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Decreased tyrosine kinase activity of insulin receptor isolated from rat adipocytes rendered insulin-resistant by catecholamine treatment *in vitro*

Hans HÄRING, Dieter KIRSCH, Bert OBERMAIER, Britta ERMEL and Fausto MACHICAO
Institut für Diabetesforschung, Kölner Platz 1, 8000 München 40, Federal Republic of Germany

Catecholamine treatment of isolated rat adipocytes decreases insulin binding and inhibits insulin stimulation of the glucose-transport system. There is increasing evidence that the insulin signal is transmitted after insulin is bound to the receptor via a tyrosine kinase, which is an intrinsic part of the receptor. To find whether the receptor kinase is modified by catecholamines, we solubilized and partially purified the insulin receptor of isoprenaline-treated adipocytes and studied the effect of insulin on its kinase activity. (1) Insulin increased the tyrosine autophosphorylation of the insulin receptor kinase from catecholamine-treated cells only 4-fold, compared with a 12-fold stimulation in control cells. (2) The rate of insulin-stimulated ^{32}P incorporation into the receptor of isoprenaline-treated cells at non-saturating [^{32}P]ATP concentrations (5 μM) was decreased to 5–8% of the values for receptor from control cells. (3) ^{125}I-insulin binding to the partially purified receptor from catecholamine-treated cells was also markedly decreased. The insulin receptor from catecholamine treated cells bound 25–50% of the amount of insulin bound by the receptor from control cells at insulin concentrations of 10 pM–0.1 μM. Part of the impaired insulin-responsiveness of the receptor kinase of catecholamine-treated cells is therefore explained by impaired binding properties; however, an additional inhibition of the kinase activity of the insulin receptor from catecholamine-treated cells is evident. (4) This inhibition of kinase activity decreased when the concentration of [γ-^{32}P]ATP in the phosphorylation assay was increased. A Lineweaver–Burk analysis revealed that the K_m for ATP of the receptor kinase from isoprenaline-treated cells was increased to approx. 100 μM, compared with approx. 25 μM for receptor of control cells. (5) We conclude from the data that catecholamine treatment of rat adipocytes modulates the kinase activity of the insulin receptor by increasing its K_m for ATP and that this is part of the mechanism leading to insulin-resistance in these cells.

INTRODUCTION

Catecholamine treatment of isolated rat adipocytes induces insulin-resistance of the glucose-transport system (Kirsch *et al.*, 1983*a*,*b*; Pessin *et al.*, 1983; Kashiwagi *et al.*, 1983). This insulin-resistance seems to be caused predominantly by an effect on the signal transmission after insulin binding (Kirsch *et al.*, 1983*a*,*b*). Binding of insulin to its receptor in the plasma membrane of intact cells stimulates phosphorylation of the β-subunit of the insulin receptor (Kasuga *et al.*, 1982*a*,*b*; Häring *et al.*, 1982). It is clear now that this insulin-stimulated phosphorylation is due to the activation of a tyrosine kinase in the β-subunit of the insulin receptor (Kasuga *et al.*, 1983; Van Obberghen *et al.*, 1983), which undergoes insulin-stimulated autophosphorylation at tyrosine residues. In intact cells, phosphorylation of the insulin receptor is also found at serine residues (Kasuga *et al.*, 1982*b*; Gazzano *et al.*, 1983; Häring *et al.*, 1984*a*), suggesting that the insulin receptor in intact cells is also the substrate for at least one so far unidentified serine kinase. Whereas tyrosine phosphorylation appears to activate the insulin-receptor kinase for other substrates (Petruzelli *et al.*, 1982; Yu & Czech, 1984), the effect of this serine phosphorylation of the insulin receptor is still not known. As the catecholamine inhibition of the insulin stimulation of D-glucose transport is a cyclic-AMP-dependent process (Kirsch *et al.*, 1983*a*), and as cyclic AMP stimulates a serine kinase, we investigated whether catecholamine treatment of isolated rat adipocytes would change binding and tyrosine kinase activity of the solubilized insulin receptor. In the present paper we report inhibition of the insulin-stimulated tyrosine autophosphorylation of the solubilized insulin receptor isolated from rat adipocytes after isoprenaline treatment *in vitro*.

MATERIALS AND METHODS

Materials

Pig insulin was purchased from Novo Industrie (Bagsvaerd, Denmark); [γ-^{32}P]ATP (2900 Ci/mmol) and Triton X-100 were from NEN (Dreieich, Germany); aprotinin, phenylmethanesulphonyl fluoride, leupeptin and pepstatin were from Sigma (Munich, Germany); isoprenaline was from Serva (Heidelberg, Germany); wheat-germ agglutinin coupled to agarose was from Miles (Munich, Germany). All reagents for SDS/polyacrylamide-gel electrophoresis were from Bio-Rad; all other reagents were of the best grade commercially available.

Methods

Fat-cells were prepared from male Sprague–Dawley rats fed *ad libitum* (180–220 g body wt.). All incubations were performed in Krebs–Ringer–Hepes buffer (131 mM-NaCl/4.8 mM-KCl/2.5 mM-$CaCl_2$/1.2 mM-KH_2PO_4/

1.2 mM-$MgSO_4$/25 mM-Hepes, pH 7.4) containing 2.5% (w/v) crystalline bovine serum albumin. High cyclic AMP concentrations were achieved as described by Kirsch *et al.* (1983*a*,*b*) by a 30 min preincubation with isoprenaline (10 μM), which was carried out in the presence of 16 mM-D-glucose to stabilize intracellular ATP concentrations (Kirsch *et al.*, 1983*b*).

