Perivenous localization of insulin receptor protein in rat liver, and regulation of its expression by glucose and oxygen in hepatocyte cultures

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Insulin stimulates glucose utilization in the liver, which occurs mainly in the less aerobic, perivenous, zone. Accordingly, the insulin receptor protein was predominantly expressed in this area, although the insulin receptor mRNA was homogeneously distributed. In hepatocyte cultures venous O₂ partial pressure (*p*O₂) induced insulin receptor protein expression. High glucose

concentrations enhanced insulin receptor protein under arterial and venous *p*O₂. The induction of insulin receptor protein by venous *p*O₂ would explain its zonated expression.

Key words: carbohydrate, hormones, hypoxia, metabolic zonation.

INTRODUCTION

Insulin, a peptide hormone composed of an A chain, with 21 amino acids, and a B chain, with 30 amino acids, linked by two disulphide bridges, activates hepatic glucose utilization via glycolysis and glycogen synthesis. Insulin acts via an ectocellular receptor, which is a transmembrane glycoprotein consisting of two α - (130 kDa) and two β - (95 kDa) chains [1] linked to each other by disulphide bridges. On binding of insulin to the two ectocellular α -chains [2] the membrane spanning β -chains of the insulin receptor are autophosphorylated [3,4]. The autophosphorylated insulin receptor is an activated protein tyrosine kinase [5] which triggers the phosphorylation of effector proteins, such as insulin receptor substrate-1 (IRS-1), at multiple tyrosine residues [6]. The phosphotyrosine residues of the IRS-1 function as docking sites for Src homology ('SH2') domains of other proteins, e.g. phospholipase C χ and phosphatidylinositol 3-kinase [1].

The major target organ for insulin is the liver, which exhibits a heterogeneous distribution of key enzymes and subcellular structures between periportal and perivenous areas. The zonal enzyme distribution in conjunction with functional studies led to the model of metabolic zonation [7–12]. Accordingly, glucose uptake for glycogen synthesis and glycolysis occurs mainly in the perivenous area; conversely, glucose release from glycogenolysis and gluconeogenesis takes place preferentially in the periportal area. Due to metabolism, concentration gradients of substrates, such as oxygen and hormones, are formed during a single passage of blood through the liver [7,11,12]. The O₂ partial pressure (*p*O₂) falls from periportal values of approx. 65 mmHg (1 mmHg $\equiv$ 133.3 Pa) to perivenous values of approx. 35 mmHg. The glucagon concentration is decreased by approx. 50% and the insulin concentration only by approx. 15% after meals. Thus during the absorptive phase the concentrations of oxygen and glucagon are relatively higher in the periportal area; conversely, the concentration of insulin is relatively higher in the perivenous zone. The oxygen gradient

was considered to be a key regulator for the zonal expression of the genes of carbohydrate-metabolizing enzymes [7,10–12]. The zonation of key enzymes and capacities of metabolism within the liver are well understood, but little is known about the distribution of hormone receptors, except that the glucocorticoid receptor was found to be homogeneously distributed [13] and that glucagon receptor mRNA was predominantly expressed periportal [14].

Since the insulin-stimulated process of glycolysis together with its rate-limiting enzyme, the insulin-dependent glucokinase, were found predominantly in the perivenous zone, the insulin receptor gene would be expected to be expressed mainly in this less aerobic area. Therefore it was the aim of the present study to investigate the zonation of insulin receptor gene expression in rat liver and to study the regulation of its expression by the substrates glucose and O₂ in rat primary hepatocyte cultures. In line with expectations the insulin receptor protein was predominantly localized perivenously and could be induced in primary hepatocyte cultures at basal glucose levels by perivenous *p*O₂. Since insulin and the insulin receptor are coupled to glucose utilization the induction of insulin receptor gene expression by glucose in the absorptive phase would be in line with expectations.

MATERIALS AND METHODS

All biochemicals and enzymes were of analytical grade and were purchased from commercial suppliers.

Animals

Male Wistar rats (200–260 g) were kept on a 12 h day/12 h night cycle (light from 07:00–19:00 h) with free access to water and food. Rats were anaesthetized with pentobarbital (60 mg/kg body weight) prior to preparation of hepatocytes between 08:00 h and 09:00 h.

