubated without (*a*, *c*, *e*) or with insulin (*b*, *d*, *f*) for 2 h at 20°C , and without protein substrates (*a*, *b*) or with either casein (*c*, *d*) or histone (*e*, *f*) for an additional 10 min at 20°C . The phosphorylation reaction was initiated by addition of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. After 15 min at 20°C the reaction was stopped with a boiling SDS-containing solution. The labelled proteins were analysed by SDS/polyacrylamide-gel electrophoresis under reducing conditions, followed by autoradiography. An autoradiograph of the gel is shown.

insulin's effect revealed a 2–3-fold increase in ^{32}P labelling as measured by scanning densitometry of the labelled bands, or by counting the radioactivity eluted from the bands corresponding to the phosphorylated substrates. It should be noted that, as shown in Fig. 3 (lanes *e* and *f*), the histone preparation used contained two minor contaminants, of M_r 105 000 and 70 000, which are phosphorylated.

We next examined the insulin-receptor kinase activity towards exogenous protein substrates present in immunoprecipitated receptors. Insulin receptors were first immunoprecipitated with anti-receptor antibodies, and then incubated with histone and casein together with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Similarly to our findings with partially purified insulin receptors, both substrates were phosphorylated by the immunoprecipitated receptors, and their phosphorylation was found to be increased several-fold by insulin (results not shown).

Identification of amino acids phosphorylated by insulin receptors in exogenous substrates

When casein was phosphorylated by partially purified insulin receptors, and the phosphoamino acids were analysed in the labelled protein, we found under basal phosphorylation conditions (i.e. absence of insulin) ^{32}P phosphoserine, ^{32}P phosphothreonine and ^{32}P phosphotyrosine (Fig. 4). Insulin induced a 3–5-fold increase in ^{32}P incorporation into phosphotyrosine, and a smaller stimulating effect on the labelling of phosphoserine, which routinely amounted to a 25–30% increment as measured by scanning densitometry. Similar results were obtained with histone as a protein substrate (results not shown). We next identified the amino acids phosphorylated by immunoprecipitated insulin receptors in casein and histone. As shown in Fig. 5, when these substrates were phosphorylated in the absence of insulin, the major labelling occurred on phosphotyrosine; only minute amounts of phosphoserine and phosphothreonine were found. Further, the stimulating action of insulin on ^{32}P incorporation was largely accounted for by an increase in phosphotyrosine. The amount of the two other amino acids was only slightly increased, and probably reflects a small contamination of the immunoprecipitated receptors with other kinases.

Discussion

Several laboratories, including our own, have reported on the phosphorylation of the insulin receptor. In brief, both in intact cells and in cell-free systems the insulin receptor can serve as a substrate for phosphorylation reactions, and the hormone itself increases the phosphorylation of its own receptor (Kasuga *et al.*, 1982*a,b*; Van Obber-

Fig. 4. Analysis of phosphoamino acids in casein phosphorylated by partially purified insulin receptors

Casein was phosphorylated by partially purified insulin receptors and analysed by SDS/polyacrylamide-gel electrophoresis as described in the legend to Fig. 3. The labelled bands corresponding to the phosphorylated casein were excised from the gel, and the radioactivity was eluted and processed for phosphoamino acid analysis. The phosphoamino acids were separated by thin-layer electrophoresis. An autoradiograph of the plate is shown. Lanes show phosphoamino acids in casein phosphorylated by partially purified insulin receptors in the absence (a) or presence of insulin (b).

ghen & Kowalski, 1982; Zick *et al.*, 1983). Further, using covalent affinity labelling we and others have identified an ATP-binding site on the receptor β -subunit, indicating that the insulin receptor is likely

Fig. 5. Analysis of phosphoamino acids in exogenous protein substrates phosphorylated by immunoprecipitated insulin receptors

Casein (a, b) and histone (c, d) were phosphorylated by immunoprecipitated insulin receptors in the absence (a, c) or presence of insulin (b, d) as described for the experiment with partially purified insulin receptors in Fig. 3. Thereafter the reaction was stopped and the proteins were separated by SDS/polyacrylamide-gel electrophoresis under reducing conditions, followed by autoradiography. The labelled bands corresponding to casein and histone were excised from the gel, and the radioactivity was eluted and processed for phosphoamino acid analysis as described in the Materials and methods section. The phosphoamino acids were separated by thin-layer electrophoresis; an autoradiograph of the plate is shown.

to be its own protein kinase (Van Obberghen *et al.*, 1983; Shia & Pilch, 1983; Roth & Cassell, 1983).

In the present study we have investigated the specificity of the kinase activity associated with the insulin receptor, and modulated by insulin. When the nature of the amino acids phosphorylated in the insulin receptor was analysed, significant and intriguing differences were observed. In intact hepatocytes we found phosphoserine and phosphothreonine, but insulin's stimulatory action on receptor phosphorylation was limited to phosphoserine. In contrast, in cell-free systems consisting of either partially purified insulin receptors or highly purified