3-*O*-Methylglucose transport in adipocytes

This was measured as described by Häring *et al.* (1981). Samples (100 μl) of the concentrated cell suspension (5×10^6 cells/ml) were taken together with 200 μl of 3-*O*-methylglucose (final concn. 0.5 mM) and a tracer of 0.1 μCi of 3-*O*-methyl-D-[^{14}C]glucose (The Radiochemical Centre, Amersham, Bucks., U.K.) into a mixing pipette (Abimed, Düsseldorf, Germany). After 4 s, the uptake was stopped by diluting the cells in 5 ml of NaCl (0.9 g/dl) which contained phloretin (1 mM). Cells and medium were separated by centrifugation through silicone oil (1000 *g* for 60 s). The cell layer was removed by a pipette, added to scintillation fluid, and the radioactivity measured. The amount of 3-*O*-methylglucose in extracellular fluid or taken up by diffusion was determined in samples which contained 1 mM-phloretin. All other uptake data were corrected by this value. 3-*O*-Methylglucose accumulation measured during 4 s is expressed as a percentage of the 3-*O*-methylglucose accumulated at equilibrium in fat-cells after 30 min incubation at saturating insulin concentrations (67 nM).

Binding of insulin to intact adipocytes

After preincubation of fat-cells (4.5×10^5–5.5×10^5 cells/ml) in the presence or absence of isoprenaline (10 μM), 33 pM A14-mono[^{125}I]iodinated insulin (radioactivity 250 μCi/μg; Novo Industrie) was added, alone or together with unlabelled insulin in concentrations between 0.01 and 100 nM. After 20 min, 400 μl samples were transferred into polyethylene centrifuge tubes and the cells were separated from the medium by centrifugation through dinonyl phthalate. The tubes were cut at the oil layer, and radioactivity of the cell layer was measured. Non-specific binding was assessed by addition of unlabelled insulin (6.7 μM) to the tracer, and that value was subtracted from other results.

Receptor solubilization and phosphorylation

For each receptor preparation 40 ml of either isoprenaline (10 μM)-treated cells or control cells (each 4.5×10^6–5.5×10^6 cells/ml) was used. Cell lysis was achieved by freezing and thawing three times in the presence of the proteinase inhibitors phenylmethanesulphonyl fluoride (5 mM), aprotinin (1200 trypsin-inhibiting units/l), leupeptin (2 μM), pepstatin (2 μM), benzamidine (10 mM), bacitracin (7500 units/l) and leucine (10 mM), in a buffer containing NaH_2PO_4 (pH 7.4, 22 °C; 10 mM), EDTA (5 mM) and sucrose (250 mM). Subsequently the cell lysate was centrifuged for 90 min at 200000 *g*. The fat layer and the supernatant were discarded. The pellet was dissolved in 25 mM-Hepes/1% Triton X-100/100 mM-NaF/10 mM-sodium pyrophosphate/2 mM-phenylmethanesulphonyl fluoride and aprotinin (1 trypsin-inhibiting unit/ml) and centrifuged for 60 min at 200000 *g* to remove insoluble material. The supernatant was applied to a column of wheat-germ agglutinin coupled to agarose. After extensive washing with 25 mM-Hepes buffer (pH 7.4) containing 0.1% Triton X-100, 10 mM-sodium pyrophosphate, and 100 mM-NaF, the bound material was eluted with 25 mM-Hepes buffer containing 0.1% Triton X-100 supplemented with 0.3 M-*N*-acetylglucosamine. For the standard phosphorylation assay, approx. 3 μg of wheat-germ-purified protein was preincubated at 22 °C for 30 min with insulin (0.1–100 nM) or without insulin, followed by an incubation with [^{32}P]ATP (5 μM if not otherwise indicated) in elution buffer containing 10 mM-$MnCl_2$ and 1 mM-vanadate at 22 °C for 2–15 min. The incubation was stopped by addition of Laemmli (1970) buffer containing 100 mM-dithiothreitol and boiling for 5 min. Subsequently phosphoproteins were separated by polyacrylamide-gel electrophoresis and identified by autoradiography. The phosphoproteins identified by the autoradiography were cut out from the gel and counted for radioactivity in a scintillation counter.

Binding to solubilized receptor

Solubilized and wheat-germ-purified receptor was prepared as described above. Samples of eluate from the wheat-germ column, containing 3 μg of protein, were incubated with A14-mono[^{125}I]iodinated insulin (30 pM) and various concentrations of unlabelled insulin for 30 min at 22 °C in 150 mM-NaCl/25 mM-Hepes, pH 7.4. Separation of the free and receptor-bound insulin was then performed by using dextran-coated charcoal (Williams & Turtle, 1979).

