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Periportal localization of glucagon receptor mRNA in rat liver and regulation of its expression by glucose and oxygen in hepatocyte cultures

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Abstract Glucagon is the major hormone activating glycogenolysis and gluconeogenesis both localized in the periportal, more aerobic zone of the liver. Accordingly, the glucagon receptor (GcgR) mRNA was found to be predominantly expressed in this area. In hepatocyte cultures high glucose concentrations as reached after a meal induced GcgR mRNA under arterial but not venous pO_2. The induction by glucose was partially antagonized by insulin and unaffected by glucagon. The modulation by O_2 of the glucose-dependent induction would contribute to the zonated expression of GcgR mRNA.

Key words: Glucagon receptor; Oxygen; Glucose; Metabolic zonation

1. Introduction

Glucagon, a 29 amino acid peptide, activates hepatic glucose production by glycogenolysis and gluconeogenesis. The rat [1], mouse [2] and human [3] glucagon receptor genes have been cloned. They encode a protein of 485 amino acids with a predicted molecular size of 55 kDa [1]. The glucagon receptor is closely related to the receptors for glucagon-like peptide-1, gastric inhibitory polypeptide, secretin and vasoactive intestinal polypeptide [1,4]. It belongs to the superfamily of G-protein-coupled receptors having seven putative transmembrane domains and activates adenylate cyclase [1].

The major target organ for glucagon 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 [5–8]. Accordingly, glucose release from glycogenolysis and gluconeogenesis takes place preferentially in the periportal area; conversely, glucose uptake for glycogen synthesis and glycolysis occurs mainly in the perivenous area. Due to metabolism and elimination, concentration gradients of substrates such as oxygen and hormones are formed during a single passage of blood through the liver [6,8]: For oxygen, glucagon and insulin a 2-fold periportal > perivenous gradient is established; for insulin the gradient is reduced to 1.2-fold during meals. Thus, during the absorptive phase the ratio of insulin versus its antagonist glucagon increases from the periportal to the perivenous zone. The gradients in oxygen, glucagon and insulin were considered to be key regulators for the zonal expression of the genes of carbohydrate-metabolizing enzymes [6–8].

Since the glucagon-stimulated processes of glycogenolysis and gluconeogenesis occur predominantly in the periportal zone, the glucagon receptor gene would be expected to be expressed mainly in this more aerobic area. Therefore, it was the aim of this study to investigate the possible zonation of GcgR mRNA in rat liver and to study the regulation of its expression by the substrates glucose and O_2 as well as the hormones glucagon and insulin in primary rat hepatocyte cultures. In line with expectations GcgR mRNA was predominantly localized periportally and could be induced in primary hepatocyte cultures by glucose only under periportal but not perivenous pO_2. Insulin partially antagonized the induction by glucose, but glucagon had no effect. The induction of GcgR mRNA by glucose in the absorptive phase would prepare the cell for the following postabsorptive phase. The glucose-antagonistic action of insulin might function to prevent an overinduction by glucose.

2. Materials and methods

2.1. Chemicals

All chemicals were of reagent grade and purchased from commercial suppliers. Collagenase, digoxigenin-UTP, the digoxigenin nucleic acid detection kit, T3 and T7 RNA polymerase, CSPD (disodium 3-(4-methoxyspiro(1,2-dioxetane-3,2′-(5′chloro) tricyclo[3.3.1.1^{3,7}] decan)-4-yl)phenyl phosphate), DIG RNA labelling mixture and fetal calf serum were from Boehringer (Mannheim), medium M199 from Gibco BRL (Eggenstein). Hormones were delivered from Serva (Heidelberg). Guanidinium thiocyanate was purchased from Fluka (Neu-Ulm). All other chemicals were from Sigma (Taufkirchen). The BLUESCRIPT vector (pBS) was from Stratagene (Heidelberg).

2.2. Cell culture experiments

Liver cells were isolated by collagenase perfusion. Cells (1×10^6 per dish) were maintained under standard conditions in an atmosphere of 16% O_2, 79% N_2, and 5% CO_2 (by vol.) in medium M199 containing 0.5 nM insulin added as a growth factor for culture maintenance, 100 nM dexamethasone required as a permissive hormone and 4% fetal calf serum for the initial 4 h of culture. After 4 h cells were cultured in serum-free M199 under 16% O_2 (mimicking arterial O_2 tensions) or 8% O_2 (mimicking venous O_2 tensions) with additions as described in the legends to figures. The O_2 values take into account the O_2 diffusion gradient from the media surface to the cells [9].

