ELSEVIER

Biochemical Pharmacology 64 (2002) 903–911

Biochemical Pharmacology

Signaling cross-talk between hypoxia and glucose via hypoxia-inducible factor 1 and glucose response elements

Thomas Kietzmann*, Anja Krones-Herzig, Kurt Jungermann

Institut für Biochemie und Molekulare Zellbiologie, Georg-August-Universität, Humboldtallee 23, D-37073 Göttingen, Germany

Received 2 February 2002; accepted 5 March 2002

Abstract

The substrates oxygen and glucose are important for the appropriate regulation of metabolism, angiogenesis, tumorigenesis and embryonic development. The knowledge about an interaction between these two signals is limited. We demonstrated that the regulation of glucagon receptor, insulin receptor and L-type pyruvate kinase (L-PK) gene expression in liver is dependent upon a cross-talk between oxygen and glucose. The periportal to perivenous drop in O_2 tension was proposed to be an endocrine key regulator for the zonated gene expression in liver. In primary rat hepatocyte cultures, the expression of the glucagon receptor and the L-PK mRNA was maximally induced by glucose under arterial pO_2 whereas the insulin receptor was maximally induced under perivenous pO_2. It was demonstrated for the L-PK gene that the modulation by O_2 of the glucose-dependent induction occured at the glucose-responsive element ($Glc_{PK}RE$) in the L-PK gene promoter. The reduction of the glucose-dependent induction of the L-PK gene expression under venous pO_2 appeared to be mediated via an interference between hypoxia-inducible factor 1 (HIF-1) and the glucose-responsive transcription factors at the $Glc_{PK}RE$. The glucose response element (GlcRE) also functioned as a hypoxia response element and, vice versa, a hypoxia-responsive element was functioning as a GlcRE. Thus, our findings implicate that the cross-talk between oxygen and glucose might have a fundamental role in the regulation of several physiological and pathophysiological processes.
© 2002 Elsevier Science Inc. All rights reserved.

Keywords: Pyruvate kinase; Oxygen; HIF-1; Glucose; Metabolic zonation

1. Introduction

Oxygen plays a major role in the energy metabolism of aerobic living organisms. Due to its low redox potential it serves as an electron acceptor in oxidation–reduction reactions which are necessary for the combustion of organic substrates such as glucose to yield energy in form of ATP. Regarding the fact that especially in mammals glucose serves as one of the major energy substrates it is necessary that constant blood glucose levels have to be maintained otherwise severe complications may arise. Besides other functions, the liver serves as the major glucostat of the body, thus being responsible for the maintainance of a constant blood glucose level.

* Corresponding author. Tel.: +49-551-395952; fax: +49-551-395960.
E-mail address: tkietzm@gwdg.de (T. Kietzmann).

Abbreviations: AMPK, adensine monophosphate stimulated protein kinase; ARNT, arylhydrocarbon receptor nuclear translocator; ChREBP, carbohydrate responsive element binding protein; CMV, cytomegalie virus promoter; DIG, digoxigenin; EMSA, electrophoretic mobility shift assay; EPO, erythropoietin; EPAS, endothelial PAS protein; GIcRE, glucose responsive element; HRE, hypoxia responsive element; HIF, hypoxia-inducible factor; HLF, HIF-like factor; HRF, HIF-related factor; MOP, member of PAS; PAS, Per-ARNT-Sim domain; PCK, phosphoenolpyruvate carboxy kinase; PK, pyruvate kinase; USF, upstream stimulatory factor.

2. O_2 as modulator of zonated gene expression in liver

A special feature of liver metabolism is that it takes place in different areas of the liver acinus. Based on the blood supply, the liver acinus represents the smallest functional unit of the liver and extents from the upstream periportal area to the downstream perivenous area [1]. Due to metabolism and elimination, respectively, concentration gradients of substrates such as oxygen and hormones are

0006-2952/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0006-2952(02)01160-7

formed during a single passage of blood through the liver [2–6]. The oxygen tension is about 65 mmHg in the periportal area and falls to about 35 mmHg in the perivenous zone. This oxygen gradient was considered to be a key regulator for the zonal expression of the genes of carbohydrate-metabolizing enzymes [2,6]. The zonal expression, in turn, contributes to a different content of key enzymes and catalytic capacities. This was the basis for the model of metabolic zonation [2–6].

Accordingly, glucose release from glycogenolysis and gluconeogenesis with the key regulatory enzyme phosphoenolpyruvate carboxykinase 1 (PCK1) takes place preferentially in the more oxygenated periportal area; conversely, glucose uptake for glycogen synthesis and glycolysis with the key enzymes glucokinase and pyruvate kinase (L-PK) occurs mainly in the less aerobic perivenous area [7]. Since glucose utilization is coupled to insulin, the insulin receptor and the insulin-stimulated process of glycolysis one would expect that glucose in the absorptive phase and perivenous O_2 tensions may exert a modulatory role in the zonal expression of the insulin receptor and the rate generating enzymes such as glucokinase and L-PK. In contrast, since glucagon, the glucagon receptor and consequently the glucagon-dependent PCK1 are functionally linked to glucose release rather than uptake, one would expect the reciprocal regulation by glucose and periportal pO_2.

The modulatory role of O_2 for the zonation of gene expression was first shown in primary rat hepatocytes with the glucagon-dependent PCK1 gene expression and the insulin-dependent glucokinase expression: glucagon activated the transcription of the PCK1 gene maximally under periportal pO_2 [8–10] and, reciprocally, insulin activated the transcription of the glucokinase gene maximally under perivenous pO_2 [11]. According to this reciprocal regulation it was then additionally described that also the glucagon receptor was predominantly expressed in the periportal area whereas the insulin receptor was expressed perivenous area [12,13].