Identification of phospho amino acids

The protein band corresponding to the 95 kDa β-subunit of the insulin receptor in the dried gel was excised and washed with dioxan, followed by methanol and 10% (v/v) methanol. The gel fragments, containing 5000–7000 c.p.m. of ^{32}P, were placed into 200–500 μl of 6 M-HCl and hydrolysed for 2 h at 110 °C. The samples were then diluted with 2 ml of water, freeze-dried and redissolved in 30–50 μl of water. Electrophoresis was performed on Whatman 3 MM paper at pH 3.5 with pyridine/acetic acid/water (1:10:189, by vol.) for 90 min at 1 kV. Phospho amino acid standards were localized with ninhydrin and ^{32}P-labelled amino acids by autoradiography.

Tryptic-peptide mapping by h.p.l.c.

Fixed, stained, destained and dried polyacrylamide-gel fragments containing the 95 kDa β-subunit of the receptor located by autoradiography were washed for 12 h at 37 °C with 20 ml of 10% (v/v) methanol. The adsorbent paper was removed from the gel fragment. The gel was dried at 70 °C for 60 min and rehydrated in 2 ml of 50 mM-NH_4HCO_3 containing 100 μg of trypsin (treated with 1-chloro-4-methyl-3-L-tosylamidobutan-2-one). The mixture was incubated for 12 h at 37 °C, the gel fragment was removed and the supernatant was clarified by centrifugation (10000 *g* for 2 min). The supernatant was freeze-dried, and the residue was dissolved in 25–50 μl of 0.1% trifluoroacetic acid and once more clarified by centrifugation and filtration. The phosphopeptides were separated in a LKB h.p.l.c. system equipped with a Ultropac column TSK ODS-120 T (5 μm column). Phosphopeptides applied to the column were eluted at a flow rate of 0.8 ml/min with a 40 min gradient of 0–40% acetonitrile (containing 0.1% trifluoroacetic acid). Radioactivity in fractions (0.10–0.11 ml) eluted

from the reversed-phase column was measured in 3 ml of scintillation mixture.

RESULTS

In previous studies (Kirsch *et al.*, 1983*a*,*b*) we had shown that isoprenaline (10 nM–10 μM) and other stimulators of the adenylate cyclase induce *in vitro* insulin-resistance of the glucose-transport system of isolated rat adipocytes. The insulin-resistance was caused by effects on the binding and at the post-binding level. In the present study we treated isolated rat adipocytes with isoprenaline (10 μM), determined insulin binding and glucose transport in the intact cell, and isolated the insulin receptor from these cells. As described by Kirsch *et al.* (1983*b*), we found a decreased maximal responsiveness and a decreased insulin-sensitivity of the glucose transport to insulin. Fig. 1(*a*) shows the effect of isoprenaline treatment on ^{125}I-insulin binding in these cells. A 30% decrease in insulin binding predominantly at low insulin concentrations occurs, whereas only small differences are present at higher insulin concentrations. We had discussed previously (Kirsch *et al.*, 1983*b*) that the decreased binding might be responsible for the lower insulin-sensitivity of the isoprenaline-treated adipocytes, whereas the lower maximal responsiveness was interpreted as consequence of a post-binding defect in insulin signal transmission. We isolated insulin receptor from control and isoprenaline-treated cells as described in the Materials and methods section and determined insulin binding and kinase activity of the solubilized receptor. Fig. 1(*b*) shows the binding of ^{125}I-insulin to solubilized and partially purified insulin receptor. There was markedly less insulin binding to the solubilized insulin receptor from catecholamine-treated cells than in the intact cell. At all insulin concentrations studied (10 pM–0.1 μM), the solubilized receptor from catecholamine-treated cells bound only approx. 25–50% of the control amounts.

For the phosphorylation assay, equal amounts of wheat-germ-purified proteins were used. Insulin-stimulated receptor kinase activity was studied with a non-saturating concentration of [γ-^{32}P]ATP and insulin as described in the Materials and methods section. Fig. 2 shows autoradiograms of phosphoyrlated glycoproteins from isoprenaline-treated (*a*) and control (*b*) fat-cells. The phosphorylated 95 kDa protein has previously been identified as the 95 kDa β-subunit of the insulin receptor from rat adipocytes (Häring *et al.*, 1982). The autoradiograms show the time course of ^{32}P incorporation into the 95 kDa protein in the absence and presence of insulin (0.1 μM). The ^{32}P incorporation into the 95 kDa band was quantified as described in the Materials and methods section, and the mean values for three experiments are shown in Fig. 3. The rate of phosphorylation of the 95 kDa protein at non-saturating ATP concentrations (5 μM) was linear up to 15 min. The phospho amino acid analysis shown in Fig. 4 revealed that the ^{32}P incorporation was exclusively on tyrosine residues of the receptor protein. To study the dose–response of the

