Stimulation of Glucose Transport by Hypoxia: Signals and Mechanisms

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Glucose transport is acutely stimulated by hypoxia through enhanced GLUT-1 and GLUT-4 glucose transporter function. GLUT-1 expression is also stimulated by hypoxia or azide. Moreover, hypoxia per se, acting through hypoxia-inducible factor 1, enhances GLUT-1 transcription. GLUT-1 is the first gene whose transcription is dually stimulated in response to hypoxia and inhibition of oxidative phosphorylation.

Studies pertaining to the adaptive response of mammalian cells to hypoxia have led to the identification of numerous genes whose expression is enhanced in response to anaerobic conditions. Some of these genes are transcribed in specific cells, and their products are secreted as paracrine and endocrine factors that promote processes such as erythropoiesis and vascularization. Other genes encode products (such as glycolytic enzymes) that are constituents of the basic metabolic machinery of cells and are induced by hypoxia in a wide range of cells and tissues. Among this latter group is the gene encoding the GLUT-1 glucose transporter, a ubiquitously expressed transmembrane glycoprotein that mediates Na⁺-independent transport of glucose into cells. The enhanced expression and function of GLUT-1 in response to hypoxia represents a fundamental adaptation that is critical to the maintenance of cellular homeostasis. It should be noted that although glucose transport by GLUT-4 is also augmented during hypoxia, the steps mediating this response have been less well studied; hence, this review is primarily focused on the regulation of GLUT-1 by hypoxia, and modulation of GLUT-4 activity will be mentioned as indicated.

The observation that hypoxic conditions enhance glucose uptake by cells and tissues is a well-established phenomenon and was first described in cardiac and skeletal muscle by H. Morgan and collaborators (reviewed in Ref. 6). In addition, it has been shown that this response can be mimicked by exposure to pharmacological inhibitors of oxidative phosphorylation. Hence it has been proposed that the decrement in mitochondrial respiration and ATP synthesis triggers the stimulation of glucose transport. During aerobic metabolism, the availability of molecular oxygen as an electron acceptor allows the bulk of the cellular energy, in the form of ATP and reducing power, to be derived by oxidative phosphorylation. Inhibition of this process,
secondary to decreased supply of oxygen, leads to an increase in the cellular demand for glucose metabolism through the glycolytic pathway to generate lactate. However, the effectiveness of the response to the increase in demand for glucose is limited because, in the majority of cells, glucose transport is rate limiting for glucose utilization, especially under conditions of high energy demand (reviewed in Ref. 6). Thus the overall effectiveness of the adaptive response to hypoxia in terms of cellular homeostasis is critically dependent on enhancement of glucose transport as the key rate-controlling step.

GLUT-1 is regulated by a variety of stimuli via multiple mechanisms

GLUT-1 belongs to the family of highly homologous facilitative Na+-independent glucose transporters (reviewed in Ref. 9). The GLUT-1 gene is active early in development, and its expression persists in virtually all tissues of the adult animal, albeit to varying degrees. It is present in most cultured cell lines, and its expression is markedly upregulated by a variety of agents and conditions including serum and growth factors, oncogenic transformation, calcium ionophores, and thyroid hormone and in response to marked reduction in external glucose concentration, hypoxia, and inhibition of oxidative phosphorylation (6). Owing to its ubiquitous expression, it is proposed that GLUT-1 mediates the basal transport of glucose as well as much of the non-insulin-dependent transport of glucose in mammals. Indeed, overexpression of GLUT-1 in skeletal muscle of transgenic mice is associated with lowered fasting blood glucose levels and enhanced tolerance to a glucose load.

The enhancement of GLUT-1 expression and function is mediated by a number of distinct mechanisms. Depending on the stimulus, GLUT-1 may be regulated acutely, chronically, or both. Acute stimulation of GLUT-1-mediated glucose transport in response to some stimuli is observed within minutes of exposure and is mediated through either translocation of the transporter to the plasma membrane or activation of GLUT-1 transporters preexisting at the cell surface. These mechanisms have been shown to increase glucose transport in the absence of an increase in total cell GLUT-1 transporter content. In addition, more prolonged exposure to some stimuli and conditions is associated with increased levels of GLUT-1 mRNA. The latter mode of regulation, which involves transcriptional and posttranscriptional mechanisms, leads to an overall increase in cell and plasma membrane GLUT-1 content. As discussed below, the regulation of GLUT-1 by hypoxia utilizes all of the above mechanisms to achieve a rapid and sustained enhancement of glucose transport in a wide range of cells and tissues.