2.3. RNA preparation and Northern analysis

Total RNA was prepared from 3×10^6 cells as described [10]. Forty μg RNA were denatured by formaldehyde and used in a typical Northern experiment. Digoxigenin (DIG)-labelled antisense GcgR, PCK1 and β-actin RNA served as hybridization probes. Respective cDNAs of GcgR (1092 bp; nucleotide 247–1338; GenBank: L04796) and β-Act (588 bp; nucleotide 69–618; EMBL: HSAC07) were cloned into pBS and transcribed into antisense RNA by using T7 RNA polymerase and DIG RNA labelling mixture. PCK1 antisense RNA was synthesized from pBS-PCK containing a 1200 bp *Pst*I cDNA fragment [11] by using T3 RNA polymerase and DIG RNA labelling

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Abbreviations: β-Act, β-actin; DIG, digoxigenin; Gcg, glucagon; GcgR, glucagon receptor; Glc, glucose; Ins, insulin; PCK1, cytosolic phosphoenolpyruvate carboxykinase

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mixture. Hybridizations were carried out with 50 ng/ml transcript at 65°C according to the DIG nucleic acid detection kit (Boehringer Mannheim). Detection of hybrids was performed by enzyme-linked immunoassay using an anti-digoxigenin alkaline phosphatase conjugate. Hybrids were visualized via chemiluminescence with CSPD. Luminescent blots were exposed to Hyperfilm-MP (Amersham, Braunschweig) and quantified with a videodensitometer (Biotech Fischer, Reiskirchen).

2.4. In situ hybridization

Liver tissue of normal fed rats was embedded in paraffin. Then 5 μm sections were prepared. Paraffin was removed from the liver sections by xylene. The sections were then rehydrated with descending ethanol concentrations and finally equilibrated in PBS. They were then permeabilized in 0.3% Triton X-100, 10 μg/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-labelled RNA transcripts were hydrolysed to a length of 200–300 bp in the presence of Na_2CO_3 buffer, pH 10.2. Hybridization was performed overnight with 25 ng RNA probes at 42°C (PCK1) or 45°C (GcgR) in 100 μl hybridization solution containing 50% formamide, 10% dextrane sulfate, 1× Denhardts (0.02% bovine serum albumin, 0.02% Polyvinilpyrrolidon, 0.02% Ficoll) and 0.5 mg/ml each *E. coli* DNA in 4× SSC (sodium chloride-sodium citrate: 0.6 M NaCl, 0.06 M sodium citrate, pH 7.0). After hybridization, sections were washed in 4× SSC and unhybridized RNA was digested by incubation with RNase A (10 μg/ml) for 30 min. Bound digoxigenin-labelled hybrids were detected by enzyme-linked immunoassay using an anti-digoxigenin alkaline phosphatase conjugate and visualized via color reaction with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates (Boehringer Mannheim).

3. Results

The possible zonated expression of GcgR mRNA in the liver of normal fed rats was studied by in situ hybridization. Primary rat hepatocyte cultures were used to study the influence of the substrates glucose, oxygen and the hormones insulin and glucagon as possible determinants for the zonated gene expression.

3.1. Periportal localization of glucagon receptor mRNA in rat liver

The GcgR mRNA abundance in livers of fed and 24 h fasted rats as revealed by Northern analysis was not significantly different (not shown). In situ hybridization with paraffin-embedded sections from livers of normal fed rats showed that GcgR mRNA was localized predominantly in the periportal zone of the liver acinus (Fig. 1). In parallel sections PCK1 mRNA, which served as a periportal marker [11], could be localized also in the same periportal area as GcgR mRNA (Fig. 1) and glutamine synthetase mRNA, which is a perivenous marker [12,13], was found to be expressed in the distal perivenous area only (not shown).

Thus, GcgR mRNA was predominantly expressed in the periportal area, in which the glucagon-activated pathways of glycogenolysis and gluconeogenesis had been localized previously [6,8,14].