Abbreviations used: cMRP, canalicular multidrug resistance protein; DIG, digoxigenin; ECL, enhanced chemiluminescence; FCS, fetal calf serum; GS, glutamine synthetase; IRS-1, insulin receptor substrate 1; PCK1, cytosolic phosphoenolpyruvate carboxykinase 1; L-PK, pyruvate kinase L; *p*O₂, O₂ partial pressure (O₂ tension).

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Cell culture experiments

Liver cells were isolated by collagenase perfusion. Cells (1×10^6 /dish) were maintained under standard conditions in an atmosphere of $O_2/N_2/CO_2$ (16:79:5, by vol.) in medium M 199 containing 0.5 nM insulin, added as a growth factor for culture maintenance, 100 nM dexamethasone, required as a permissive hormone, and 4% (v/v) fetal calf serum (FCS) for the initial 4 h of culture. After 4 h, cells were cultured in serum-free M 199 at 16% O_2 (mimicking arterial pO_2) or 8% O_2 (mimicking venous pO_2) with additions as described in the Figure legends. The O_2 values take into account the O_2 diffusion gradient from the media surface to the cells [15].

RNA preparation, cDNA synthesis and competitive PCR

Total RNA was prepared from 3×10^6 cells using a NucleoSpin Kit (Macherey and Nagel, Düren, Germany). For cDNA synthesis 2 μ g of RNA was incubated with 500 μ g/ml Oligo-dT₁₂₋₁₈ (Pharmacia Biotech, Freiburg, Germany) and 1 μ l of $10 \times$ hexanucleotide mix (Roche, Mannheim, Germany) in a volume of 12 μ l at 68 °C for 10 min, and was subsequently chilled on ice for 10 min. To this, a total of 13 μ l of cDNA synthesis mix, containing 5 μ l of $5 \times$ reaction buffer, 2.5 μ l of 0.1 mM dithiothreitol, 1.5 μ l of 10 mM dNTP mix, 40 units of RNasin and 200 units of Superscript[®] II RT (Gibco BRL, Eggenstein, Germany), was added. Incubations were carried out for 90 min at 42 °C, 30 min at 52 °C, 15 min at 95 °C and finally the mixture was cooled on ice. A 1 μ l sample of this cDNA preparation was used in competitive PCR. The principle of competitive PCR is that target cDNA and a competitor DNA compete for the use of the same primers. The amount of the target cDNA can then be quantified via gel electrophoresis by comparing the intensity of the stained target PCR product with the intensity of the PCR product amplified from the known concentration of competitor DNA. The DNA competitor was prepared using a Competitive DNA Construction Kit (Takara, Heidelberg, Germany) as outlined in the manufacturer's protocol. The 5'-end and the 3'-end of the competitor can bind the primers which are used to amplify the insulin receptor cDNA. The competitor PCR product was distinguishable from the insulin receptor PCR product, because the size and internal sequence were different. The competitor was constructed by using hybrid primers containing sequences of the target cDNA (insulin receptor) and sequences of the competitor DNA: sense primer, 5'-CAGGCATGCAATC-AGGACTCGTACGGTCATCATCTGACAC-3'; antisense primer 5'-AACGTATACGGAAGATGGAAGACACTGCGTGTGAGCTTGA-3' (underlining indicates the insulin receptor sequence; the rest of the sequence corresponds to the λ DNA sequence). The competitor had a size of 489 bp.

This competitor DNA was added in various concentrations to the PCR, while the amount of cDNA remained constant. Competitive PCR was performed by using 1 μ l of cDNA preparation, various concentrations of competitor DNA (as described in the Figure legends), 20 pmol of sense primer [bp 2817–2836 from rat insulin receptor cDNA (GenBank accession number M290145); 5'-CAGGCATGCAATCAGGACTC-3'], 20 pmol of antisense primer (bp 3363–3386 from rat insulin receptor cDNA; 5'-AACGTATACGGAAGATGGAAGACACTGCGTGTGAGCTTGA-3'), 1 μ l of DMSO, 4 μ l of 2.5 mM dNTP mix, 5 μ l of $10 \times$ buffer and 1 unit of *Taq* polymerase (Takara, Heidelberg, Germany) in a final volume of 50 μ l. PCR reactions (35 cycles) were carried out at 95 °C for 1 min, 53 °C for 1 min and 72 °C for 2 min. A 570 bp product (insulin receptor cDNA) and a 487 bp product (competitor DNA) were expected; the PCR products

were analysed by electrophoresis on a 2% agarose gel and their identities were checked by sequencing.