What is hypoxia and what are the mediating signals?

The elucidation of the signal transduction pathways mediating the effects of hypoxia has been challenging in part because hypoxia is a complex signal. The complexity arises from the fact that many cellular processes are oxygen dependent. This means that a host of oxygen-consuming reactions could potentially serve as the source of distinct second messengers that trigger the hypoxic response. Thus the ensuing signal(s) are most likely generated by more than one intracellular perturbation and act, independently or in concert, to modulate specific changes in gene expression and/or gene product function. In light of such complexity, the use of chemical agents that can mimic hypoxia by selectively interacting with specific O2-dependent reactions has been of some value in our efforts to understand the regulation of GLUT-1 gene expression and function by hypoxia. In fact, employing such a strategy, it has been possible to show that the stimulation of GLUT-1 gene expression by hypoxia follows activation of at least two independent cell signaling pathways (Fig. 1). One pathway appears to be activated by the inhibition of oxidative phosphorylation secondary to hypoxia and leads to both acute stimulation of glucose transport and a delayed induction of GLUT-1 gene expression. In addition, lowered oxygen concentrations per se, operating through a distinct “oxygen-sensing” pathway, also stimulate GLUT-1 gene transcription. Although the specific molecules involved in the signal transduction pathways mediating the effects of hypoxia remain largely unknown, a number of recent studies have increased our understanding of the nature of the signaling system and, in some cases, have excluded some potential candidate intermediaries.

It makes teleological sense that the regulation of glucose transport is coupled to the energy charge of the cell. Among the many mammalian gene products that are induced by hypoxia, GLUT-1 expression is thus far unique in that it can also be stimulated by exposing cells to chemical inhibitors (e.g., azide, cyanide, rotenone) or uncouplers [e.g., dinitrophenol (DNP), salicylate, oligomycin] of oxidative phosphorylation (6, 10). In the cell systems examined, the stimulation by these chemicals, much like hypoxia, is associated with the transient fall in intracellular ATP levels. Indeed, it has been hypothesized that ATP levels...
may negatively regulate glucose transporter function. According to one proposed model, normal cell ATP levels repress glucose transport. The transient fall in cell ATP concentration after exposure to hypoxia or inhibition of oxidative phosphorylation would inhibit the Na-K pump. The ensuing rise in cytosolic calcium concentration ([Ca^{2+}]), resulting from plasma membrane depolarization, reduces Ca-ATPase action and/or enhances Na^+/Ca^{2+} exchange and could stimulate glucose transport. In line with this premise are results that demonstrate that exposure to hypoxia or chemical inhibitors of oxidative phosphorylation raises cytosolic [Ca^{2+}]. Moreover, the calcium ionophore-induced rise in cytosolic [Ca^{2+}], is associated with a marked enhancement of GLUT-1 function and expression (8). Although this is an intriguing model, observations have yet to establish a direct link between ATP concentration and glucose transport regulation. In addition, not all cells exposed to hypoxia exhibit a dramatic early fall in ATP levels, raising the possibility either that ATP concentration is not involved in triggering the response or that localized, rather than global, changes in intracellular ATP levels modulate glucose transport.

With regard to the role of ATP in triggering the stimulation of GLUT-1 gene expression during the chronic phase of the response, these same paradigms apply. In this case, it has been shown that both DNP and rotenone increase GLUT-1 transporter content in fat and muscle cells (Ref. 15 and references therein). Because both these agents transiently decrease cell ATP concentration but have an opposite effect on cellular NADPH-to-NADP^+ ratio, those groups concluded that the energy charge rather than the redox state serves as the signal in the response. Stimulation of GLUT-1 mRNA expression by uncouplers of oxidative phosphorylation has also been described in Clone 9 cells. Extending this premise, it is tempting to speculate as to whether a rise in [Ca^{2+}], is the more proximal signal for the regulation of GLUT-1 expression during the chronic phase of the response. Against this possibility is a recent study that showed that preventing the azide-induced rise in [Ca^{2+}], did not block the stimulation of GLUT-1 gene expression by this inhibitor in Clone 9 cells (8). Thus a definitive role for changes in ATP (and calcium) concentration in mediating the glucose transport response to hypoxia still remains to be determined.