3.2. Induction of glucagon receptor mRNA by glucose under periportal but not perivenous pO_2 in primary hepatocyte cultures

In 4 h rat hepatocyte cultures the expression of GcgR mRNA was induced within 24 h by increasing glucose concentrations from 5 mM up to 50 mM only under periportal pO_2. There was no induction by glucose of GcgR mRNA under perivenous pO_2 (Fig. 2). Twenty-five mM glucose induced GcgR mRNA by about 2.3-fold and 50 mM by about 3.5-fold. The increase of GcgR mRNA by 25 mM glucose was essentially linear with time within 24 h (Fig. 3). A glucose concentration of 25 mM was then chosen for further experiments. The same results were obtained (not shown), when cells were cultured for 24 h and then induced for the following 24 h with the same glucose concentrations and under the same oxygen partial pressures. As a control the induction by glucose of pyruvate kinase L was studied [15]. 25 mM glucose increased pyruvate kinase L mRNA by 2-fold within 24 h (not shown).

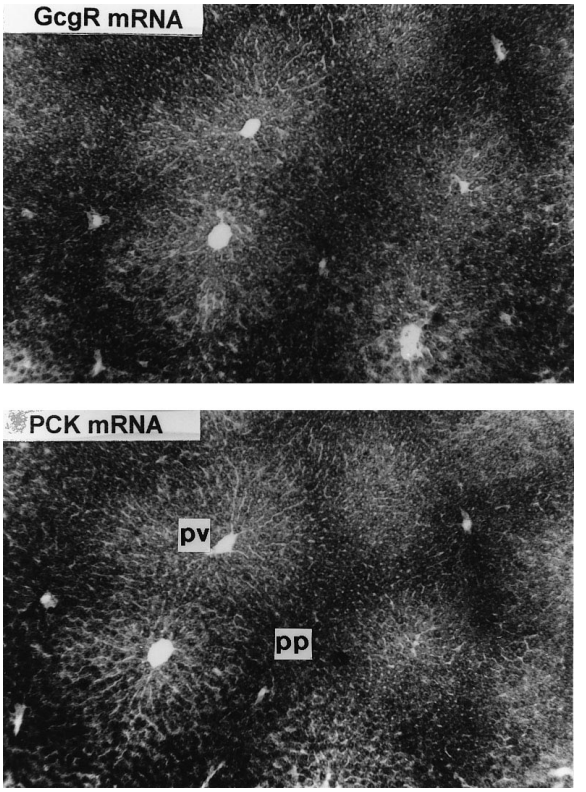

Fig. 1. Periportal zonation of glucagon receptor mRNA in rat liver. Five μm parallel sections were prepared from livers of rats kept under a normal daily feeding cycle. mRNAs were localized by in situ hybridization using a DIG-labelled GcgR and PCK1 antisense RNA probe (cf. Section 2); dark precipitates indicate high levels of GcgR mRNA and PCK mRNA, respectively, in the periportal area. pp, periportal; pv, perivenous.

Thus, increasing glucose concentrations as reached in the portal vein after a meal enhanced GcgR mRNA in hepatocyte cultures only under periportal pO_2 in accord with the periportal expression of GcgR mRNA in rat liver (Fig. 1).

3.3. Modulation by insulin and glucagon under periportal pO_2 of the glucose-dependent induction of GcgR mRNA in primary hepatocyte cultures

In another series of experiments with primary rat hepatocyte cultures the influence of insulin and glucagon on GcgR mRNA expression was investigated. Twenty-five mM glucose induced GcgR mRNA maximally to about 350% within 24 h. Addition of 100 nM insulin reduced the glucose-dependent induction of GcgR mRNA to about 200%, while the presence of 10 nM glucagon was without effect. However, 10 nM glu-

cagon given in combination with 100 nM insulin abolished the impairment by insulin of the glucose-dependent induction of GcgR mRNA (Fig. 4).

Thus, insulin partially inhibited the glucose-dependent induction of GcgR mRNA and glucagon as the major insulin antagonist counteracted the insulin action.

4. Discussion

The mRNA of the GcgR was predominantly expressed in the periportal more oxygenated zone of the rat liver acinus (Fig. 1). In rat hepatocyte cultures high absorptive glucose concentrations increased the GcgR mRNA levels only under high periportal pO_2 but not under perivenous pO_2. This pO_2-dependent induction might explain the periportal expression of the GcgR gene.