In situ hybridization

Liver tissue of normal fed rats was embedded in paraffin wax, 5 μ m sections were prepared and paraffin wax was removed from the liver sections with xylene. The sections were then rehydrated with descending concentrations of ethanol and finally equilibrated in PBS. They were subsequently permeabilized in 0.3% Triton X-100, 10 μ g/ml proteinase K and 4 mg/ml glucosidase for 15 min at 37 °C, and finally incubated in 0.1 M triethanolamine (pH 8.0) containing 0.25% acetic acid anhydride for 10 min. For hybridization, digoxigenin (DIG)-labelled antisense RNA transcripts served as hybridization probes. The cDNA of the insulin receptor (537 bp; nt 1171–1707) was cloned into pBS and transcribed into antisense RNA by using T3 RNA polymerase and DIG RNA labelling mixture (Roche). Cytosolic phosphoenolpyruvate carboxykinase 1 (PCK1) antisense RNA was synthesized from pBS-PCK containing a 1200 bp *Pst*I cDNA fragment [20] by using T3 RNA polymerase and DIG RNA labelling mixture. The DIG-labelled antisense insulin receptor RNA and PCK1 antisense RNA were hydrolysed to lengths of 200–300 bp in the presence of sodium carbonate buffer (pH 10.2). Hybridization was performed overnight with 25 ng of RNA probes at 42 °C in 100 μ l of hybridization solution, containing 50% (v/v) formamide, 10% (w/v) dextran sulphate, 1 $\times$ Denhardt's solution (0.02% BSA, 0.02% polyvinylpyrrolidone and 0.02% Ficoll) and 0.5 mg/ml *Escherichia coli* DNA in $4 \times$ SSC (where $1 \times$ SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0). After hybridization, sections were washed in $4 \times$ SSC and non-hybridized RNA was digested by incubation with RNaseA (10 μ g/ml) for 30 min. Bound DIG-labelled hybrids were detected by enzyme-linked immunoassay using an anti-DIG alkaline phosphatase conjugate, and visualized via a colour reaction with Nitroblue Tetrazolium and 5-bromo-4-chloroindol-3-yl phosphate as substrates (Roche).

Western-blot analysis

Cultured primary hepatocytes in 60 mm diameter dishes were washed with 0.9% NaCl and then frozen for 10 min at -20 °C. The cells were scraped in 1 ml of boiled SDS buffer [10 mM Tris/HCl (pH 7.5)/0.1% SDS], boiled for 5 min, homogenized through a 25 G needle and centrifuged at 15800 g for 5 min. Protein content in the supernatant was determined using the Bradford method. Protein samples (30–50 μ g), dissolved in 27 μ l of SDS sample buffer [16], were loaded on to an SDS-10% polyacrylamide gel and after electrophoresis blotted on to nitrocellulose membranes. Non-specific binding sites were blocked with blocking buffer [10 mM Tris/HCl (pH 7.5), 100 mM NaCl, 0.1% Tween 20 and 10% (w/v) milk powder]. Blots were incubated with the primary polyclonal rabbit antibody against the β -chain of the human insulin receptor (Affiniti, Exeter, Devon, U.K.) at a 1:250 dilution in blocking buffer overnight at 4 °C. Washing was performed with blocking buffer without the milk powder. The secondary antibody was an anti-rabbit IgG from goat (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and was used at a 1:2000 dilution for 1 h. After washing for 30 min the enhanced chemiluminescence (ECL) Western-blotting system (Amersham, Braunschweig, Germany) was used for detection. Under these conditions, the insulin receptor β -chain was visible as two bands, of 95 kDa and 69 kDa, caused by different glycosylation status.