Irrespective of the inhibitory effects of hypoxia on oxidative phosphorylation, a decrease in the concentration of oxygen per se enhances GLUT-1 gene expression and glucose transport. In this

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FIGURE 1. The enhancement of GLUT-1-mediated glucose transport by hypoxia is mediated by at least 2 independent pathways (I vs. II) utilizing multiple mechanisms. A decrease in oxygen concentration per se activates putative intracellular heme-protein sensors that, via hypoxia-inducible factor 1 (HIF-1), lead to the stimulation of GLUT-1 gene transcription (Ia). Independently, the attendant inhibition of oxidative phosphorylation stimulates GLUT-1 gene transcription (IIa) and increases GLUT-1 mRNA stability (IIb). Inhibition of oxidative phosphorylation can also stimulate glucose transport through activation (IIc) or translocation (IId) of preexisting GLUT-1 molecules.
model, oxygen can be viewed as a ligand capable of binding reversibly to intracellular receptors. Under normoxic conditions, these putative receptors would be maintained in an inactive state. However, in the absence of oxygen, the receptors would become activated to transmit the hypoxic signal. Such an oxygen-"sensing" pathway has been described in virtually all mammalian cells, and the presence of heme-containing molecules serving as oxygen sensors has been postulated (5). Besides oxygen, these receptors bind tightly to certain divalent cations such as Co²⁺, Ni²⁺, and Mn²⁺. The substitution of iron by these metal ions is postulated to maintain the putative receptor in an active state, thereby mimicking hypoxic conditions. This ubiquitous oxygen-sensing system and signaling pathway, which regulates a number of hypoxia-inducible genes in a variety of cell types, also controls the expression of the GLUT-1 gene through what appears to be primarily a transcriptional mechanism (1). The pathway can be activated in the absence of any inhibition of oxidative phosphorylation or a decrement in cell ATP concentration and, in some systems, has an absolute requirement for de novo protein synthesis (1). As discussed below, a key player in this scheme is a recently cloned member of the bHLH-PAS family of transcription factors called hypoxia-inducible factor 1 (HIF-1) (see Ref. 4 for review).

### Acute regulation

In several insulin-responsive tissues and cells, the translocation of both GLUT-1 and GLUT-4 from intracellular membrane vesicles to the plasma membrane in response to acute hypoxia has been observed. In L6 myotubes, cardiac and skeletal muscle, and 3T3-L1 adipocytes, translocation of GLUT-1 and GLUT-4 from low-density microsomal fractions to the plasma membrane is observed after exposure to hypoxia or rotenone. Although insulin treatment of these cells leads to the phosphatidylinositol 3-kinase-dependent translocation of GLUT-1, the translocation in response to hypoxia appears to be distinct because it is wortmannin insensitive and, in some cases, is additive to the effects of insulin. Interestingly, in skeletal muscle the intracellular pool of GLUT-4 transporters in response to hypoxia (or repeated plasma membrane depolarization) appears to be distinct from the GLUT-4 pool that is translocated by the action of insulin. In the absence of translocation, activation of GLUT-1 transporters preexisting in the plasma membrane has been proposed to account for the increase in glucose transport in avian erythrocytes and Clone 9 cells, a nontransformed rat liver cell line (2, 11).

These latter cells are particularly useful because they only express the GLUT-1 isoform. In avian erythrocytes, the stimulation of glucose uptake in response to cyanide and uncouplers of oxidative phosphorylation appears to occur through "derepression" or "unmasking" of cell surface GLUT-1 molecules (2). Likewise, during the early phase (1–2 h) of the response of Clone 9 cells to cyanide or azide, enhancement of glucose transport is observed in the absence of any significant increase in whole cell or plasma membrane GLUT-1 content (11). On the basis of recent experimental results in Clone 9 cells, it has been proposed that under basal conditions, a cytosolic factor is bound to GLUT-1 (perhaps at its COOH-terminus) and inhibits the transporter's substrate binding and transport function. It is further proposed that on exposure to inhibitors of oxidative phosphorylation, GLUT-1 is released from this interaction and the inhibitory protein appears in the cytosol (12). Thus, in addition to translocation, acute regulation of GLUT-1 by hypoxia or inhibition of oxidative phosphorylation is possible through the modulation of the activity of GLUT-1 transporters preexisting at the plasma membrane. Whether the apparent activation of GLUT-1 reflects a conversion of previously inactive sites to functionally active GLUT-1 sites or is instead mediated by an increase in the intrinsic activity of previously functional sites remains to be determined. Moreover, it remains to be determined whether this mechanism of GLUT-1 activation exists in other cells and in tissues.

