Glucose-induced stimulation of human insulin-receptor mRNA and tyrosine kinase activity in cultured cells

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The effects of high glucose on insulin-receptor tyrosine kinase activity and gene expression were investigated in 3T3-HIR cells. Cells incubated for 48 h in the presence of 25 mM glucose showed a 5-fold increase in the amount of insulin receptors per cell, receptor autophosphorylation and phosphorylation of the exogenous substrate poly(Glu/Tyr) compared with cells grown in the absence of glucose but in the presence of 25 mM fructose. These effects were associated with a 4-fold stimulation in steady-state levels of insulin-receptor mRNA. Significant cellular glucose utilization and lactate production were observed in the presence of high glucose in the culture medium, indicating a functional glycolytic pathway in glucose-treated cells, but not in cells treated with fructose. Such a differential response to hexoses favours the hypothesis of a carbohydrate regulation via a glycolytic intermediate. This was further supported by a similar glucose-induced increase in mRNA levels of the enzyme glyceraldehyde-3-phosphate dehydrogenase. To test the hypothesis that the stimulatory effect of glucose on amount of insulin receptors and phosphorylation state could result from post-transcriptional modifications, cells exposed to glucose were incubated with actinomycin D, a potent inhibitor of gene transcription. In cells challenged with high glucose plus inhibitor, insulin-receptor mRNA half-life was increased from 1 to 3 h, indicating that post-transcriptional mechanisms are involved in these processes of glucose regulation. Inhibition of protein synthesis by cycloheximide induced an overexpression of insulin-receptor mRNA levels in the presence of glucose, suggesting that labile repressor protein(s) could be implicated in the effects of glucose. We conclude that (1) long-term culture with high glucose increases the amount of insulin receptors and their tyrosine kinase activity and (2) the glucose-induced increase in insulin-receptor mRNA levels can be accounted for, at least in part, by post-transcriptional events.

INTRODUCTION

On binding to its cell-surface receptor, insulin activates its receptor tyrosine kinase. The biological effects induced by the hormone require phosphorylation of the three tyrosine residues, 1157, 1162, 1163, located in the receptor tyrosine kinase domain [1,2]. This activation is essential for the generation of the signals that produce the final effects of insulin [3]. Defective tyrosine kinase activity is generally associated with insulin-receptor and post-receptor alterations [4–6]. Among the metabolic and hormonal stimuli known to modify the activity of the insulin receptor, the most common include variations *in vivo* in glucose homoeostasis caused by starvation and changes in diet composition [7,8]. In addition, pathological situations usually associated with insulin resistance such as obesity [9,10] or diabetes [11–13] are characterized by altered insulin-receptor tyrosine kinase activity. Experimental diabetes induced by injection of streptozotocin increases insulin-receptor mRNA levels [14]. However, the molecular mechanisms underlying these modifications of insulin action have yet to be identified, as insulinopenic diabetes induces multiple hormonal and metabolic perturbations. Analysis of the insulin-receptor gene structure indicates that it belongs to the family of housekeeping genes. Further, it has been shown that insulin exerts a permissive effect on glucose to increase the transcription of genes encoding insulin in the pancreatic islet [15] or L-pyruvate kinase in the liver [16]. To the best of our knowledge, no data are available concerning the metabolic regulation of the human insulin-receptor gene, although its promoter region is now well characterized [17,18].

The present study was undertaken to investigate the effects of glucose on insulin-receptor activity and to search for possible glucose effects on insulin-receptor gene expression. We provide here evidence that prolonged incubation of 3T3-HIR cells with high glucose concentrations in the absence of insulin increases mRNA levels and kinase activity of the human insulin receptor.

EXPERIMENTAL

Materials

Hepes, ATP, Triton X-100, *N*-acetyl-D-glucosamine, dithiothreitol (DTT), BSA, protease inhibitors and poly(Glu_4/Tyr_1) were purchased from Sigma (St. Louis, MO, U.S.A.). cDNA probes encoding the human insulin receptor were provided by Dr. A. Ullrich (Munich, Germany), poly(A)-binding protein was provided by Dr. T. Grange (Paris, France) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by Dr. P. Jeanteur (Montpellier, France). 3T3-HIR cells were a gift from Dr. J. Whittaker (Stony Brook, NY, U.S.A.). $Na^{125}I$ was obtained from CEA (Saclay, France). The random primer extension kit, [γ-^{32}P]ATP (6000 Ci/mmol) and [α-^{32}P]dCTP (3000 Ci/mmol) were purchased from Amersham International (Amersham, Bucks., U.K.). Agarose–what-germ agglutinin–glycaminosylex (WGA) was purchased from Biomaker (Rehovot, Israel). Rabbit anti-(insulin-receptor β-subunit) antibodies were raised and purified by affinity chromatography at INSERM U145 (Nice). Reagents for SDS/PAGE and RNA analysis were purchased from Bio-Rad (Richmond, CA, U.S.A.) or Serva (Heidelberg,