Immunohistochemistry

Liver tissue of normal fed rats was embedded in paraffin wax, 5 μm sections were prepared and paraffin wax was removed from liver sections with xylene. The sections were then rehydrated with descending ethanol concentrations and finally equilibrated for 10 min in TBS [50 mM Tris/HCl (pH 7.5)/150 mM NaCl]. For detection of insulin receptor protein after rehydration, the sections were washed three times with H_2O and boiled for 2.5 min in citrate buffer (1.8 mM mono-citrate and 8.2 mM trisodium-citrate, pH 6) at 360 W in a microwave oven. The sections were subsequently treated with 100 μl of pepsin [0.4% (w/v) pepsin in 0.01 M HCl] for 5 min at 37 °C and were washed again in TBS for 10 min. To prevent non-specific binding of the primary antibody, the sections were blocked with 20% (v/v) FCS in TBS for 30 min at 37 °C. The diluted primary antibody was applied in 0.1% BSA in TBS overnight at 37 °C. The following were used as primary antibodies: insulin receptor β -chain antibody (Affiniti) at 1:100 dilution and glutamine synthetase (GS) antibody (Affiniti) at 1:100 dilution were used as perivenous markers [17,18] and EAG15 polyclonal antibody at 1:100 dilution, raised against the C-terminus of the rat canalicular multidrug resistance protein (cMRP), was used as a membrane marker [19]. The peroxidase-conjugated secondary antibodies were used at 1:100 dilution in 0.1% BSA in TBS for 30 min at 37 °C. After washing, the sections were incubated with a 1:10 dilution of diaminobenzidine (Pierce, Rockford, IL, U.S.A.) in 1 $\times$ peroxidase buffer (Pierce) for 10 min. The reaction was stopped in TBS and the sections were covered with DePex (Serva, Heidelberg, Germany) and photographed.

RESULTS

The possible zoned insulin receptor gene expression in rat liver was studied by *in situ* hybridization and immunohistochemistry. Primary rat hepatocyte cultures were used to study the influence of the substrates glucose and oxygen as possible determinants for the zoned gene expression.

Homogeneous distribution of insulin receptor mRNA in rat liver

In situ hybridization with paraffin-embedded sections from rat liver showed that insulin receptor mRNA was equally distributed over the liver acinus (Figure 1). In parallel sections, PCK1 mRNA, which served as a periportal marker [20], was localized in the periportal area (Figure 1). GS mRNA, which is a perivenous marker [17,18], was found to be expressed in the distal perivenous area only (results not shown). Since there was no zonation of insulin receptor mRNA expression in rat liver, the regulation of a possible zonation of the insulin receptor might occur at the post-transcriptional level.

Perivenous zonation of insulin receptor protein in rat liver

The distribution of the insulin receptor protein was studied by immunohistochemistry using sections from paraffin-embedded rat liver, which were incubated with an antibody against the β -subunit of the insulin receptor. The insulin receptor protein was predominantly localized in the perivenous zone (Figure 2A), mainly in the plasma membranes (Figure 2B). In parallel sections, GS protein was detected in the distal perivenous area (Figure 2A). In contrast to the GS protein, which was expressed only in the last two cell layers around the central vein (distal perivenous zone), the insulin receptor protein

Figure 1 Homogenous distribution of insulin receptor mRNA in rat liver

Parallel sections (5 μm) were prepared at the end of the feeding period from livers of rats kept under a normal daily feeding cycle. mRNAs were localized by *in situ* hybridization using DIG-labelled insulin receptor (InsR) and PCK1 antisense RNA probes, and an alkaline phosphatase-conjugated antibody against DIG (see the Materials and methods section). Dark precipitates indicate high levels of InsR mRNA throughout the tissue, and of PCK1 mRNA in the periportal area. pp, periportal; pv, perivenous. Bar, 200 μm .

Figure 2 Perivenous zonation of insulin receptor protein in rat liver

(A) Localization of insulin receptor protein in the tissue. Parallel sections (5 μm) were prepared at the end of the feeding period from livers of rats kept under a normal daily feeding cycle. Proteins were localized by immunohistochemistry using a polyclonal rabbit antibody raised against the β -chain of the insulin receptor (InsR) or a mouse antibody raised against sheep GS, as primary antibodies, and a peroxidase-conjugated secondary antibody against rabbit or mouse IgG (see the Materials and methods section). Dark precipitates indicate high levels of InsR protein and GS protein. pp, periportal; pv, perivenous. Bar, 200 μm . (B) Localization of insulin receptor protein on the basolateral cell membrane. Immunohistochemistry was performed as described in (A). A polyclonal antibody against the C-terminus of the rat cMRP was used as a membrane marker [19]. Bar, 20 μm .