3. Hypoxia-inducible factor 1 as a central component of the O_2 signallig pathway

The modulation by O_2 appears to be mediated by H_2O_2, which could be formed by a CO-sensitive heme-containing oxidase as the oxygen sensor [9,14,15], an iron-dependent Fenton reaction [16] and specific iron-dependent prolyl hydroxylases [17] as intracellular components of the O_2 signaling pathway. One of the main targets in this pathway is the transcription factor hypoxia-inducible factor 1 (HIF-1) [18,19]. HIF-1 was initially identified as the transcription factor permitting the induction of the erythropoietin (EPO) gene by hypoxia and is now considered to be a main regulator of an increasing number of physiologically important oxygen sensitive genes [19–22] among them the angiogenesis mediator vascular endothelial growth factor, several genes of the glycolytic pathway and the glucose transporter family. HIF-1 is a heterodimer composed of a HIF-1α and a HIF-1β-(ARNT) subunit and binds to the consensus sequence 5′-BACGTSSK-3′ (B = C/G/T, S = C/G, K = G/T) [23] with the core sequence 5′-RCGTG-3′ (R = purine, A, G) [20] which partially constitutes an E-Box sequence 5′-CANNTG-3′ (N = any nucleotide) (Fig. 1). Both HIF-1α [24] and ARNT [25] are members of the basic helix-loop-helix-PAS family of transcription factors. While ARNT is the constitutively expressed protein, HIF-1α is the O_2-sensitive subunit. Meanwhile, two other HIF α-subunits have been cloned from human mouse and rat: HIF-2α (EPAS/HLF/HRF/MOP2) [26–29] and HIF-3α [30–32].

4. Glucose response elements (GlcRE) overlap with hypoxia response elements

A carbohydrate-rich diet and especially glucose is known to induce the expression of the glycolytic enzyme gene pyruvate kinase L and the Spot14 gene in liver as well as the lipogenic enzyme genes acetyl-CoA carboxylase and fatty acid synthase in liver and adipose tissue [33–36] thereby promoting long-term storage of sugars in the form of triglycerides [37,38].

The intracellular mediators of the glucose response have not been completely characterized, although glucose-6-phosphate generated via the insulin-dependent glucokinase in hepatocytes and xylulose-5-phosphate, a metabolite of the pentose phosphate pathway, as well as the AMP-activated protein kinase have been shown to be involved [36] (Fig. 1). The glucose signaling chain ends up with the transcriptional activation of a target gene and it appeared that the glucose-dependent activation of the L-PK gene is mediated via glucose-responsive transcription factors (USF, upstream stimulating factors [39,40] or the newly identified ChREBP) [41] binding to the L4 (−168/−145) element in the L-PK promoter consisting of two imperfect palindromic E-Boxes [34,42,43]. Similar glucose-responsive elements were found also to be responsible for the glucose-dependent induction of the Spot14 gene [44,45], the glucagon receptor gene, the hexokinase II gene [46] and the fatty acid synthetase gene [47].

The HIF-1 binding consensus sequence resembles a partial E-Box sequence and thus it might be that beside the glucose-responsive transcription factors HIF-1 may bind to the glucose-responsive E-Boxes. Within the L-PK gene, this transcription factor cross-talk between the signals oxygen and glucose might contribute to the zonated L-PK expression.

The glycolytic enzyme L-PK catalyzes the formation of pyruvate and ATP from phosphoenolpyruvate and ADP. Although the L-PK is present in the kidney, small intestine, and pancreatic β-cells, it is predominantly expressed in the