Abbreviations used: DMEM, Dulbecco's modified Eagle's medium; DTT, dithiothreitol; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IGF-I, insulin-like growth factor I; SSC, standard saline citrate (0.15 M NaCl + 0.015 M sodium citrate); WGA, wheat-germ agglutinin.

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Germany). Plasticware for tissue culture was obtained from Nunc (Copenhagen, Denmark), culture media from Gibco (Grand Island, NY, U.S.A.) and fetal calf serum (FCS) from Institut J. Boy (Reims, France). Nitrocellulose membranes were bought from Schleicher and Schuell (Cera Labo, France).

Cell culture

Mouse embryo fibroblasts (NIH-3T3) transformed with an expression vector containing the human insulin-receptor-coding region (3T3-HIR cells) express 6×10^6 receptors/cell [19]. The expression vector consists of human insulin-receptor cDNA under the control of mouse metallothionein promoter ligated to a 5.5 kb subgenomic transforming fragment of the bovine papilloma virus [19]. NIH-3T3 cells transformed with an expression vector containing human insulin-like growth factor I (IGF-I) receptor cDNA [20] were provided by Dr. P. de Meyts and Dr. J. D. Groffin (Copenhagen, Denmark). Both cell lines were grown to confluence in standard Dulbecco's modified Eagle's medium (DMEM) containing 12.5 mM glucose supplemented with 10% FCS and were fed every other day. Cultures were passaged weekly (when they reached confluence) at a ratio of 1:4 using trypsin/EDTA (Gibco).

Inhibition of protein synthesis

Cycloheximide was added at a concentration of 1 μg/ml to the culture media and left for either 8 h and 24 h in the presence of glucose. This concentration inhibits L-[4,5-^{3}H]leucine incorporation into trichloroacetic acid-precipitable proteins by 85 ± 4% at both times studied compared with control cells (results not shown).

RNA isolation and Northern-blot analysis

Total cellular RNA was isolated by repeated low-temperature guanidinium thiocyanate/phenol/chloroform extraction followed by precipitation with cold ethanol [21]. After size fractionation on a 1% agarose/0.67 M formaldehyde gel, RNA was transferred by capillary action to nitrocellulose membranes in $10 \times$ SSC ($1 \times$ SSC = 150 mM NaCl/15 mM sodium citrate) and fixed by baking at 80 °C for 2 h. The integrity of the blotted RNAs was assessed by visualization of the ethidium bromide-stained 28S and 18S ribosomal bands by u.v. shadowing. Hybridizations were performed for 16 h at 42 °C with [α-^{32}P]dCTP-labelled cDNA probes, in $4 \times$ SSC/10% dextran sulphate/40% formamide. Filters were washed four times in $1 \times$ SSC/0.1% SDS at room temperature and once in $0.1 \times$ SSC/0.05% SDS at 55 °C, then autoradiographed (Kodak X-Omat S films) for 4–24 h at −80 °C with two intensifying screens. The intensity of the signals was measured with a scanning densitometer (Hoefer GS-300 scanning densitometer; San Francisco, CA, U.S.A.) connected to a Macintosh SE computer. After hybridization with the insulin-receptor probe, the blots were rehybridized with a GAPDH probe. A poly(A)-binding protein cDNA probe or an oligonucleotide specific for 18S RNA [22] was used to correct for the exact amount of total RNA transferred to the filters. In the numbering of the amino acid residues of the insulin receptor we took into account the residues encoded by exon 11 [23].