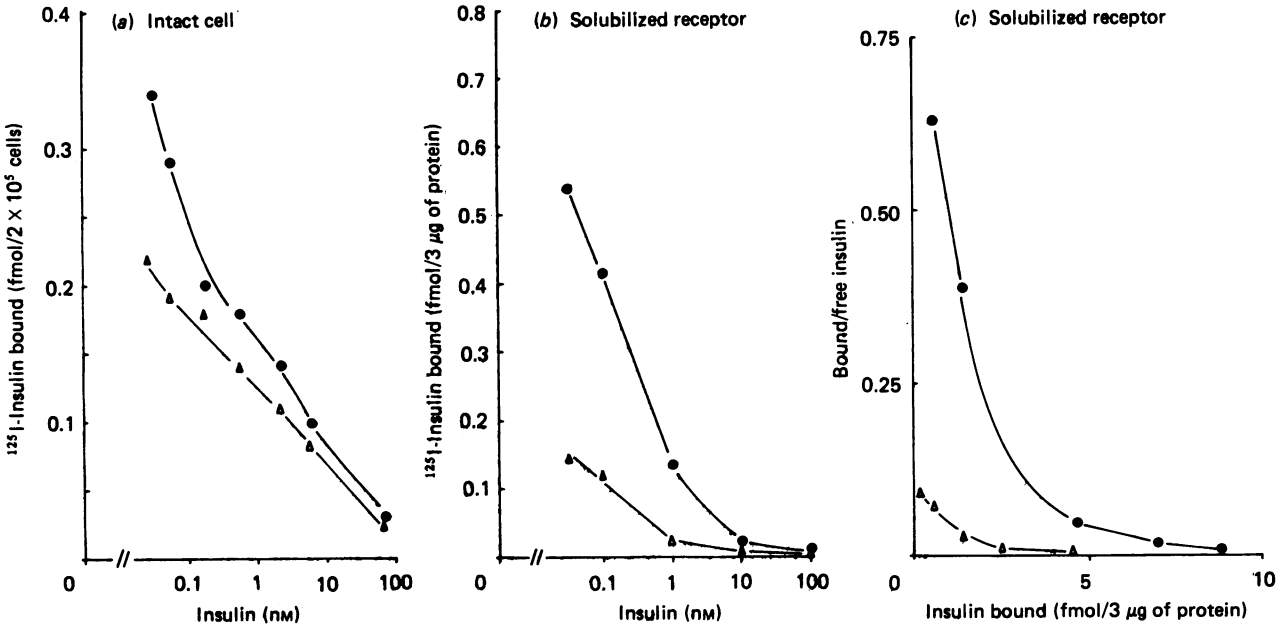

Fig. 1. Isoprenaline-induced inhibition of insulin binding

(*a*) Intact adipocytes were preincubated in the absence (●) or the presence (△) of isoprenaline (10 μM) for 30 min at 37 °C. Subsequently, 30 pM-^{125}I-insulin together with increasing amounts of unlabelled insulin as indicated on the abscissa were added, and cells were incubated for 20 min at 37 °C. Specific insulin binding was measured as described in the Materials and methods section. The data represent the means of three independently performed experiments. Displacement curves are shown. (*b*) Solubilized and partially purified insulin receptor (3 μg of protein) from isoprenaline (10 μM)-treated cells (△) or control cells (●) was incubated with 30 pM-^{125}I-insulin together with increasing amounts of unlabelled insulin as described in the Materials and methods section. The data represent the means of three independently performed experiments. (*c*) Scatchard analysis of the binding data for solubilized receptor shown in part (*b*).