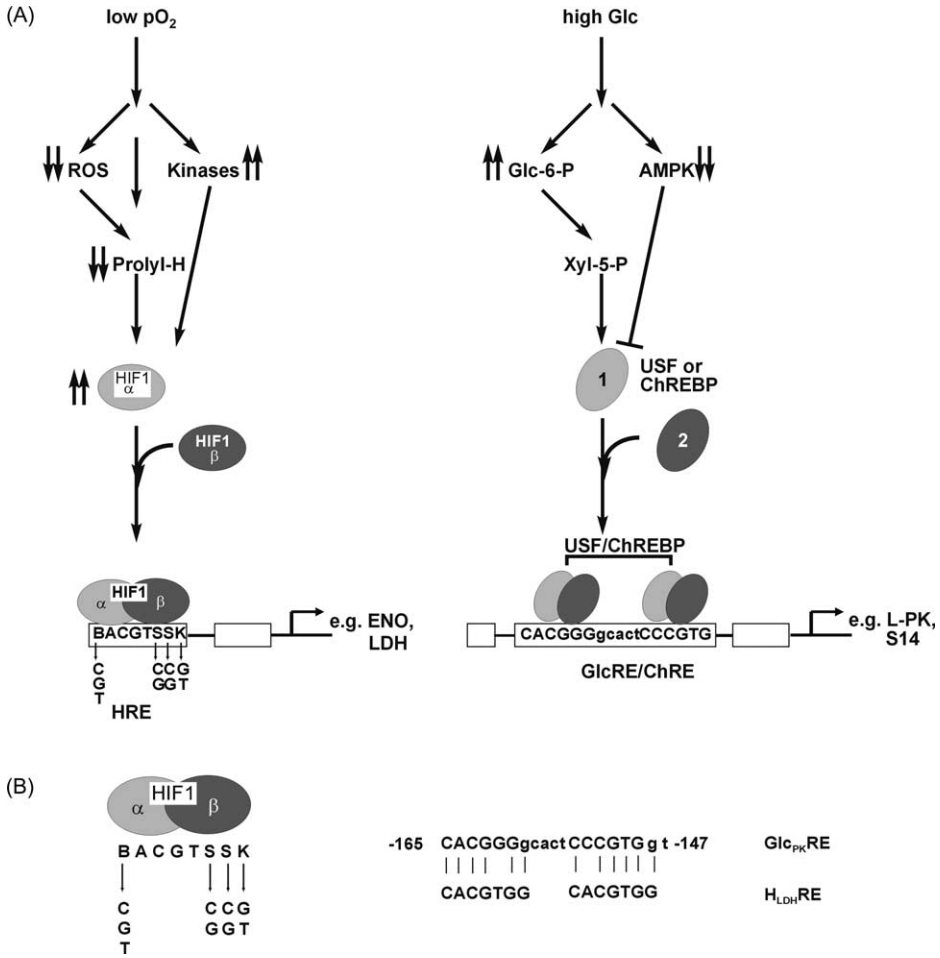

Fig. 1. Role of oxygen and glucose in the regulation of gene expression. (A) *Left*: Low pO_2 values decrease the availability of O_2 as substrate for the iron-dependent prolyl hydroxylases, a mechanism which leads to a stabilization of the α-subunit of the hypoxia-inducible factor 1 (HIF-1α). The decreased O_2 tensions also lower the levels of reactive oxygen species (ROS) within the cells or they may activate kinases either of the MAPK family or the proteinkinase B. Both, lowering of ROS levels and kinase activation, are mechanisms which contribute to the stabilization of HIF-1α. After nuclear translocation, HIF-1α dimerizes with its partner HIF-1β (ARNT) forming the active HIF-1 molecule which in turn binds to hypoxia response elements (HRE) within regulatory gene sequences. This mechanism then leads to the hypoxia-dependent activation of genes such as enolase (ENO) or lactate dehydrogenase (LDH). *Right*: When cultured under high glucose genes may be regulated via signaling molecules such as glucose-6-phosphate (Glc-6-P) generated from glucose by the action of glucokinase. The subsequent steps remain not completely resolved but it was proposed that other glucose metabolites such as xylitol or xylulose-5-phosphate (Xyl-5P) may have a role as signaling molecules. It appeared also that high glucose decreased the activity of the AMP-activated protein kinase (AMPK) which then leads to the activation of an activator such as USF or ChREBP or to the deactivation of a repressor [36]. With the L-type pyruvate gene (L-PK) and Spot14 gene (S14) the glucose-sensitive transcription factors interact with a glucose response element (GlcRE) consisting of two imperfect E-Boxes separated by five nucleotides which then leads to the glucose-dependent activation of these genes. (B) Hypoxia response element. Comparison with $Glc_{PK}RE$. The consensus sequence of the hypoxia response element (HRE) [23] and the model of HIF-1 binding to this sequence. The base B can be C, G or T, the base S can be C or G and the base K can be G or T. Comparison between the HRE of lactate dehydrogenase A promoter (LDH-A) [54] and the GlcRE of L-PK promoter.

liver [48]. Beside L-PK, several isoenzymes which are referred to as R-, M1- and M2-PK, respectively, have been described [49]. The R-PK expression is restricted to erythrocytes and M1-PK is expressed in skeletal muscle, heart and brain. The M2-PK is widely distributed and the only detectable isoenzyme in early fetal tissues [49].

Thus, with regard to the possibility that O_2 via HIF-1 and glucose via USF or ChREBP may target the same DNA-response element in the L-PK gene promoter we investigated the influence of oxygen and glucose on L-PK gene expression in primary cultured rat hepatocytes in more detail.

5. Venous pO_2 enhanced L-PK gene expression

The expression of L-PK mRNA and L-PK protein was investigated in rat hepatocytes cultured in the presence of 5 mM glucose and under venous (8%) pO_2 and arterial (16%) pO_2. L-PK mRNA was induced within 24 hr by about 4-fold under venous (8%) pO_2 compared to arterial (16%) pO_2. The increase of L-PK mRNA by venous pO_2 was relatively fast and already maximal after 4 hr incubation under venous pO_2. The L-PK protein levels were also enhanced by about 3-fold under venous pO_2 (Fig. 2) [50]. Thus, the results indicated that physiologically occuring