Isolation of insulin receptors

Insulin receptors were purified as previously described [24]. Briefly, cells were washed twice with ice-cold PBS and scraped into Hepes/NaCl solubilization buffer containing 1% Triton X-100, in the presence of inhibitors of both proteases and phosphatases (100 units/ml aprotinin, 2 mM phenylmethanesulphonyl fluoride, 5 mM EDTA, 100 mM NaF, 10 mM sodium pyrophosphate, 1 mg/ml bacitracin, 1 mM benzamidine). After solubilization by stirring on ice for 90 min the cell lysate was passed over a WGA affinity column, washed and eluted with 0.3 M *N*-acetylglucosamine in the presence of protease inhibitors. Partially purified insulin receptors were kept frozen at −80 °C until use. Insulin-receptor concentration in the WGA preparations obtained from control and glucose-treated cells was quantified by measuring insulin binding. Specific radioactivity was calculated by subtracting the radioactivity (^{125}I c.p.m.) obtained in the presence of 1 μM unlabelled insulin and was expressed per mg of protein.

Immunoblot analysis of insulin receptors

Proteins resolved on SDS/polyacrylamide gels were transferred on to a nitrocellulose membrane using a custom-made transfer apparatus and a one-buffer system containing 48 mM Tris, 40 mM glycine, 0.05% SDS and 20% methanol [23]. Transfers were carried out for 3 h at room temperature at constant current and the filters were blocked overnight at 4 °C in Tris-buffered saline containing 0.2% Tween and 4% non-fat dried milk. Filters were incubated at room temperature with a human antibody directed against the β-subunit of the insulin receptor (residues 1247–1261 and 1297–1316) at a 1:100 dilution in the same buffer for 2 h. After several washes, ^{125}I-Protein A (30 mCi/mg) was added for 1 h at room temperature. Filters were washed again and subjected to autoradiography.

Insulin-receptor autophosphorylation and tyrosine kinase assays

Preparations of partially purified receptors were diluted to contain the same amount of insulin receptors, based on specific binding radioactivity (c.p.m./mg of protein). Receptor samples were preincubated for 60 min at 4 °C in the absence or presence of 1 μM insulin in 50 mM Hepes/0.1% Triton X-100, pH 7.4. The phosphorylation reaction was initiated by the addition of 15 μM [γ-^{32}P]ATP, 8 mM $MgCl_2$ and 4 mM $MnCl_2$, continued for 30 min at 22 °C and stopped by the addition of an ice-cold stopping solution containing 80 mM NaF and 30 mM EDTA. Samples were immunoprecipitated with anti-(insulin-receptor) antibodies. After precipitation with Protein A, the pellets containing the immunoadsorbed proteins were washed three times with 1 ml of ice-cold 30 mM Hepes buffer (pH 7.4) containing 30 mM NaCl and 0.1% Triton X-100 and subsequently solubilized in 70 μl of $1 \times$ Laemmli sample buffer containing 100 mM DTT. All samples were boiled for 3 min before electrophoresis [25]. The proteins were then separated and analysed by one-dimensional SDS/PAGE on 7.5%-acrylamide resolving gels under reducing conditions [24]. The gels were stained with Coomassie Blue in 50% trichloroacetic acid and destained in 6% acetic acid. The M_r values of the standards used were: 200000 (myosin); 116000 (β-galactosidase); 93000 (phosphorylase *b*); 66000 (BSA); 45000 (ovalbumin); 31000 (carbonic anhydrase). The intensity of the signals corresponding to the autophosphorylated phosphoproteins shown on the autoradiograms was quantified by scanning densitometry.

For phosphorylation of the exogenous substrate, the receptor preparations were preincubated in the presence of 1 μM insulin for 30 min at 22 °C, then incubated for an additional 30 min in a phosphorylation buffer containing 8 mM $MgCl_2$, 4 mM $MnCl_2$ and 15 μM [γ-^{32}P]ATP (2500 mCi/mmol). Finally, 0.2 mg of the poly(Glu_4/Tyr_1) synthetic substrate/ml was added and the incubation was continued for 45 min at 22 °C. The phosphorylation reaction was stopped by spotting a portion of each sample on

Whatman filter paper (Whatman 3M) and immersing it in 10% trichloroacetic acid containing 10 mM pyrophosphate. Filters were washed three times in the same solution, rinsed with ethanol and allowed to dry. Radioactivity incorporated into trichloroacetate-precipitable proteins was measured in a liquid-scintillation counter.

Analytical methods

Culture media were stored at −20 °C. Glucose levels were measured by the glucose oxidase method (Boehringer, Meylan, France) and fructose was assessed enzymically [26]. Lactate concentrations were measured by the method of Hohorst [27]. Protein concentrations were determined as described by Bradford [28].