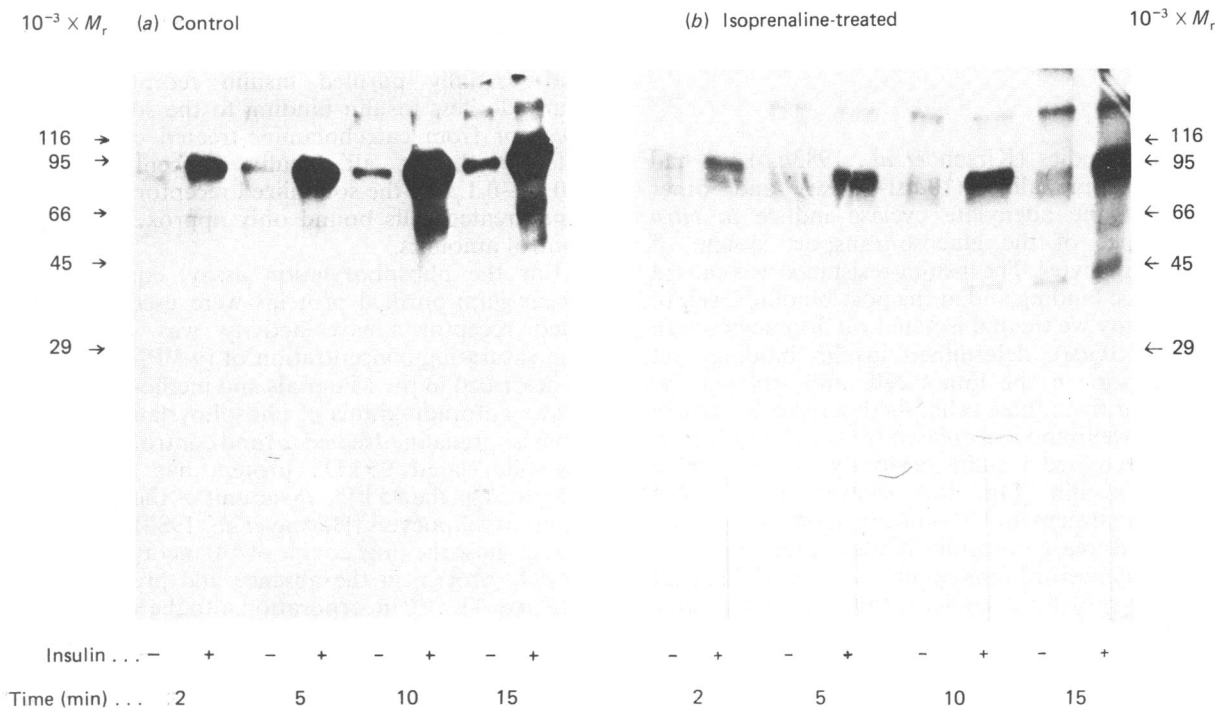

Fig. 2. Insulin-receptor autophosphorylation

Samples (3 μg) of wheat-germ-agglutinin-purified insulin receptor were incubated for 30 min in the presence (+) or absence (−) of 100 nM-insulin. Then 5 μM-[γ-^{32}P]ATP was added for the indicated time intervals. The reactions were terminated by heating with dithiothreitol (0.1 M) in Laemmli (1970) buffer for 5 min. Fig. 2(*a*) shows phosphoproteins from control cells and Fig. 2(*b*) those from isoprenaline-treated cells.

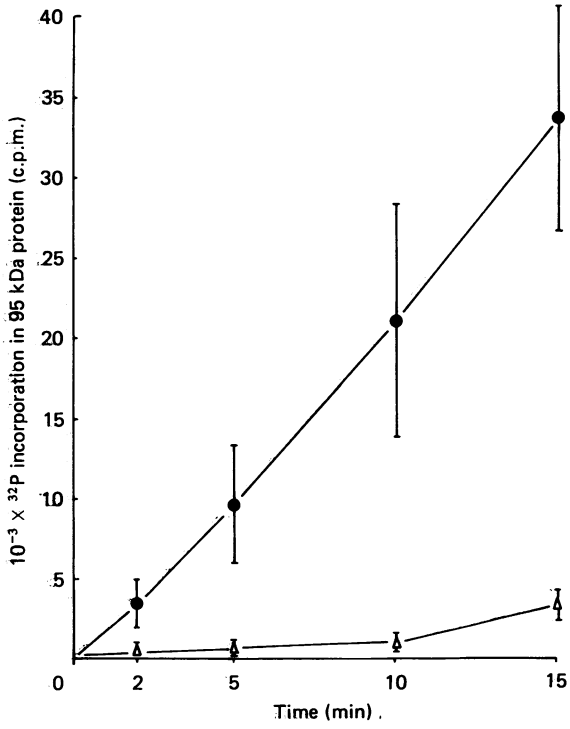

Fig. 3. Time course of insulin-receptor autophosphorylation

The insulin (100 nM)-stimulated ^{32}P incorporation into the 95 kDa receptor protein from control cells (●) and isoprenaline-treated cells (△) was quantified as described in the Materials and methods section. The values represent means ± S.E.M. for three experiments.

insulin effect on receptor phosphorylation, 10 min values were chosen. In the absence of insulin, the mean ^{32}P labelling of the 95 kDa band in seven experiments in control and isoprenaline-treated cells was 1423 ± 287 and 234 ± 31 c.p.m. (± S.E.M.; $n = 7$) respectively. At the maximal effective insulin concentration (0.1 μM), 15424 ± 3563 and 944 ± 192 c.p.m. (± S.E.M.; $n = 7$) were found in the 95 kDa band in control and isoprenaline-treated cells respectively. The quantification of the dose–response curve of the insulin effect on receptor phosphorylation is shown in Fig. 5(*a*). The insulin-stimulated ^{32}P incorporation into the 95 kDa protein from isoprenaline-treated cells was only 5–10% of that found with the insulin receptor from control cells at all insulin concentrations tested. To determine whether the decreased ^{32}P incorporation into the receptor from isoprenaline-treated cells can be explained by the decreased binding ability of this receptor alone, we calculated the insulin-induced increase in the ^{32}P incorporation per amount of bound insulin at each insulin concentration. The results are given in Fig. 5(*b*), showing that this increase is still 50–70% lower at all insulin concentrations tested, suggesting a decrease in the tryosine kinase activity in addition to the lower binding ability of the insulin receptor from isolated isoprenaline treated cells.

To analyse further the modification of the receptor that causes the decreased receptor kinase autophosphorylation, we prepared tryptic-peptide maps of the receptor protein by h.p.l.c. In a previous study with an insulin-resistant melanoma cell line (Häring *et al.*, 1984*b*), we also had found a decreased receptor kinase autophosphorylation. H.p.l.c. chromatograms of tryptic peptides of the

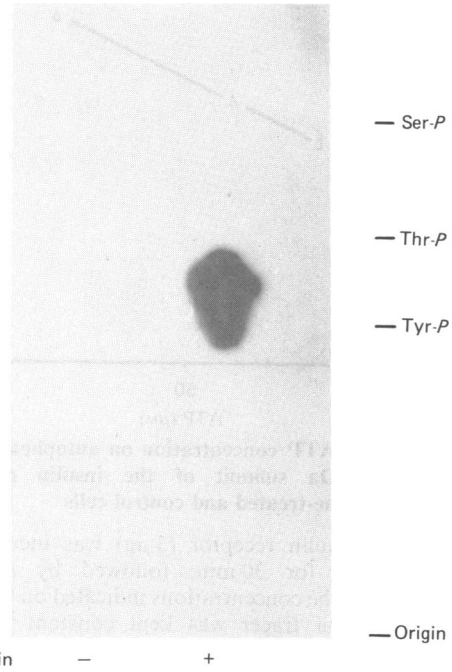

Fig. 4. Identification of phospho amino acids in the β-subunit of the insulin receptor