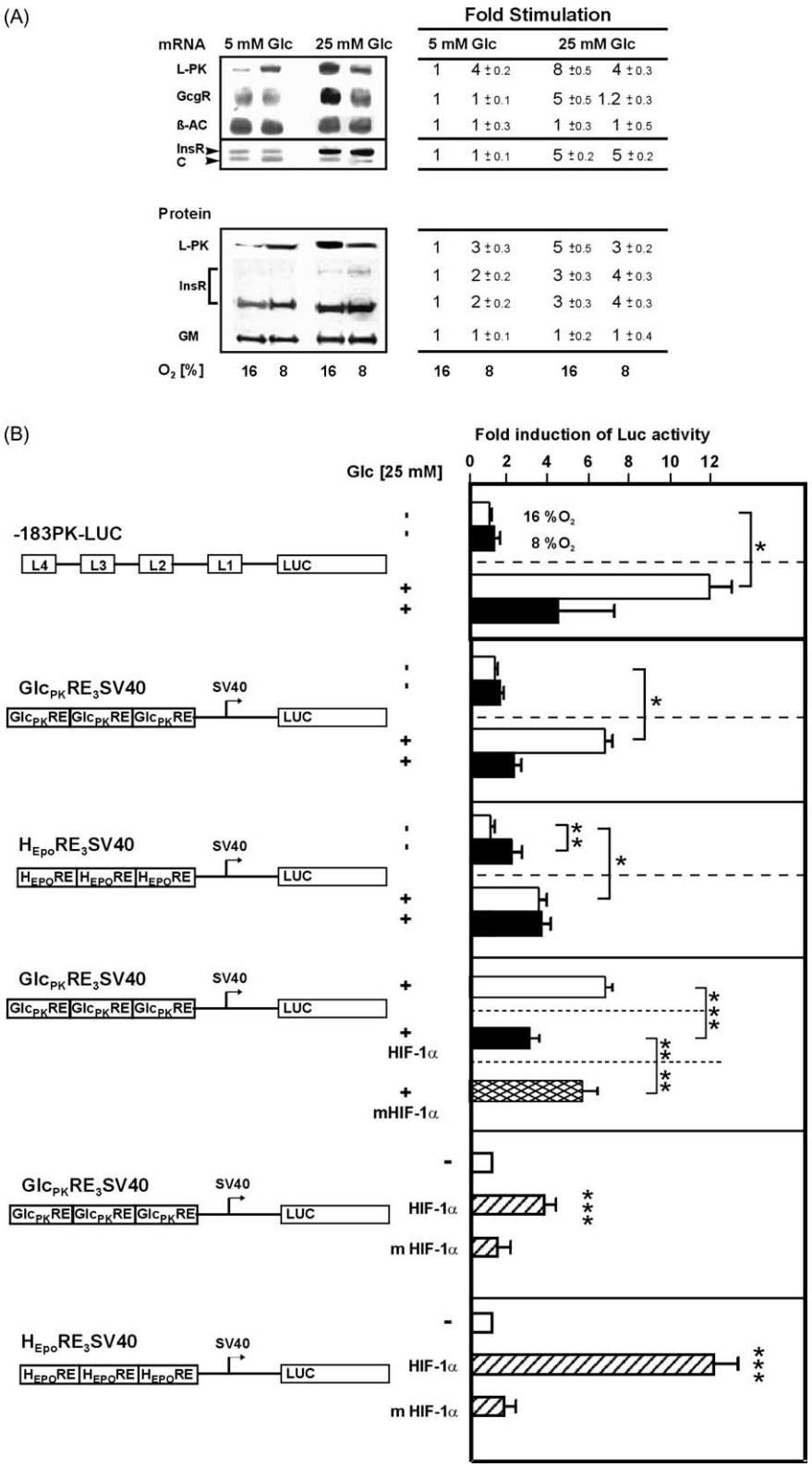

Fig. 2. Induction of L-PK expression by perivenous pO_2 and by glucose under periportal but not perivenous pO_2 in primary rat hepatocyte cultures. Modulation by hypoxia-inducible factor 1. (A) Primary rat hepatocytes were cultured for 24 hr under arterial pO_2 (16% O_2) at basal glucose levels (5 mM) 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 hr. Northern blots with RNA from cultured rat hepatocytes were hybridized to a DIG-labeled L-PK, GcgR or β-actin (β-AC) antisense RNA probe. Insulin receptor (InsR) mRNA levels were estimated by quantitative RT-PCR analysis (for details, see [12,13,50]). For Western analysis cells were cultured as described above. Total protein was analyzed by blotting using an antibody against L-PK, InsR or as control with an antibody against Golgi membrane (GM), cf. [50]. Values are means ± SEM of three independent culture experiments. L-PK was visible as a band of 60 kDa, the InsR β-chain was visible as two bands of 95 and 69 kDa, GM was visible as a band of 94 kDa. C: competitor; L-PK: L-type pyruvate kinase; GM: Golgi membrane;

mild hypoxic conditions, i.e. venous pO_2 as observed in the pericentral area of the liver acinus may activate the expression of genes encoding glycolytic enzymes, e.g. L-PK.

6. High glucose enhanced L-PK gene expression only under periportal pO_2

In rat hepatocyte cultures increasing glucose concentrations from 5 up to 50 mM were able to enhance L-PK mRNA expression about 7-fold and about 12-fold, respectively, but only under arterial pO_2. No induction by glucose was observed under perivenous pO_2. The results on L-PK mRNA level were consistent with the results obtained on protein level. L-PK protein expression was induced about 5-fold in the presence of 25 mM glucose but only under arterial pO_2 (Fig. 2A) [50].

A similar pattern of regulation was observed with the glucagon receptor mRNA. The same increasing glucose concentrations which enhanced L-PK expression and which can be reached in the portal vein after a meal enhanced GcgR mRNA in hepatocyte cultures only under periportal pO_2 in accordance with the periportal expression of GcgR mRNA in rat liver (Fig. 2A) [12].

Thus, glucose appeared to act as an activator of gene expression mainly under periportal conditions.

7. A glucose response element within the L-PK promoter was responsible for the modulation by O_2 of its glucose-dependent induction

Several experiments using actinomycin and cycloheximide as transcriptional and translational inhibitors, respectively, revealed that with the L-PK gene both the response to O_2 and to glucose are mediated on the transcriptional level. As mentioned above, the GlcRE of the L-PK gene promoter has been characterized [34] to consist of two imperfect palindromic E-Boxes [34] which might be candidate sites to be responsible for an O_2-regulated transcription factor.

To test whether this GlcRE is also responsible for the modulation by O_2 of the glucose-dependent induction of the L-PK gene expression transient transfections of hepatocytes with L-PK promoter luciferase gene constructs were performed. In hepatocytes transfected with the L-PK promoter construct −183PK-LUC containing the minimal glucose-responsive L-PK promoter glucose-induced Luc activity by about 12-fold under arterial pO_2 and only by about 4-fold under venous pO_2 indicating that the modulation by O_2 was mediated within the minimal glucose-responsive L-PK gene promoter (Fig. 2B). Thus, these results supported a cross-talk between the signals hypoxia and glucose at the glucose-responsive element [50].

8. Activation of the glucose-responsive promoter and the hypoxia-responsive promoter by HIF-1

The GlcRE within the promoter of the L-PK gene (−165/−149) [42], which was responsible for the induction by glucose, revealed high identity to the binding site of HIF-1. Sequence comparison between the HIF-1 consensus binding site and the GlcRE within the L-PK promoter showed only a one base pair mismatch between the first E-Box of the GlcRE and the HIF-1 consensus binding site. This was also found for the second E-Box (Fig. 1B). Therefore, it might be possible that HIF-1 can interfere with the glucose-dependent-induction of the L-PK gene or that glucose can influence a HIF-1-dependent promoter.

To test this, gene constructs containing three GlcREs in front of the SV40 promoter and the luciferase gene ($Glc_{PK}RE_3$SV40-LUC) were transfected into hepatocytes and it was found again that glucose-induced Luc activity predominantly under arterial pO_2 (Fig. 2B). Thus, the GlcRE within the L-PK gene promoter was sufficient to confer the modulation by O_2 of the glucose-dependent induction of the L-PK gene expression. Reciprocally, transfection of hepatocytes with luciferase gene constructs containing three hypoxia response elements in front of the SV40 promoter ($H_{EPO}RE_3$SV40-LUC) displayed an induced Luc activity by about 2-fold under venous pO_2 (Fig. 2B). Addition of glucose abolished the modulation by O_2 and enhanced Luc activity to the same levels of about 3-fold under arterial and venous pO_2 (Fig. 2B).