Statistics

Results are expressed as means ± S.E.M. Unpaired Student's *t* test was used to establish the statistical significance of the differences observed between two groups. Statworks software (Calabasas, CA, U.S.A.) was used for all statistical analyses.

RESULTS

Effect of glucose on the regulation of insulin-receptor content and tyrosine kinase activity

Cells were cultured for 48 h in the presence of either 25 mM glucose (high glucose) or 25 mM fructose (controls). Glucose stimulation was assessed by comparing cells cultured in high glucose with control cells. The following parameters were measured: (1) insulin-receptor content (Figure 1), (2) autophosphorylation of the insulin receptor in the basal state and after insulin stimulation (Figure 2a) and (3) phosphorylation of an exogenous substrate by the insulin receptor (Figure 2b). We observed that high glucose stimulates all three parameters by 4–5-fold.

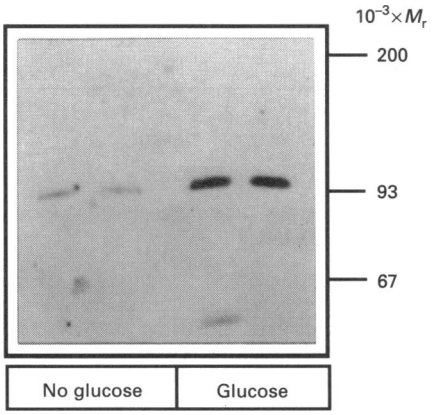

Figure 1 Immunoblot analysis of receptors obtained from HIR cells treated with 25 mM fructose or glucose over 48 h

WGA-purified insulin receptors (25 μg of protein) were subjected to SDS/PAGE on a 7.5% polyacrylamide gel before being transferred to a nitrocellulose membrane. Immunodetection was performed using an antibody directed towards residues 1247–1261 and 1297–1316 of the insulin-receptor β-subunit; the blots were incubated with ^{125}I-Protein A and autoradiographed. Three separate analyses which gave similar results were performed using two different receptor preparations.

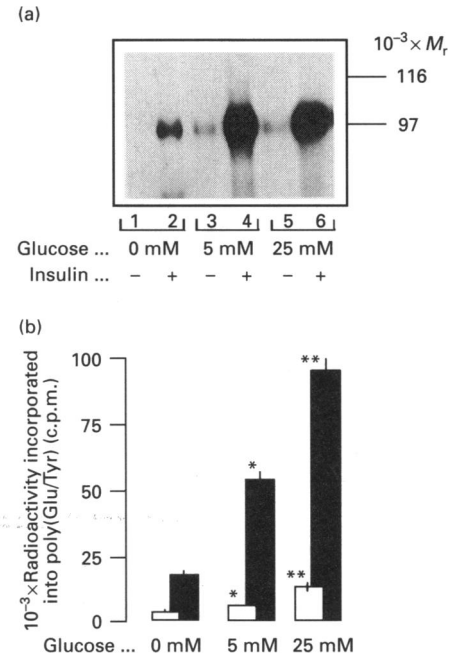

Figure 2 Insulin-receptor tyrosine kinase activity after incubation of intact HIR cells in high-glucose medium

Cells grown to confluence in standard DMEM were deprived of serum and insulin for 24 h before the experiments. The media were then supplemented with the glucose concentrations shown and cells were incubated for a further 24 h before receptor isolation. Glucose-free medium was supplemented with 25 mM fructose. (**a**) Autophosphorylation of insulin receptors partially purified from glucose-treated cells. Portions of WGA-purified insulin receptors (3–5 μg of protein) prepared from the different treated cells were incubated *in vitro* in the absence (lanes 1, 3, 5) or presence of 1 μM insulin (lanes 2, 4, 6). They were then phosphorylated in the presence of 15 μM [γ-^{32}P]ATP, immunoprecipitated with anti-(insulin receptor) antibody and subjected to SDS/PAGE. The fixed and dried gels were autoradiographed at −80 °C for 16 h. Shown is an autoradiograph representative of three independent experiments yielding similar results. (**b**) Tyrosine kinase activity towards poly(Glu_4/Tyr_1). Portions of WGA-purified receptors (5–9 μg of protein) were preincubated with (■) or without (□) 1 μM insulin, phosphorylated in the presence of 15 μM [γ-^{32}P]ATP for 30 min and then with 0.2 mg/ml poly(Glu_4/Tyr_1) for 45 min at 22 °C. Radioactivity incorporated into trichloroacetate-precipitable proteins was counted. Results are expressed as mean ± S.E.M. of three independent experiments. Differences are statistically significant (**$P < 0.003$ and *$P < 0.04$) relative to the respective controls with or without insulin.