4.1. Zonation of glucagon receptor gene expression

According to the model of 'metabolic zonation' the glucagon-stimulated glycogenolysis and gluconeogenesis occur mainly in the more aerobic periportal zone of the liver acinus [6–8]. Thus, the mainly periportal expression of GcgR mRNA was in line with expectations. However, in a previous examination using antibodies against partially purified rat liver GcgR, which recognized surface structures on liver, kidney and adipocyte but not lung or erythrocyte membranes and which did not inhibit the action of glucagon, the receptor protein was localized mainly in the perivenous zone [16]; yet, in another study the same authors reported the periportal localization of the GcgR protein [17]. If the antibodies were specific, it is difficult to explain the apparently conflicting results.

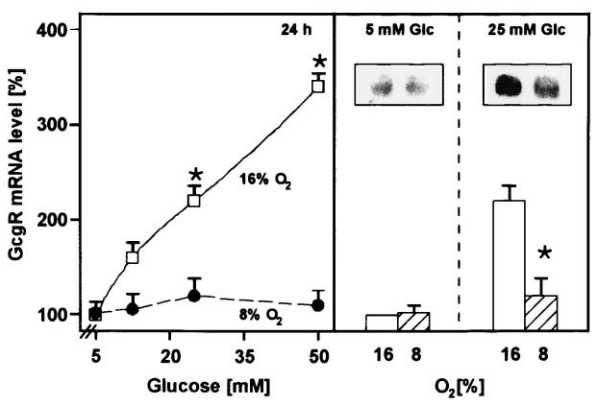

Fig. 2. Induction of glucagon receptor mRNA by glucose under periportal but not perivenous pO_2 in primary rat hepatocyte cultures. Cells were cultured for 4 h under arterial pO_2 (16% O_2) and basal glucose (5 mM). The basal 5 mM glucose concentration is that of the culture medium M199. Then glucose was added to the final indicated concentrations and the cells were further cultured under arterial and venous pO_2 (8% O_2) for another 24 h. In each experiment the mRNA level with basal glucose under arterial pO_2 measured by Northern blotting was set to 100%. Northern blots with 40 μg of total RNA from cultured rat hepatocytes were hybridized to a DIG-labelled GcgR and β-actin antisense RNA probe (cf. Section 2). Chemiluminescent signals were quantified by videodensitometry. Values are means ± S.E.M. of three independent culture experiments. Statistics: Student's *t*-test for paired values: $^*P \leq 0.05$.

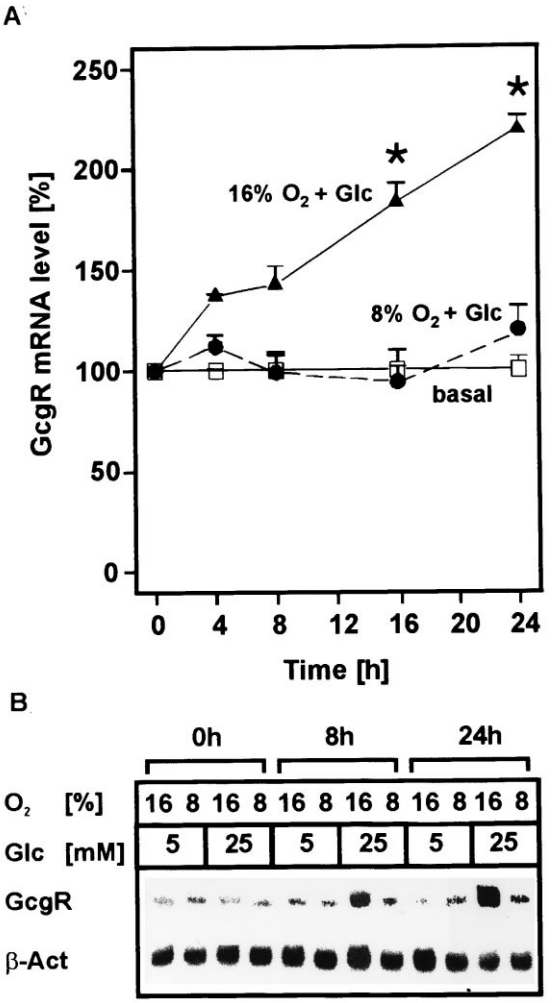

Fig. 3. Time course of the induction by glucose of glucagon receptor mRNA under periportal pO_2 in primary rat hepatocyte cultures. A: Cells were cultured as described in Fig. 2 with the glucose concentration raised from 5 to 25 mM. mRNA was analyzed by Northern blotting as in Fig. 2. In each experiment the basal mRNA level with 5 mM Glc under arterial pO_2 at each time point was set to 100%. Values are means ± S.E.M. of three independent culture experiments. Statistics: Student's *t*-test for paired values: $^*P \leq 0.05$. B: Representative Northern blot; 40 μg total RNA was analyzed.