Glc: glucose. (B) Primary hepatocytes were transiently transfected with luciferase gene constructs containing the −183 bp L-PK promoter sequence in front of the luciferase gene (−183PK-LUC) or with luciferase gene constructs containing three glucose response elements ($Glc_{PK}RE$), from the L-PK promoter or three hypoxia response elements ($H_{EPO}RE$) from the EPO gene in front of the SV40 promoter and the luciferase gene. Inside the L4 site of the L-PK promoter the two imperfect palindromic E-Boxes (−165/−149) were designated $Glc_{PK}RE$ of the L-PK promoter. When indicated the glucose concentration was raised up to 25 mM and the cells were incubated for 24 hr at 16% O_2 or 8% O_2. In cotransfection assays, hepatocytes were transiently transfected with the LUC constructs indicated and an expression vector for HIF-1α (CMV-HIF-1α) or a vector encoding a mutated form of HIF-1α (CMV-mHIF-1α). The values represent means ± SEM of three independent experiments. Statistics, Student's *t*-test for paired values: (*) significant differences 16% O_2 vs. 16% O_2 + 25 mM glucose ($P \leq 0.05$); (**) significant differences $H_{EPO}RE_3$SV40-LUC 16% O_2 vs. $H_{EPO}RE_3$SV40-LUC 8% O_2 ($P \leq 0.05$); (***) significant differences $Glc_{PK}RE_3$SV40-LUC or $H_{EPO}RE_3$SV40-LUC vs. $Glc_{PK}RE_3$SV40-LUC + HIF-1α or $H_{EPO}RE_3$SV40-LUC + HIF-1α ($P \leq 0.05$); (****) significant differences $Glc_{PK}RE_3$SV40-LUC + HIF-1α vs. $Glc_{PK}RE_3$SV40-LUC + mHIF-1α ($P \leq 0.05$). L: liver-specific; L1: binding site for hepatocyte nuclear factor 1; L2: binding site for nuclear factor 1; L3: binding site for hepatocyte nuclear factor 4; L4: binding site for upstream stimulating factor [42,43].