The insulin receptor, partially purified on wheat-germ agglutinin, was phosphorylated in the presence (+) or absence (−) of insulin (100 nM). The β-subunit was separated by SDS/polyacrylamide-gel electrophoresis under reducing conditions and located by autoradiography. The phospho amino acids were identified as described in the Materials and methods section.

receptor from this cell line had suggested that the phosphorylation of one specific site was inhibited. H.p.l.c. tryptic-peptide maps of the 95 kDa receptor subunit of the insulin receptor from control cells and isoprenaline-treated cells are shown in Fig. 6. Six major phosphopeptides are found in both preparations. The relative phosphorylation of each peak is almost identical in both preparations, suggesting that the decreased phosphorylation of the 95 kDa subunit from isoprenaline-treated cells is not due to inhibition of one specific autophosphorylation site as was found in the insulin-resistant melanoma cell, but reflects a proportionate inhibition of the phosphorylation of all phosphorylated tyrosine residues of the receptor.

To test if the decreased autophosphorylation of the receptor from isoprenaline-treated cells might reflect a decreased affinity of the receptor kinase for ATP, we studied the effect of the ATP concentration in the phosphorylation assay on the phosphorylation of receptor from control and isoprenaline-treated cells; 2 min phosphorylation intervals were used to measure initial rates. Fig. 7 shows that the degree of inhibition of receptor kinase from isoprenaline-treated cells decreases when increasing ATP concentrations are used in the phosphorylation assay. Therefore it appears that the inhibition of receptor kinase in isoprenaline-treated cells is due to an inreased K_m of the kinase for ATP. Fig. 8 shows a Lineweaver–Burk plot of the insulin-stimulated tyrosine phosphorylation of the 95 kDa receptor subunit. The $V_{max.}$ of both receptor preparations is similar, whereas the K_m for ATP of the receptor kinase from isoprenaline-treated cells is over 100 μM, compared with 25 μM for the receptor of control cells.

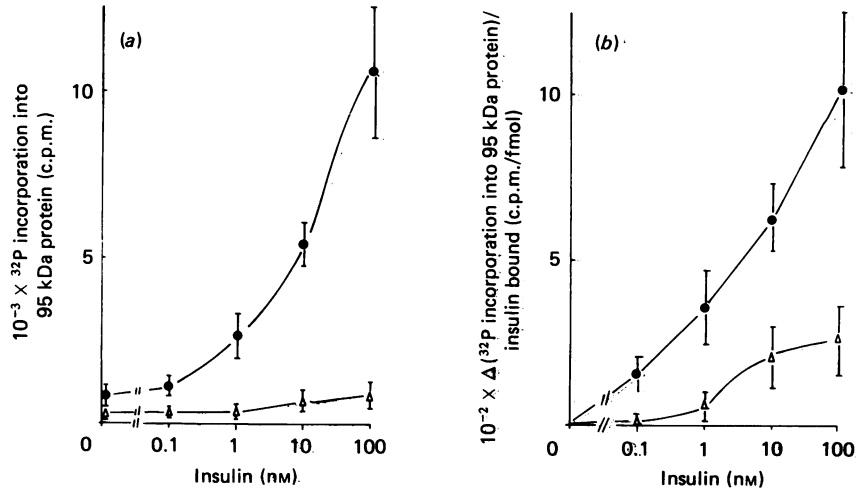

Fig. 5. Dose–response curve of insulin action on ^{32}P incorporation into the 95 kDa receptor protein from isoprenaline-treated cells (△) and control cells (●)

Equal amounts of wheat-germ-purified protein were used (3 μg). In (*a*) absolute values of ^{32}P incorporation into the 95 kDa protein are shown. The values represent means ± S.E.M. for four to six experiments. In (*b*) the insulin-induced increase (△) in ^{32}P incorporation into the 95 kDa protein is shown. As the receptor occupancy at the respective insulin concentrations is lower in the receptor from isoprenaline-treated cells (Fig. 1), the amount of ^{32}P incorporation was normalized for equal amounts of insulin bound at each insulin concentration.