Effect of glucose on insulin-receptor mRNA levels

To evaluate the effect of glucose on insulin-receptor mRNA levels, HIR cells were incubated in DMEM in the absence of FCS and insulin, but in the presence of increasing concentrations of glucose. Total RNA (20 μg) was analysed by Northern blotting (Figure 3) and insulin-receptor mRNA levels were quantified (Figure 4).

Insulin-receptor mRNA levels were increased 2–3-fold after incubation of the cells in the presence of a physiological concentration of glucose (5 mM), and there was a further increase when cells were incubated in the presence of higher (12.5 and 25 mM) glucose concentrations (Figure 4). This stimulation was associated with a 6-fold increase in GAPDH mRNA levels, whereas the levels of poly(A)-binding protein mRNA remained unchanged (results not shown). Similarly, an increase in the amount of IGF-I-receptor mRNA was observed in response to high glucose concentrations in NIH-3T3 cells transformed with the human IGF-I-receptor cDNA (results not shown). To determine if protein synthesis is required for the glucose-stimulated increase in insulin-receptor mRNA levels, we treated

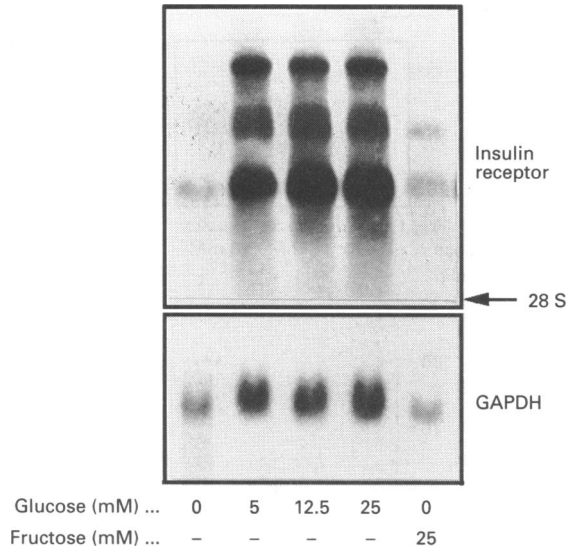

Figure 3 Regulation of human insulin-receptor mRNA levels by glucose

Cells were grown to confluence in standard DMEM containing 10% FCS, then incubated in serum-free and insulin-free DMEM for 24 h. Various concentrations of hexose (0–25 mM glucose and 25 mM fructose) were then added to the medium, and after a 24 h incubation period, cells were harvested for RNA isolation. Samples of total RNA (20 μg/lane) were analysed by Northern blotting. The same blots were hybridized with insulin receptor and GAPDH cDNA probes. Blots were autoradiographed for 12 h at −80 °C. The position of 28S rRNA is indicated on the autoradiogram. The sizes of the major insulin-receptor mRNA transcripts identified were 5.1, 7.9 and 9.5 kb. Three independent experiments yielded similar results.

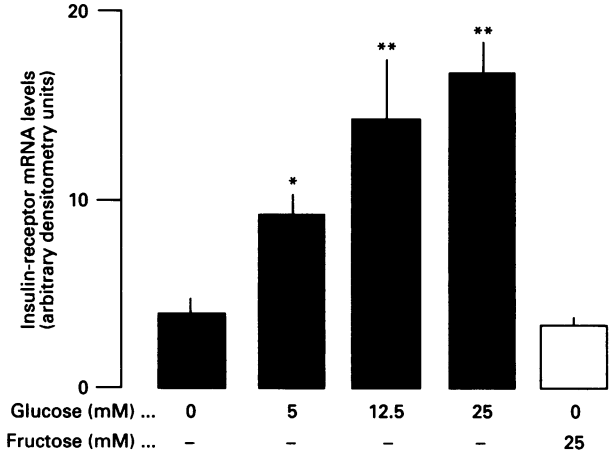

Figure 4 Analysis of mRNA levels by scanning densitometry

Cells were grown in standard DMEM with 10% FCS, then incubated for 4 h in the absence of serum and insulin. Increasing concentrations of glucose or 25 mM fructose were then added to the medium for 24 h. Results are expressed as means ± S.E.M. of three analyses performed on independent Northern blots. $^*P < 0.01$, $^{**}P < 0.002$, compared with cells grown in the absence of glucose.