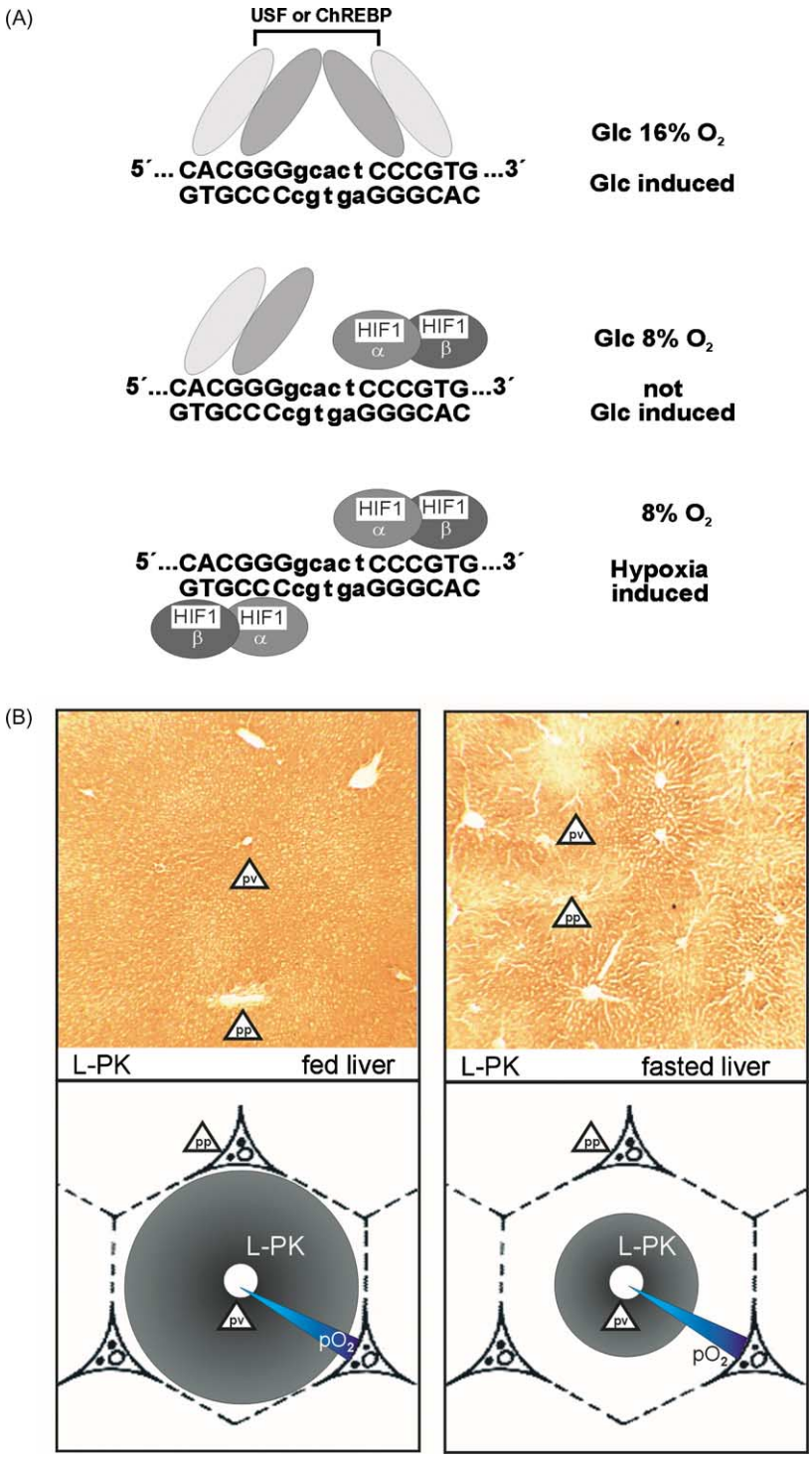

Fig. 3. O_2 modulated dynamic zonation of L-PK expression in the liver of fasted and fed rats. (A) Model of the competition between HIF-1 and the glucose-responsive transcription factors at the glucose-responsive element of the L-PK gene. High glucose at arterial pO_2 induces binding of the glucose-sensitive transcription factors to the $Glc_{PK}RE$ and thus mediates the glucose-dependent activation of L-PK gene expression. In the presence of high glucose under venous pO_2 it is likely that a HIF-1α-containing complex could replace an USF or ChREBP protein complex. This may then disturb the glucose-responsive complex which subsequently results in a reduction of the glucose-dependent L-PK gene activation. In the absence of glucose under venous pO_2 the $Glc_{PK}RE$ might act as a low affinity HIF-1 binding site regulating the induction of the L-PK gene expression by venous pO_2. Glc: glucose. (B) Five micrometer parallel sections were prepared from livers of fed or fasted rats. L-PK was localized by immunohistochemistry using a monoclonal mouse L-PK antibody (for details, see [50]). Dark brown precipitates indicate high levels of L-PK protein. Scheme of the dynamic zonation of L-PK expression in the liver acinus. The O_2 tension drops by about 50% from the periportal (pp) to the perivenous (pv) area. In the fed state (high glucose) the predominant L-PK gene activation by glucose occures under periportal pO_2 and allows expression of L-PK also in the periportal region as observed in the livers of fed rats. This results in a diminution of the zonation pattern. In the fasted state (low glucose) the glucose-dependent induction of the L-PK gene expression in the periportal area is no longer present leading to the predominant L-PK gene activation by venous pO_2 and to a more pronounced perivenous zonation [50]. pp: periportal; pv: perivenous.

The modulation by O_2 of the glucose-dependent induction could be mimicked by cotransfection of hepatocytes with $Glc_{PK}RE_3SV40$-LUC and an expression vector for HIF-1α. Cotransfection of the HIF-1α vector reduced the glucose-dependent induction of Luc activity, whereas cotransfection of vectors for ARNT or vectors for an HIF-1α mutant lacking the first 51 amino acids of the DNA binding domain had no effect (Fig. 2B). This further showed that the reduction of the glucose-dependent L-PK gene activation is dependent of HIF-1α DNA binding activity. Thus, in primary rat hepatocytes the reduction of the glucose-induced L-PK expression under venous pO_2 was mediated by interaction of HIF-1 with the GlcRE within the L-PK promoter.

Thus, it seems likely that in the absence of glucose the GlcRE resembles a low affinity HIF-1 binding site and can regulate the induction of L-PK gene expression by venous pO_2. To support this, cotransfections of the glucose-responsive $Glc_{PK}RE_3SV40$-LUC and the hypoxia-responsive $H_{EPO}RE_3SV40$-LUC gene constructs with different amounts of a HIF-1α expression vector were performed. It was indeed found that cotransfection of hepatocytes with $Glc_{PK}RE_3SV40$-LUC and increasing amounts of the HIF-1α expression vector enhanced Luc activity in a concentration-dependent manner. In contrast, the induction of Luc activity with the $H_{EPO}RE_3SV40$-LUC construct was more robust compared to the $Glc_{PK}RE_3SV40$-LUC construct (Fig. 2B). However, these experiments clearly indicated that HIF-1α was able to mediate the induction of gene expression at the GlcRE of the L-PK gene even in the absence of glucose.

The binding of HIF-1 to the GlcRE of the L-PK promoter was verified by EMSA and it was shown that HIF-1α as well as the USF-1 and USF-2 which also belong to the family of basic-helix-loop-helix proteins [51] bound to the GlcRE. Thus, the GlcRE could also act as a low affinity hypoxia response element and it might be that in the presence of glucose and low oxygen a HIF-1 complex binds to the GlcRE within the L-PK promoter and replaces the binding of the USF complex thereby reducing the glucose-dependent induction of L-PK gene expression under low oxygen (Fig. 3).

A similar mode of action was proposed also from experiments with AS-30D hepatoma cells and the hexokinase type II promoter. In the distal hexokinase type II promoter two HIF-consensus binding sites −3809/3803 5′-CACGTCTG-3′ and −3765/3758 5′-CACGTGCT-3′ were identified which overlap E-Boxes known to be regulated by glucose [46]. Furthermore, the interaction of the signals glucose and oxygen (hypoxia) was shown in experiments with mouse embryonic stem cells in which hypoglycaemia and hypoxia induced the expression of phosphoglycerate kinase-1, vascular endothelial growth factor, lactate dehydrogenase and glucose transporter-1. The induction by hypoxia as well as the induction by hypoglycaemia was abolished in HIF-1α deficient embryonic stem cells [52,53].

9. Posttranscriptional regulation of gene expression by glucose and O_2

Glucose and oxygen do not only appear to regulate gene expression via transcriptional mechanisms but also by posttranscriptional processes. This was especially demonstrated for the insulin receptor (InsR) expression. In primary hepatocytes, glucose concentrations occurring in the portal vein after a meal enhanced InsR mRNA to about the same levels under periportal and perivenous pO_2. This was in accordance with the homogenous expression of InsR mRNA in rat liver. In contrast, the InsR protein was induced by perivenous pO_2 about 2-fold and more strongly in the presence of glucose (Fig. 2A). Thus, the zonation of InsR protein appeared to be regulated mainly at the posttranscriptional level by O_2 [13].

10. Physiological role of the interaction between the signals glucose and oxygen

Thus, it is tempting to propose the following model in which HIF-1 might be able to interfere with the glucose response complex. In the presence of high glucose under arterial pO_2 the glucose-sensitive transcription factors bind the $Glc_{PK}RE$ and mediate the glucose-dependent induction of the L-PK gene. In the presence of high glucose under venous pO_2, it is likely that a HIF-1α-containing complex could replace an USF or ChREBP protein complex, thus disturbing the glucose-responsive complex which results in a reduction of the glucose-dependent L-PK gene activation (Fig. 3). This would explain why glucose induces L-PK mRNA and protein predominantly only under arterial pO_2. The interaction of HIF-1α with the $Glc_{PK}RE$ might play a role in the induction of the L-PK gene by venous pO_2. In line with the results was the finding that cotransfection of HIF-1α could induce Luc activity in $Glc_{PK}RE_3SV40$-LUC transfected hepatocytes.