was found in all zones, with a predominance in the intermediate and the distal perivenous zones. These results were identical with the results obtained with an antibody against the α -subunit of the insulin receptor (results not shown). As a positive control for the presence of the insulin receptor protein on the outer cell

Figure 3 Induction of insulin receptor mRNA by glucose under periportal and perivenous pO_2 in rat primary hepatocyte cultures

(A) Cells were cultured for 4 h under arterial pO_2 (16% O_2) at the basal glucose levels (5 mM) of the culture medium M 199. Glucose was then added to the final indicated concentrations and the cells were cultured under arterial or venous pO_2 (8% O_2) for a further 24 h. Cells were harvested and total RNA was prepared. Total RNA (2 μ g) was used to generate cDNA by reverse transcription, which was used with different concentrations of competitor DNA (1.75–1750 fg), sense primer and antisense primer in competitive PCR (see the Materials and methods section). In each experiment with basal glucose under arterial pO_2 the insulin receptor (InsR) mRNA levels were set to 100% when equimolar amounts of InsR cDNA and competitor DNA were present (17.5 fg = 100%). Values are means $\pm$ S.E.M. of three independent culture experiments. Statistics: Student's *t* test for paired values: *significant difference between basal glucose versus high glucose, $P \leq 0.05$. (B) Competitive PCR: PCR products with a size of 570 bp (InsR) and 489 bp (Competitor DNA) were separated in a 2% agarose gel. Glc, glucose; Comp, competitor.

membranes, parallel sections were treated with an antibody against the isoform of the cMRP, which is localized on the canalicular membranes (Figure 2B). Thus the insulin receptor protein was predominantly expressed in the perivenous area, in which the insulin-activated pathways of glycolysis and glycogen synthesis have been localized previously [7,11].

Induction of insulin receptor mRNA by glucose in primary hepatocyte cultures

To study the influence of the substrates oxygen, as a key regulator of the zoned gene expression, and glucose, hepatocyte cultures were treated with increasing glucose levels and incubated under arterial or venous pO_2 . In 4-h rat hepatocyte cultures the expression of insulin receptor mRNA was induced within 24 h by increasing glucose concentrations from 5 mM up to 50 mM to the same extent under periportal pO_2 and perivenous pO_2 (Figure 3). Glucose (25 mM) induced insulin receptor mRNA

Figure 4 Induction of insulin receptor protein by perivenous pO_2 at basal glucose levels and by high glucose concentrations under periportal and perivenous pO_2 in rat primary hepatocyte cultures

(A) Cells were cultured for 4 h under arterial pO_2 (16% O_2) at the basal glucose levels (5 mM) of the culture medium M199. The cells were cultured under arterial or venous pO_2 (8% O_2) for a further 24 h with the glucose concentration being raised to 25 mM, when indicated. Total protein was analysed by Western blotting using a polyclonal rabbit antibody raised against the β -chain of the insulin receptor (InsR) as primary antibody and an anti-rabbit IgG as secondary antibody (see the Materials and methods section); the immune complex was detected by ECL. Blots were quantified with a videodensitometer. Values are means $\pm$ S.E.M. of three independent experiments. Statistics: Student's *t* test for paired values: *significant differences between 16% O_2 basal glucose versus 8% basal glucose; 16% O_2 basal glucose versus 16% high glucose; 16% O_2 basal glucose versus 8% high glucose, $P \leq 0.05$. (B) Representative Western-blot; 40 μ g of total protein was analysed. Glc, glucose.

by approx. 5-fold and with 50 mM glucose it was induced by approx. 5.2-fold. The increase in insulin receptor mRNA by 25 mM glucose was linear with time over 24 h (results not shown) and this concentration was chosen for further experiments. As a control, the induction by glucose of pyruvate kinase L (L-PK) was studied [21]. Glucose (25 mM) increased L-PK mRNA by 2-fold within 24 h (results not shown). Thus since the difference in glucose concentration from periportal to perivenous is rather shallow, the increase in glucose concentrations, as occurs in the portal vein after a meal, enhanced insulin receptor mRNA to approx. the same levels in hepatocyte cultures under periportal and perivenous pO_2 . This is in accord with the homogenous expression of insulin receptor mRNA in rat liver (Figure 1).