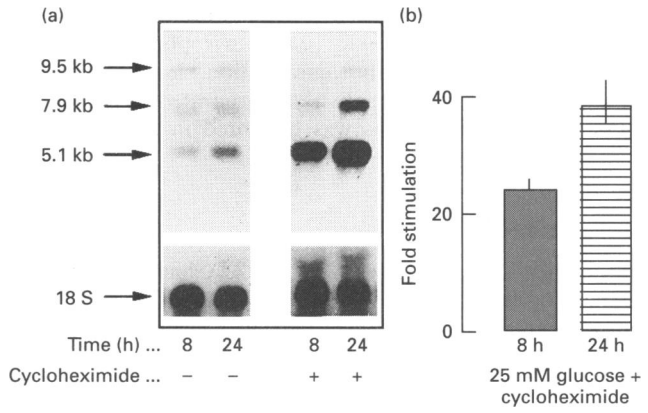

Figure 5 Effect of protein synthesis inhibition by cycloheximide on glucose-induced insulin-receptor mRNA levels

Cells were incubated in standard DMEM for 20 h, then 1 μg/ml cycloheximide was added to the plates for 8 and 24 h. Total RNA was extracted and Northern-blot analysis was performed; 20 μg RNA samples were applied per lane. (**a**) Insulin-receptor transcripts after exposure of autoradiograms for 3 h. The lower bands correspond to 18S RNA; 18S RNA is not affected by cycloheximide treatment. (**b**) Quantification of the effect of cycloheximide on the 5.1 kb transcript by scanning densitometry. Results are expressed as mean values ± S.E.M. obtained from three independent experiments.

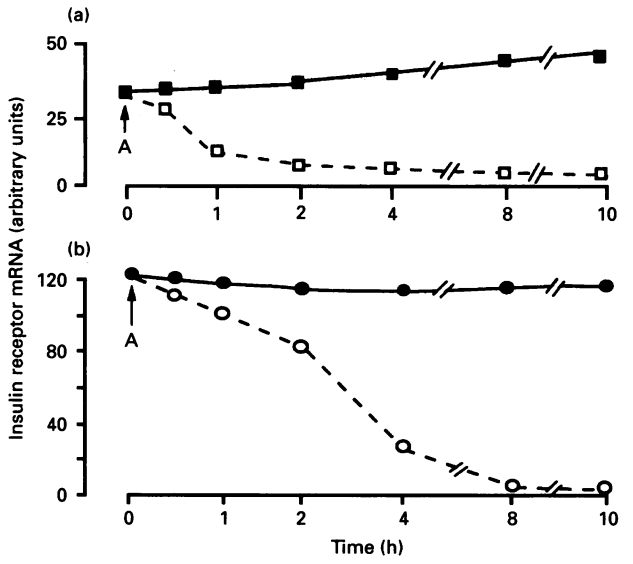

Figure 6 Effect of actinomycin D on glucose-induced increase in steady-state insulin-receptor mRNA levels

Cells were incubated with (**a**) fructose (25 mM) or (**b**) glucose (25 mM) for 20 h, then actinomycin D (A; 5 μg/ml) was added to the culture media for the indicated times. Total RNA was extracted and mRNA levels were quantified by Northern-blot analysis followed by scanning densitometry of the autoradiographs. Each value, expressed in arbitrary densitometry units, represents the average of two experiments, each performed in duplicate. ■, ●, −actinomycin; □, ○, +actinomycin.

cells exposed to 25 mM glucose for 8 and 24 h with cycloheximide. Under these conditions, in which protein synthesis was inhibited by 85%, a 40-fold increase in insulin-receptor mRNA levels was observed whereas the level of 18S RNA remained unchanged (Figure 5).