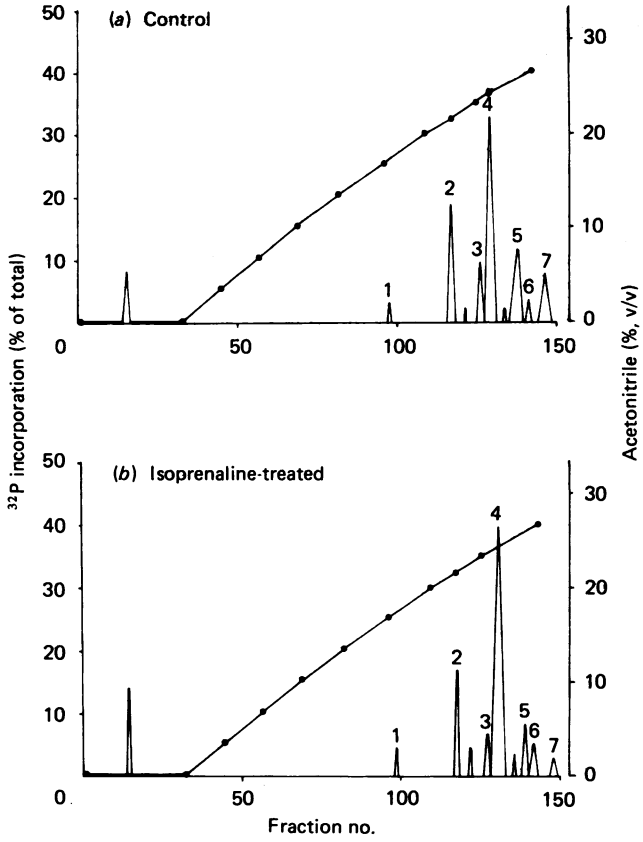

Fig. 6. H.p.l.c. tryptic-peptide maps of the 95 kDa receptor subunit from (*b*) isoprenaline-treated and (*a*) control adipocytes

Solubilized insulin receptor (3 μg) was incubated with 100 nM-insulin for 30 min, followed by the addition of 5 μM-[γ-^{32}P]ATP. After 10 min the reaction was terminated by heating. The phosphorylated β-subunit of the insulin receptor was identified by autoradiography. The gel fragments containing the β-subunit were incubated with trypsin for 12 h and further processed as described in the Materials and methods section. Phosphopeptides from isoprenaline-treated cells are shown in (*b*) and those from control cells in (*a*). The phosphopeptides were separated by h.p.l.c. and the relevant regions of the chromatograms are shown. The ^{32}P incorporation in each peak is expressed as a percentage of the total radioactivity found in all fractions of the chromatogram. The acetonitrile gradient (●) used was 0% (5 min) and 0–40% (40 min).

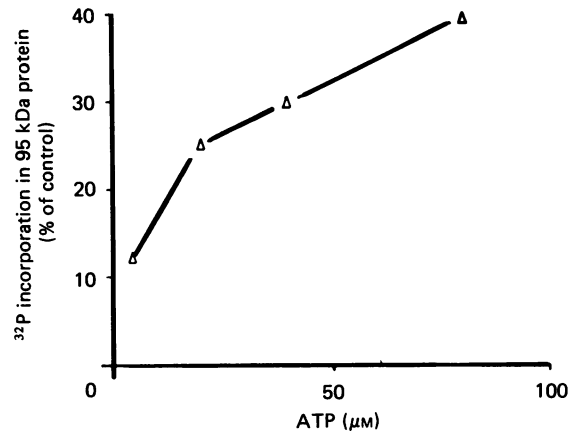

Fig. 7. Effect of ATP concentration on autophosphorylation of the 95 kDa subunit of the insulin receptor from isoprenaline-treated and control cells

Solubilized insulin receptor (3 μg) was incubated with 100 nM-insulin for 30 min, followed by addition of [γ-^{32}P]ATP in the concentrations indicated on the abscissa. The amount of tracer was kept constant at all ATP concentrations. The reaction was stopped after 2 min, and the ^{32}P incorporation into the 95 kDa protein was determined as described in the Materials and methods section. The insulin-stimulated phosphorylation of the 95 kDa protein from isoprenaline-treated cells is expressed as a percentage of the value found for receptor of control cells at the corresponding ATP concentration. Means of three experiments are shown.

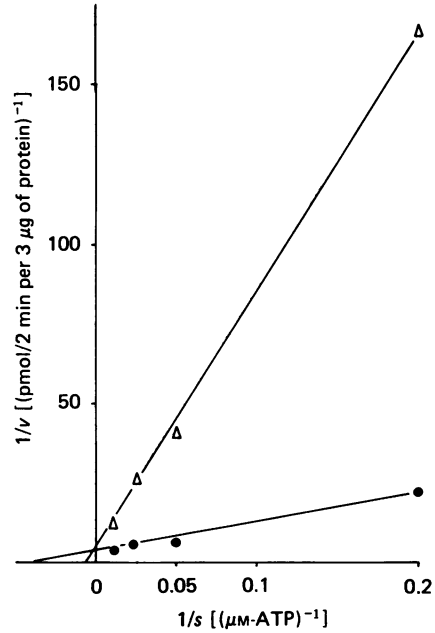

Fig. 8. Lineweaver–Burk plot of insulin-stimulated tyrosine kinase activity

The values are the means of three experiments with insulin receptor from isoprenaline-treated cells (△) or control cells (●).

DISCUSSION

The insulin receptor isolated from isoprenaline-treated fat-cells behaves differently from the receptor of normal control cells, as follows.

(1) Insulin binding is decreased.

(2) The affinity of the receptor kinase for the substrate, ATP, is also decreased, and this explains the low rate of receptor phosphorylation at non-saturating ATP concentrations.