Induction of insulin receptor protein by perivenous pO_2 at basal glucose levels and by high glucose concentrations under periportal and perivenous pO_2 in primary hepatocyte cultures

In 4-h rat hepatocyte cultures insulin receptor protein was induced at basal 5 mM glucose within 24 h by perivenous pO_2 , and more strongly by 25 mM glucose under both periportal and perivenous pO_2 (Figure 4). Perivenous pO_2 induced insulin

receptor protein approx. 2-fold, 25 mM glucose enhanced insulin receptor protein expression approx. 3-fold under periportal pO_2 and approx. 4-fold under perivenous pO_2 (Figure 4). Thus the zonation of insulin receptor protein appeared to be regulated mainly at the post-transcriptional level by O_2 (Figure 2).

DISCUSSION

Zonation of insulin receptor gene expression

According to the model of 'metabolic zonation' insulin-stimulated glycolysis and glycogen synthesis occur mainly in the less aerobic perivenous zone of the liver acinus [7,10–12]. Therefore it was expected that the insulin receptor would be expressed in the perivenous area. It was found that the insulin receptor mRNA was distributed homogeneously and that the insulin receptor protein was predominantly localized in the perivenous zone (Figures 1 and 2). The equal expression of insulin receptor mRNA and the mainly perivenous localization of insulin receptor protein corresponds to the homogenous distribution of the insulin-inducible glucokinase mRNA [22], and the preferentially perivenous occurrence of glucokinase protein [23] in the liver of normal fed rats [24]. However, the glucagon receptor mRNA [14] and glucagon-inducible PCK1 mRNA and protein [25] were located in the more aerobic periportal zone of the liver acinus, where gluconeogenesis and glucagon-stimulated glycogenolysis mainly occur [7,11,12]. Thus the perivenous expression of the insulin receptor protein is in good agreement with the model of metabolic zonation.

Modulation of insulin receptor gene expression by oxygen and glucose

The periportal to perivenous pO_2 gradient was proposed to be a key regulator for the zonation of gene expression [7,10–12]. In rat primary hepatocyte cultures the glucose-inducible insulin receptor mRNA expression was not modulated by O_2 . This pO_2 -independent induction might explain the equal expression of the insulin receptor mRNA, and implies that the regulation of the zonation of insulin receptor protein might occur mainly at the post-transcriptional level. Indeed, insulin receptor protein expression was induced at basal 5 mM glucose levels by venous pO_2 (Figure 4). Insulin receptor protein was also induced by high glucose concentrations; but then the modulation by O_2 became less dominant (Figure 4). Thus the insulin receptor might be an example of a role for O_2 to modulate zonation of gene expression at the post-transcriptional level. Conversely, the modulatory role of O_2 in the zonation of gene expression was shown to occur at the transcriptional level in rat primary hepatocytes with the gluconeogenic key enzyme, PCK1, and the glucose-utilizing enzyme, glucokinase; glucagon activated the transcription of the PCK1 gene maximally under periportal pO_2 [26,27] and, reciprocally, insulin activated the transcription of the glucokinase gene maximally under perivenous pO_2 [28].

Glucose is known to induce the expression of the glycolytic enzyme gene L-PK in hepatocytes and the lipogenic enzyme genes, acetyl-CoA carboxylase and fatty acid synthase, in liver and adipose tissue [29–31]. Insulin and consequently the insulin receptor are functionally linked to glucose uptake. Therefore one would have expected an inductive effect of glucose, which was indeed the case (Figures 3 and 4). The mechanism by which glucose induced insulin receptor mRNA is not completely understood. A post-transcriptional mechanism such as stabilization of insulin receptor mRNA may be responsible in part for the glucose-induced insulin receptor mRNA increase. This is

indicated by a previous study with mouse embryo fibroblasts transformed with a vector containing the coding region of the human insulin receptor [31]. In these transformed cells, 25 mM glucose increased insulin receptor mRNA levels, the amount of insulin receptor protein and tyrosine kinase activity after 24–48 h. These alterations were associated with an increased half-life of insulin receptor mRNA in the presence of 25 mM glucose [32].

The positive modulation by perivenous pO_2 of the insulin receptor protein would confirm the predominant perivenous localization of the insulin receptor, which is in good agreement with the model of metabolic zonation, and is further evidence that the periportal to perivenous pO_2 gradient appears to be a major determinant in the zonation of gene expression.

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