receptors obtained by immunoprecipitation, phosphotyrosine was present in a vast majority over phosphothreonine and phosphoserine. Further, for both receptor preparations the insulin-induced increase in phosphorylation of the receptor β -subunit can largely be accounted for by an increment in phosphotyrosine without significant change in the other two amino acids. Although we have not detected differences in the nature of the phosphoamino acids modified in the receptor itself when partially purified receptors were compared with immunoprecipitated receptors, we did find important differences in the amino acids phosphorylated in protein substrates, depending on whether partially purified receptors or immunoprecipitated receptors were used as a protein kinase source. Indeed, partially purified receptors catalyse the phosphorylation of casein and histone, mainly on serine residues, but also on tyrosine residues. Insulin has a stimulating effect on the labelling of tyrosine residues predominantly, but in addition a distinct and reproducible effect on serine residues. In contrast, when immunoprecipitated insulin receptors were used, insulin's stimulatory action on substrate phosphorylation was strictly limited to tyrosine residues. Taking the data on receptor phosphorylation and substrate phosphorylation together, we suggest the following model for the insulin receptor and its associated kinase activities. We envisage an insulin-receptor kinase complex with two distinct protein kinases, a tyrosine-specific and a serine-specific kinase. The tyrosine-specific kinase would be a constituent of the insulin receptor, and, on the basis of the identification of an ATP-binding site on the receptor β -subunit, this kinase could be an integral part of the β -subunit itself. In contrast, the serine-specific kinase would be closely associated with the insulin receptor in the cell membrane, but would not be an integral part of the receptor structure. This view of the insulin-receptor kinase complex easily enables us to explain our data on receptor phosphorylation in intact cells and cell-free systems. Insulin binds to the α -subunit of the insulin receptor, and then, probably through a conformational change, activates both the tyrosine-specific and the serine-specific kinases. Inclusive in this model is the idea that the nature of the phosphoamino acid(s) modified by the insulin-stimulated kinases does not depend on the hormone itself, but is exclusively dictated by the conformation of the receptor. Thus, in intact hepatocytes, although the binding of insulin activates both the tyrosine- and serine-specific kinase, only the serine residues are phosphorylated in the β -subunit of the insulin receptor, because the tyrosine residues in the receptor β -subunit are not accessible to the tyrosine kinase. This does not mean, of course, that the activated tyrosine-specific kinase will not catalyse

the phosphorylation of cellular proteins other than the receptor in the intact cell. With insulin receptors solubilized in detergent, insulin binding leads again to activation of both kinases, but now, owing to a conformational change in the receptor itself, mainly tyrosine residues are available for phosphorylation reactions.

As expected from our model, the nature of the amino acid residues phosphorylated by insulin receptors in exogenous protein substrates depends on the insulin receptor preparation. Indeed, partially purified receptors catalyse the phosphorylation of protein substrates on both serine and tyrosine residues. This is easily explained by the idea that the tyrosine kinase-containing receptor and the serine kinase, which are closely associated in the intact cell membrane, remain in close neighbourhood in detergent micelles. Insulin-induced stimulation of the two kinases associated with partially purified receptors results therefore in the phosphorylation of both serine and tyrosine residues in protein substrates. However, when the same substrates are phosphorylated by immunoprecipitated insulin receptors, only tyrosine residues are found to be modified in the absence or presence of insulin. This is totally compatible with the concept that the serine kinase is not an integral component of the insulin receptor, and consequently is not precipitated by anti-receptor antibodies.

The biological importance of the phosphorylation of the insulin receptor is not established. We favour the idea that this insulin-induced covalent modification of the insulin receptor is an early step in insulin action. It is an appealing idea to envisage that the increased kinase activity of the insulin receptor evoked by the hormone itself would in turn lead to phosphorylation of other cellular proteins. These phosphorylations could then generate a cascade of reactions resulting in the final effects of insulin. Further, the precise relationship between the two protein kinase activities associated with the insulin receptor and their function remain to be elucidated. At present one can only speculate. At least two situations are possible, one in which both kinases serve totally independent cellular functions, and a second one in which one kinase activates the other, but only one leads to insulin's effects. Regarding the first possibility, tyrosine-specific protein kinases seem to be implicated in the cellular responses to growth factors, such as epidermal growth factor (Ushiro & Cohen, 1980; Cohen *et al.*, 1982), platelet-derived growth factor (Ek *et al.*, 1982) and tumour growth factor (Reynolds *et al.*, 1981), and in cell transformation induced by oncogenic viruses (Hunter & Sefton, 1980; Collett *et al.*, 1980). It is therefore tempting to conceive that this tyrosine-specific enzyme activity plays a role in the generation of signals regulating the mitogenic action

of insulin. The serine-specific protein kinase, in contrast, would play a role in the metabolic functions mediated by insulin. Indeed, all the known enzymes playing a role in cellular intermediary metabolism and whose activity is modulated by insulin through phosphorylation-dephosphorylation reactions are modified on serine residues. At the other extreme of the situation just discussed, where both kinases have totally distinct functions, there exists another possibility, where one kinase activates the other, and this last one is responsible for the generation of the cellular response to insulin. As the tyrosine-specific kinase seems to be an integral part of the insulin receptor structure, it is likely that, in this model with sequential activation of kinases, the tyrosine kinase is activated by binding of insulin to its receptor, which in turn leads to activation of the receptor-associated serine kinase. The existence of the two different kinases, one contained in and one associated with the receptor, provides for functional regulation at each kinase step.

In conclusion, although it is clear now that the insulin receptor is an insulin-activated protein kinase, it remains to be shown whether the kinase activity of the receptor is both required and sufficient to initiate the complex array of the cellular responses to insulin. A major advance will be the identification of natural substrates for this receptor kinase activity.

We thank G. Visciano for preparing illustrations, and A. Rainaud, L. Capolongo and J. Duch for typing work. Dr. A. Le Cam is gratefully acknowledged for his help in setting up the phosphoamino acids analysis.

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