Since in the liver the difference in glucose concentration from the periportal to the perivenous area is rather shallow, the predominant L-PK gene activation by glucose as in the fed state under periportal pO_2 would allow expression of L-PK also in the periportal region as observed in the livers of fed rats and result in a diminution of the zonation pattern. In the fasted state, as in between meals, the glucose-dependent induction of the L-PK gene expression in the periportal area is no longer present so that the L-PK gene activation by venous pO_2 would be predominant and lead to the perivenous zonation of the enzyme as it was shown in fasted rats [50] (Fig. 3).

Our results for the first time demonstrate the mutual molecular interaction of the signals oxygen and glucose which might represent a more common mechanism of oxygen- and glucose-dependent gene regulation as in metabolism, tumorigenesis or embryonic development.

Acknowledgments

This study was supported by the Deutsche Forschungsgemeinschaft SFB 402 Teilprojekt A1 and GRK 335. We are indebted to Axel Kahn (Institut Cochin de Génétique Moléculaire, INSERM, Paris, France) for the gift of the −183PK/CAT plasmid.

References

[1] Sasse D, Spornitz UM, Maly IP. Liver architecture. Enzyme 1992; 46:8–32.
[2] Jungermann K, Katz N. Functional specialization of different hepatocyte populations. Physiol Rev 1989;69:708–64.
[3] Haussinger D, Lamers WH, Moorman AF. Hepatocyte heterogeneity in the metabolism of amino acids and ammonia. Enzyme 1992;46: 72–93.
[4] Gebhardt R, Gaunitz F. Cell–cell interactions in the regulation of the expression of hepatic enzymes. Cell Biol Toxicol 1997;13:263–73.
[5] Jungermann K, Kietzmann T. Zonation of parenchymal and nonparenchymal metabolism in liver. Annu Rev Nutr 1996;16:179–203.
[6] Jungermann K, Kietzmann T. Oxygen: modulator of metabolic zonation and disease of the liver. Hepatology 2000;31:255–60.
[7] Zierz S, Katz N, Jungermann K. Distribution of pyruvate kinase type L and M2 in microdissected periportal and perivenous rat liver tissue with different dietary states. Hoppe Seylers Z Physiol Chem 1983; 364:1447–53.
[8] Hellkamp J, Christ B, Bastian H, Jungermann K. Modulation by oxygen of the glucagon-dependent activation of the phosphoenolpyruvate carboxykinase gene in rat hepatocyte cultures. Eur J Biochem 1991;198:635–9.
[9] Kietzmann T, Schmidt H, Unthan FK, Probst I, Jungermann K. A ferro-heme protein senses oxygen levels, which modulate the glucagon-dependent activation of the phosphoenolpyruvate carboxykinase gene in rat hepatocyte cultures. Biochem Biophys Res Commun 1993;195:792–8.
[10] Kietzmann T, Freimann S, Bratke J, Jungermann K. Regulation of the gluconeogenic phosphoenolpyruvate carboxykinase and glycolytic aldolase A gene expression by O_2 in rat hepatocyte cultures. Involvement of hydrogen peroxide as mediator in the response to O_2. FEBS Lett 1996;388:228–32.
[11] Kietzmann T, Roth U, Freimann S, Jungermann K. Arterial oxygen partial pressures reduce the insulin-dependent induction of the perivenously located glucokinase in rat hepatocyte cultures: mimicry of arterial oxygen pressures by H_2O_2. Biochem J 1997;321:17–20.
[12] Krones A, Kietzmann T, Jungermann K. Periportal localization of glucagon receptor mRNA in rat liver and regulation of its expression by glucose and oxygen in hepatocyte cultures. FEBS Lett 1998;421:136–40.
[13] Krones A, Kietzmann T, Jungermann K. Perivenous localization of insulin receptor protein in rat liver and regulation of its expression by glucose and oxygen in hepatocyte cultures. Biochem J 2000;348: 433–8.
[14] Goldberg MA, Dunning SP, Bunn HF. Regulation of the erythropoietin gene: evidence that the oxygen sensor is a heme protein. Science 1988;242:1412–5.
[15] Bunn HF, Poyton RO. Oxygen sensing and molecular adaptation to hypoxia. Physiol Rev 1996;76:839–85.
[16] Kietzmann T, Fandrey J, Acker H. Oxygen radicals as messengers in the oxygen-dependent gene expression. News Physiol Sci 2000;15: 202–8.
[17] Epstein AR, Gleadle JM, McNeill LA, Hewitson KS, O' RJ, Mole DR, Mukherji M, Metzen E, Wilson MI, Dhanda A, Tian YM, Masson N, Hamilton DL, Jaakkola P, Barstead R, Hodgkin J, Maxwell PH, Pugh CW, Schofield CJ, Ratcliffe PJ. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. Cell 2001;107:43–54.
[18] Semenza CL. Hypoxia-inducible factor 1: control of oxygen homeostasis in health and disease. Pediatr Res 2001;49:614–7.
[19] Semenza GL. HIF-1 and human disease: one highly involved factor. Genes Dev 2000;14:1983–91.
[20] Semenza GL. Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1. Annu Rev Cell Dev Biol 1999;15:551–78.
[21] Semenza GL. HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 2000;88:1474–80.
[22] Wenger RH, Gassmann M. Oxygen(es) and the hypoxia-inducible factor 1. Biol Chem 1997;378:609–16.
[23] Kvietikova I, Wenger RH, Marti HH, Gassmann M. The transcription factors ATF-1 and CREB-1 bind constitutively to the hypoxia-inducible factor 1 (HIF-1) DNA recognition site. Nucleic Acids Res 1995;23:4542–50.
[24] Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA 1995;92:5510–4.
[25] Hoffman EC, Reyes H, Chu FF, Sander F, Conley LH, Brooks BA, Hankinson O. Cloning of a factor required for activity of the Ah (dioxin) receptor. Science 1991;252:954–8.
[26] Tian H, McKnight SL, Russell DW. Endothelial PAS domain protein 1 (EPAS1), a transcription factor selectively expressed in endothelial cells. Genes Dev 1997;11:72–82.
[27] Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y, Fujii KY. A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development. Proc Natl Acad Sci USA 1997;94:4273–8.
[28] Flamme I, Frohlich T, von RM, Kappel A, Damert A, Risau W. HRF, a putative basic helix-loop-helix-PAS-domain transcription factor is closely related to hypoxia-inducible factor 1 alpha and developmentally expressed in blood vessels. Mech Dev 1997;63:51–60.
[29] Hogenesch JB, Chan WK, Jackiw VH, Brown RC, Gu YZ, Pray GM, Perdew GH, Bradfield CA. Characterization of a subset of the basic-helix-loop-helix-PAS superfamily that interacts with components of the dioxin signaling pathway. J Biol Chem 1997;272:8581–93.
[30] Z Y, Moran SM, Hogenesch JB, Wartman L, Bradfield CA. Molecular characterization and chromosomal localization of a third alpha-class hypoxia-inducible factor subunit, HIF-3alpha. Gene Expr 1998;7: 205–13.
[31] Kietzmann T, Cornesse Y, Brechtel K, Modaressi S, Jungermann K. Perivenous expression of the mRNA of the three hypoxia-inducible factor alpha-subunits, HIF-1alpha, HIF-2alpha and HIF-3alpha, in rat liver. Biochem J 2001;354:531–7.
[32] Hara S, Hamada J, Kobayashi C, Kondo Y, Imura N. Expression and characterization of hypoxia-inducible factor (HIF)-3alpha in human kidney: suppression of HIF-mediated gene expression by HIF-3alpha. Biochem Biophys Res Commun 2001;287:808–13.
[33] Towle HC. Metabolic regulation of gene transcription in mammals. J Biol Chem 1995;270:23235–8.
[34] Kahn A. Transcriptional regulation by glucose in the liver. Biochimie 1997;79:113–8.
[35] Girard J, Ferre P, Foufelle F. Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. Annu Rev Nutr 1997;17:325–52.
[36] Vaulont S, Vasseur-Cognet M, Kahn A. Glucose regulation of gene transcription. J Biol Chem 2000;275:31555–8.
[37] Kahn A. Transcriptional regulation by glucose in the liver. Biochimie 1997;79:113–8.
[38] Girard J, Ferre P, Foufelle F. Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. Annu Rev Nutr 1997;17:325–52.