The decreased binding of insulin to the receptor from isoprenaline-treated cells could reflect a regulatory inhibition of the binding site of the receptor as well as a loss of specific binding sites. To decide between these two possibilities on the basis of the binding data is impossible. On the basis of the phosphorylation data, however, the latter possibility can be excluded. The Lineweaver–Burk plot shown in Fig. 8 gives similar values of $V_{max.}$ for the receptor preparations from control and isoprenaline-

treated cells, and this is incompatible with the possibility of a decreased receptor number in the preparation from isoprenaline-treated cells. Therefore a regulatory inhibition of the binding function of the receptor has to be assumed. The inhibitory effect of isoprenaline treatment is significantly more pronounced if the solubilized receptor is studied than it is if the intact cell is used (Fig. 1). It might be speculated that the underlying mechanism is a conformational change at the binding site, which is more expressed as soon as the receptor is solubilized from its stabilizing membrane environment.

Concerning the effect of isoprenaline treatment on the tyrosine kinase characteristics of the insulin receptor, two major questions arise. (1) Is it likely that the isoprenaline-induced increase in the K_m for ATP of the receptor kinase found *in vitro* would lead to a decreased tyrosine kinase activity of the receptor in the intact cell? (2) What is the mechanism by which isoprenaline treatment modulates the K_m for ATP of the receptor kinase? The ATP concentration calculated from the water space of intact adipocytes is in the millimolar range (Kono *et al.*, 1977; Häring *et al.*, 1981). Considering this ATP concentration in the intact cell and the K_m values found here for the receptor kinase *in vitro*, it seems unlikely that the isoprenaline-induced increase in the K_m for ATP leads to a decreased receptor kinase activity in the intact cell. However, the K_m values were determined *in vitro* in the presence of 10 mM-Mn^{2+}, which specifically decreases the K_m for ATP (White *et al.*, 1984). Higher K_m values are found at low Mn^{2+} concentrations, which are more likely to resemble the situation in the intact cell (White *et al.*, 1984), and therefore it seems possible that the modulation of the K_m of the receptor kinase by isoprenaline treatment could lead to decreased receptor kinase activity in the intact adipocyte and that this is part of the mechanism that causes insulin-resistance of catecholamine-treated cells. Catecholamine-treated adipocytes are another example of insulin-resistant cells in which a decreased receptor kinase activity was found (Häring *et al.*, 1984*b*; Grigorescu *et al.*, 1984; Kadowaki *et al.*, 1984). The association of insulin-resistance in these cells and the inhibition of receptor kinase activity lends further support to the model that activation of the receptor kinase is indeed a crucial post-binding step in insulin-signal transmission. In this context it is noteworthy that glucagon can induce insulin-resistance of a plasma-membrane cyclic AMP phosphodiesterase (Heyworth & Houslay, 1983), and it will be important to test if an analogous modification of the receptor kinase can be found in glucagon-treated cells.

The mechanism by which catecholamine stimulation might modulate the K_m for ATP of the receptor kinase is not clear at present. However, it might be speculated that it occurs through serine phosphorylation of the insulin receptor in the intact cell. In intact hepatoma cells and hepatocytes, phosphorylation of the insulin receptor at serine residues was found as well as the insulin-stimulated tyrosine phosphorylation of the insulin receptor (Kasuga *et al.*, 1982*b*; Häring *et al.*, 1984*a*; Gazzano *et al.*, 1983). The identity of the serine kinase that phosphorylates the insulin receptor and the meaning of serine phosphorylation of the β-subunit of the insulin receptor is not yet known. It seems possible that isoprenaline causes a cyclic-AMP-kinase-dependent phosphorylation of the insulin receptor at a serine residue and that this serine phosphorylation inhibits insulin binding and tyrosine kinase activity. As the serine phosphorylation of the receptor would occur in the intact cell, this hypothesis requires the assumption that no dephosphorylation of serine residues of the insulin receptor occurs through the preparation of the partially purified receptor. Even so, we know that dephosphorylation of the insulin receptor in the intact cell occurs very rapidly (Häring *et al.*, 1984*a*); we know on the other hand that serine phosphorylation is stable throughout the procedure of receptor isolation if the isolation is performed in the presence of phosphatase inhibitors and at low temperature (Kasuga *et al.*, 1982*b*; Häring *et al.*, 1984*a*). Therefore it can be assumed that an isoprenaline-induced serine phosphorylation of the receptor that had occurred in the intact cell is still present when we test the kinase activity of the isolated receptor in the assay *in vitro* and that it is indeed the cause of the altered characteristics of the receptor kinase from isoprenaline-treated cells.

Very recently we had seen that a similar post-receptor insulin-resistance of glucose transport to that caused by catecholamine treatment of isolated fat-cells can also be produced by phorbol ester treatment of isolated fat-cells (Kirsch *et al.*, 1985). As phorbol esters are known stimulators of protein kinase C (Niedel *et al.*, 1983), it is very likely that the phorbol ester effect is caused by activation of protein kinase C in fat-cells. Furthermore, it was shown that phorbol ester treatment of human lymphocytes (Jacobs *et al.*, 1983) and rat hepatoma cells (Takayama *et al.*, 1984) leads to a serine phosphorylation of the insulin receptor in these cells. Preliminary data obtained with insulin receptor kinase isolated from phorbol-ester-treated fat-cells in our laboratory revealed a similar picture to that presented here for the insulin receptor isolated from catecholamine-treated cells. Taking these data together with the results of the present paper, it may be speculated that phosphorylation of the insulin-receptor kinase on serine residues is a means to decrease its activity and that serine phosphorylation of the receptor is one mechanism that causes insulin-resistance in the intact cell.

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