4.2. Modulation of the zonated glucagon receptor gene expression by the substrates O_2 and glucose

4.2.1. Modulation by O_2 of zonated gene expression. The periportal to perivenous pO_2 gradient was proposed to be a key regulator for the zonation of gene expression [6–8]. In primary rat hepatocyte cultures glucose induced the GcgR mRNA to maximal levels under periportal pO_2 but not under perivenous pO_2 (Figs. 2 and 3). Thus, during the postabsorptive phase at normal 5 mM glucose GcgR mRNA would be expressed homogeneously to basal levels in periportal and perivenous cells, but during the absorptive phase at postprandial >10 mM glucose in the portal vein GcgR mRNA expression would be enhanced in the periportal O_2-rich zone only, so that on average a periportal > perivenous zonation of GcgR mRNA results (Fig. 1). The modulatory role of O_2 is in line with the previous observations that glucagon activated the gluconeogenic key enzyme gene PCK1 maximally under periportal pO_2 [10,18] and, reciprocally, that insulin activated

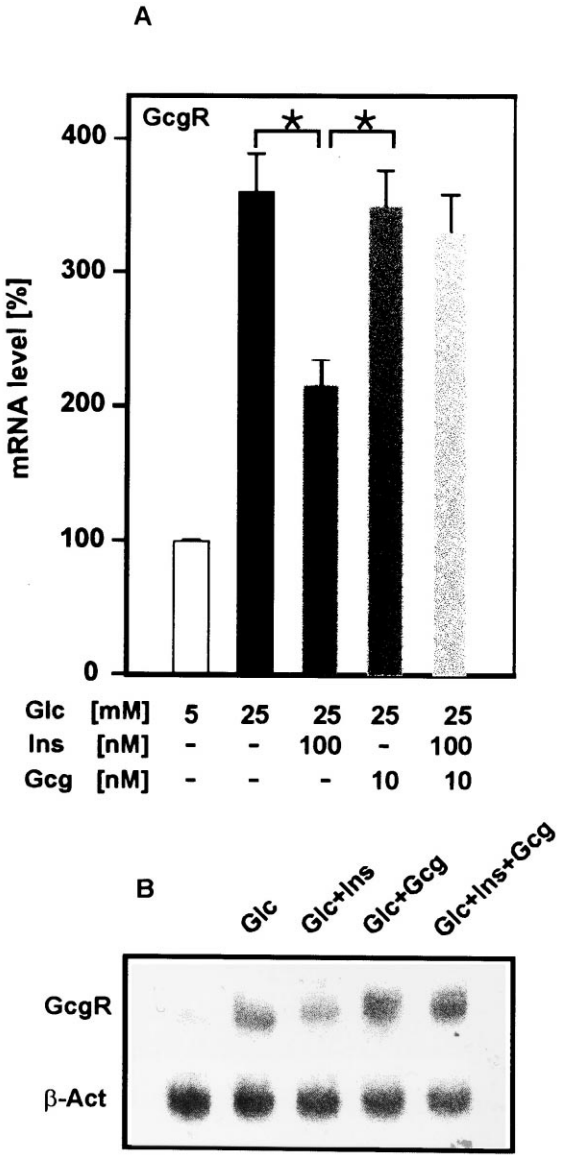

Fig. 4. Induction of glucagon receptor mRNA by glucose under periportal pO_2 in primary rat hepatocyte cultures. Modulation of insulin and glucagon. A: Cells were cultured as described in Fig. 2 with elevation of the glucose concentration from 5 to 25 mM and the addition of 100 nM insulin or 10 nM glucagon, when indicated. An additional 10 nM glucagon was added at 20 h. mRNA was quantified by Northern blotting as in Fig. 2. In each experiment the mRNA level with basal 5 mM Glc under arterial pO_2 was set to 100%. Values are means ± S.E.M. of three independent culture experiments. Statistics: Student's *t*-test for paired values: $*P \leq 0.05$. B: Representative Northern blot; 40 μg total RNA was analyzed.

the glycolytic key enzyme gene glucokinase maximally under perivenous pO_2 [19].