[39] Vallet VS, Casado M, Henrion AA, Bucchini D, Raymondjean M, Kahn A, Vaulont S. Differential roles of upstream stimulatory factors 1 and 2 in the transcriptional response of liver genes to glucose. J Biol Chem 1998;273:20175–9.

[40] Vallet VS, Henrion AA, Bucchini D, Casado M, Raymondjean M, Kahn A, Vaulont S. Glucose-dependent liver gene expression in upstream stimulatory factor 2−/− mice. J Biol Chem 1997;272:21944–9.

[41] Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K. Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element binding protein. Proc Natl Acad Sci USA 2001;98:13710–5.

[42] Bergot MO, Diaz GM, Puzenat N, Raymondjean M, Kahn A. *cis*-Regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. Nucleic Acids Res 1992;20:1871–7.

[43] Vaulont S, Puzenat N, Levrat F, Cognet M, Kahn A, Raymondjean M. Proteins binding to the liver-specific pyruvate kinase gene promoter. A unique combination of known factors. J Mol Biol 1989;209:205–19.

[44] Shih HM, Towle HC. Definition of the carbohydrate response element of the rat S14 gene. Evidence for a common factor required for carbohydrate regulation of hepatic genes. J Biol Chem 1992;267:13222–8.

[45] Shih H, Towle HC. Definition of the carbohydrate response element of the rat S14 gene. Context of the CACGTG motif determines the specificity of carbohydrate regulation. J Biol Chem 1994;269:9380–7.

[46] Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells—identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. J Biol Chem 2001;276:43407–12.

[47] Latasa MJ, Moon YS, Kim KH, Sul HS. Nutritional regulation of the fatty acid synthase promoter *in vivo*: sterol regulatory element binding protein functions through an upstream region containing a sterol regulatory element. Proc Natl Acad Sci USA 2000;97:10619–24.

[48] Noguchi T, Iritani N, Tanaka T. Molecular mechanism of induction of key enzymes related to lipogenesis. Proc Soc Exp Biol Med 1992;200:206–9.

[49] Yamada K, Noguchi T. Nutrient and hormonal regulation of pyruvate kinase gene expression. Biochem J 1999;337:1–11 (GENERIC) Ref Type: Generic.

[50] Krones A, Jungermann K, Kietzmann T. Cross-talk between the signals hypoxia and glucose at the glucose response element of the L-type pyruvate kinase gene. Endocrinology 2001;142:2707–18.

[51] Gregor PD, Sawadogo M, Roeder RG. The adenovirus major late transcription factor USF is a member of the helix-loop-helix group of regulatory proteins and binds to DNA as a dimer. Genes Dev 1990;4:1730–40.

[52] Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshert E. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumor angiogenesis (vol. 394, p. 485, 1998). Nature 1998;395:525–.

[53] Ryan HE, Lo J, Johnson RS. HIF-1alpha is required for solid tumor formation and embryo nic vascularization. EMBO J 1998;17:3005–15.

[54] Firth JD, Ebert BL, Pugh CW, Ratcliffe PJ. Oxygen-regulated control elements in the phosphoglycerate kinase 1 and lactate dehydrogenase A genes: similarities with the erythropoietin 3′ enhancer. Proc Natl Acad Sci USA 1994;91:6496–500.