As in our experiments insulin-receptor cDNA expression is directed by a heterologous promoter (metallothionein), we thought that the stimulation by glucose probably occurred at the post-transcriptional level. We therefore tested the effect of actinomycin D, a potent inhibitor of RNA polymerase II, on glucose-stimulated expression of insulin-receptor cDNA. Data presented in Figures 6 and 7 show that the half-life of the mRNA was increased from 1 to 3 h by addition of 25 mM glucose to actinomycin D-treated cells. It is clear that high glucose leads to stabilization of the insulin-receptor mRNA. The large increase in

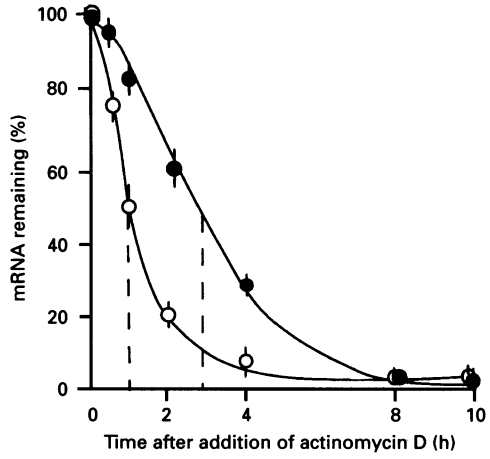

Figure 7 Effect of glucose on insulin-receptor mRNA stability

After overnight incubation without glucose, cells were incubated with 25 mM glucose in the presence of actinomycin D (5 μg/ml). At the indicated incubation times, total RNA was extracted and insulin-receptor mRNA levels were measured as described in the Experimental section. Data obtained in three separate experiments are presented as percentage of remaining insulin-receptor mRNA relative to the amount at time 0 (taken as 100%). Values are normalized for the total amount of RNA loaded on the gel assessed by hybridization with a poly(A)-binding protein cDNA probe. Broken lines indicate the insulin-receptor mRNA half-lives calculated for 0 mM (○) and 25 mM (●) glucose respectively.

Table 1 Metabolic parameters in HIR cells

Cells were cultured in serum-deprived DMEM supplemented with 25 mM glucose or fructose. After 20 h, media were collected and kept frozen at −20 °C until substrate measurement. Values are means ± S.E.M. of four plates/group in two separate experiments. Results are expressed as μmol of substrate/μg of cellular protein.

Culture medium	Glucose utilization	Fructose utilization	Lactate production
Glucose (25 mM)	90.51 ± 8.22	0	14.06 ± 1.12
Fructose (25 mM)	0	12.79 ± 3.46	2.46 ± 0.36

insulin-receptor mRNA observed in the presence of cycloheximide suggests that one or more proteins might be responsible for the short half-life of insulin-receptor mRNA.

Finally, the importance of hexose metabolism in the regulation of insulin-receptor gene expression and kinase activity was examined by measuring glucose and fructose utilization in the culture media. As shown in Table 1, a significant rate of glycolysis, assessed by glucose utilization and lactate production, was observed in cells cultured with 25 mM glucose, whereas a very low rate of glycolysis was observed in cells cultured with 25 mM fructose and no glucose.

DISCUSSION

The regulation of enzyme activity by glucose and other carbohydrates has been recognized for many years [29,30]. Increasing evidence indicates that the expression of several housekeeping genes is under the positive control of metabolic substrates. Such a control has been observed for enzymes of the glycolytic and lipogenic pathways, e.g. aldolase B [31], L-pyruvate kinase [32], fatty acid synthase [33] and the hepatic spot 14 gene [34]. Stimulation of these enzymes by glucose requires the permissive effect of insulin, and it has been demonstrated that the increase in their mRNA levels is due in large part to increased transcription of the corresponding genes. In the case of L-pyruvate kinase, a 'carbohydrate responsive element' has been identified in the 5′ flanking region of the gene [16], and it has been proposed that the glycolytic intermediate glucose 6-phosphate could bind to this element [35]. Similarly, glucose 6-phosphate is thought to be involved in triggering transcription of the fatty acid synthase gene [34].

The regulation of insulin-receptor signal transduction is likely to be under the control of multiple hormonal and metabolic stimuli. However, the molecular events implicated in the regulation of insulin-receptor gene expression are not fully understood. The present study was designed to investigate the effects of high glucose on the protein content, tyrosine kinase activity and mRNA levels of the insulin receptor. We report here for the first time that high glucose concentrations maintained for 24–48 h in cultured cells increase three parameters known to represent key steps in the transmission of insulin action. These are the amount of insulin receptor (Figure 1), tyrosine kinase activity (Figure 2) and insulin-receptor mRNA levels (Figures 3 and 4).