The modulation by O_2 of the induction of the PCK1 and glucokinase genes appears to be mediated by H_2O_2 as the intracellular mediator [18,19], which could be formed by a CO-sensitive heme-containing oxidase as the oxygen sensor [20,21].

Thus, the positive modulation by O_2 of the glucose-dependent GcgR mRNA induction and the glucagon-dependent PCK1 gene activation would explain the predominant periportal localization of GcgR mRNA and PCK mRNA in rat liver.

4.2.2. Modulation by glucose of zonated gene expression.

Glucose plays an important role in the regulation of gene expression in mammals [22–24]. It is known to induce the expression of the glycolytic enzyme gene pyruvate kinase L in hepatocytes and the lipogenic enzyme genes acetyl-CoA carboxylase and fatty acid synthase in liver and adipose tissue. In primary hepatocyte cultures glucose induced the GcgR mRNA (Figs. 2 and 3). Since glucagon and consequently the GcgR are functionally linked to glucose release rather than uptake, one would have expected a repressive rather than an inductive effect of the 'product' glucose. However, a similar paradoxical effect of glucose was found previously: (1) An about 2-fold induction of GcgR mRNA in the presence of 22.5 mM glucose was reported in cultured rat pancreatic islets within 48 h [25] and rat hepatocytes within 24 h [26]. (2) Also, an about 2-fold increase in luciferase activity by 25 mM glucose was observed in the insulinoma cell line INS-1 within 45 h after transfection with a construct driven by the gene promoter of the glucose releasing, periportally localized glucose-6-phosphatase [27]. In none of these studies were the time dependences nor the O_2 tensions, under which the cells were cultured, reported so that a comparison of the effectiveness of the gene activation by glucose between the previous studies and this work is not possible.

Glucose-dependent gene induction does not appear to be mediated by a glucose sensor; it is believed to be mediated through intermediates of glycolysis, glycogen synthesis or the pentose phosphate pathway. It was suggested that glucose induced gene expression through glucose-6-phosphate [24] or metabolites of the nonoxidative branch of the pentose phosphate pathway, perhaps xylulose-5-phosphate [15,23].

A proposal for the physiological importance of the glucose-dependent induction of the 'glucogenic' GcgR and glucose-6-phosphatase genes has not been made [25–27]. Maybe, the induction by glucose of GcgR mRNA in the absorptive phase would prepare the cells for the following postabsorptive phase.

4.3. Modulation of the zonated glucagon receptor gene expression by the hormones insulin and glucagon

In liver and adipose tissue insulin increases the glucose-dependent expression of glycolytic and lipogenic enzyme genes indirectly by stimulating glucose uptake via an increase in glucokinase or in plasma membrane glucose transporter-4 levels, respectively [23,24,28,29], while glucagon enhances the expression of gluconeogenic enzyme genes directly [29]. In primary rat hepatocyte cultures insulin inhibited the glucose-dependent induction of GcgR mRNA, glucagon alone had no effect but was antagonistic to insulin (Fig. 4). Since insulin is functionally linked to glucose uptake and glucagon as well as the GcgR to glucose release, one might have expected a repressive action of insulin on GcgR expression. However, the observed antagonism between insulin and glucose is surprising; yet, as outlined above, the underlying paradox is the inductive action of glucose.

In the previous study with cultured rat hepatocytes [26] the effects of insulin, glucagon or both together on the induction of GcgR mRNA by high glucose levels have not been investigated. Only the action of glucagon alone via cAMP, and only at basal glucose concentrations, was studied and found to be inhibitory. Thus, a comparison with the present work is again not possible.

Since in the absorptive phase insulin is degraded to a much lesser extent than glucagon during a single passage of blood through the liver, the insulin/glucagon ratio is higher in the perivenous area [6–8]. Thus, the repression of the glucose-dependent induction by higher insulin/glucagon ratios could contribute to the lower levels of GcgR mRNA expression in the perivenous zone. Moreover, it might function to prevent an overinduction by glucose.

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