Isolation and purification of insulin receptors under conditions that preserve their phosphorylation state enables analysis *in vitro* of insulin-receptor kinase activity which is thought to reflect activity *in vivo* at the time of isolation from intact cells [36]. Under these conditions, we show that high glucose increases insulin-receptor protein content (Figure 1) and stimulates its tyrosine kinase activity at the level of autophosphorylation and phosphorylation of an exogenous substrate (Figure 2). Taken as such, these data do not allow determination of whether the effect mediated by the increase in ambient glucose on insulin-receptor content and kinase activity is direct or indirect. However, they are in agreement with our recent findings that prolonged hyperglycaemia in streptozotocin-treated diabetic rats is associated with stimulation of the tyrosine kinase activity of placental insulin and IGF-I receptors [37,38]. These results are also in line with the observation that acute hyperglycaemia stimulates insulin-receptor kinase activity towards an endogenous substrate in rat muscle [39] and that high glucose concentration increases insulin-receptor affinity in cultured adipocytes [40]. Finally, an effect of glucose on the phosphorylation/dephosphorylation status of the receptor seems worthy of consideration. We have shown that tyrosine phosphatase activity towards the insulin receptor was modified in several tissues of streptozotocin-diabetic rats that developed marked hyperglycaemia after severe insulinopenia [37].

The 4-fold increase in insulin-receptor mRNA induced by high glucose (Figures 3 and 4) was associated with an increased half-life of insulin-receptor mRNA in the presence of 25 mM glucose (Figures 6 and 7). These data indicate that post-transcriptional mechanisms such as stabilization of insulin-receptor mRNA or a decrease in its degradation are partly responsible for the glucose-induced stimulation. This finding is in agreement with data showing that several genes, including immunoglobulin, aP2 and insulin receptor, are regulated, at least in part, via post-transcriptional mechanisms [41–43]. However, regulation of insulin-receptor gene expression at the transcriptional level cannot be ruled out, as it has recently been shown that the human insulin-receptor gene is also regulated by multiple protein–DNA interactions within the promoter region [44]. The large increase in the levels of insulin-receptor mRNA observed in the presence of the protein-synthesis inhibitor, cycloheximide, favours the idea that insulin-receptor gene expression is tightly regulated in the basal state. Such regulation has been reported for immediate early genes such as the proto-oncogenes c-*fos* and

c-*myc*, the activation of which occurs through pre-existing transcription factors [45,46]. Cycloheximide has also been shown to stabilize IGF-binding protein 1 mRNA and to inhibit transcription of this protein [47]. These observations indicate that inhibition of protein synthesis can affect gene expression by different mechanisms and lend support to the following hypothesis. One or several repressor protein(s) may act at transcriptional or post-transcriptional steps to turn off insulin-receptor gene expression, and glucose may prolong the half-life of the mRNA by modifying the amount and/or the activity of such proteins.

As proposed for other genes [34,35], the effects of glucose on insulin-receptor mRNA levels and protein expression could be mediated by glucose *per se* or by glycolytic metabolites. The lack of effect of high fructose concentrations on insulin-receptor mRNA levels (Figures 3 and 4) indicates that hyperosmolarity is not responsible for the effects observed. The hypothesis that a glycolytic intermediate might modulate the effects of glucose is strengthened by (1) the good correlation between the increased rate of glycolysis and the levels of receptor mRNA in glucose-treated cells (Table 1, Figure 3) and (2) by the concomitant increase in the levels of GAPDH mRNA (Figure 3), since this enzyme is implicated in the metabolism of glucose and fructose.

The physiological significance of the glucose-induced increase in the amount of insulin receptor, tyrosine kinase activity and mRNA levels leads to several hypotheses. In addition to the known effect of glucose, in stimulation of pancreatic insulin secretion, it is conceivable that it might contribute to activation of insulin signalling in target cells. *In vitro*, an increase in IGF-I mRNA levels has been observed in diabetic mesangial cells cultured in high glucose concentrations [48]. *In vivo*, an increase in insulin-receptor mRNA, number and kinase activity under conditions of hyperglycaemia would in turn lead to a modification of insulin-stimulated glucose transport and metabolism in insulin-responsive tissues. Such glucose-induced mechanisms could also play a role in the impaired insulin action observed in peripheral tissues of hyperglycaemic type-I or type-II diabetic patients [49,50].

In summary, this study provides direct evidence that the content, tyrosine kinase activity and mRNA levels of the human insulin receptor are all increased by prolonged incubation of cultured cells in the presence of high glucose and in the absence of insulin. These effects of glucose require protein synthesis and involve stabilization of the mRNA. Such regulation is likely to contribute to the control of the cascade of events leading from stimulation of insulin-receptor gene expression to receptor phosphorylation and eventually transduction of the intracellular signal of insulin.

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