### Chronic regulation

Whereas the above acute stimulation of glucose transport is independent of the biosynthetic machinery, chronic exposure to hypoxia, or to inhibitors and uncouplers of oxidative phosphorylation in the presence of oxygen, can lead to elevations of GLUT-1 mRNA and total cell content of GLUT-1 protein. This has been demonstrated in L6 muscle cells, 3T3-L1 fibroblasts and adipocytes, C₂C₁₂ cells, HepG₂ cells, Clone 9 cells, and rat brain endothelium and parenchymal cells. In keeping with the premise that independent signaling pathways impact glucose transport during hypoxic conditions, two recent studies examined the mechanism of the transcriptional regulation of the GLUT-1 gene by hypoxia in greater detail (1, 3).

Earlier studies performed on the regulation of the erythropoietin (EPO) gene have demonstrated that the expression of the EPO gene is greatly enhanced in response to hypoxia. In sharp contrast to the GLUT-1 gene, however, EPO gene expression is not augmented by exposure to
inhibitors or uncouplers of oxidative phosphorylation (chemical hypoxia). These findings suggested that the response to hypoxia is mediated by oxygen-sensing mechanisms involving putative intracellular heme-containing sensors. More recently, it was shown that exposure of a variety of cells to hypoxia leads to the induction of a transcription factor (HIF-1) that, in the kidney, stimulates the transcription of the EPO gene by binding to a specific DNA element (hypoxia-inducible element; HIE) located in the 3′-flanking region of the EPO gene (4). The effect of hypoxia on HIF-1 expression is also mimicked by divalent cations such as cobalt and manganese chloride, presumably because these metal ions can substitute for iron in the heme moiety of the sensors and maintain the molecule in the deoxy or active state (5). The unresponsiveness of the EPO gene to mitochondrial inhibitors on one hand, and the induction of HIF-1 activity by hypoxia in many cell types on the other, has led to the proposal that oxygen concentration per se stimulates the expression of specific genes including those encoding EPO, VEGF, and several glycolytic enzymes. With regard to GLUT-1, these results raised the possibility that the induction of GLUT-1 mRNA by hypoxia could be mediated in part through oxygen-sensing mechanisms, independent of the attendant inhibition of oxidative phosphorylation. Indeed, using a heterologous reporter assay, Ebert et al. (3) found that distinct cis-acting elements located in the 5′-flanking region of the mouse GLUT-1 gene are necessary for the positive transcriptional response to hypoxia and inhibitors of oxidative phosphorylation. In a similar approach, but using a structurally analogous to the HIE of the EPO gene (4), the GLUT-1 transcription start site was found to be necessary for the full response to azide. Although the reason for this discrepancy at present remains unknown, the studies concur on the dual regulation of GLUT-1 gene expression by hypoxia.

Although the above-described studies on the dual regulation of GLUT-1 by hypoxia were performed in liver-derived cell lines, these findings can probably be extended to other cell types as well, particularly with regard to the oxygen-sensing pathway mediated by HIF-1. However, additional studies are required to identify specific signaling molecules that mediate the effects of hypoxia on GLUT-1 gene expression. In defining the potential role of mitogenic and environmental stress pathways in the stimulation of GLUT-1 expression by DNP in L6 muscle cells, Taha et al. (15) found that the increase in cell GLUT-1 protein in response to DNP did not depend on the 70-kDa ribosomal protein S6 kinase, p38 mitogen-activated protein kinase (MAPK), or MAPK/extracellular signal-regulated protein kinase (ERK). Likewise, in a recent study we found that the magnitude of the induction of the stress-activated protein kinase (SAPK) in Clone 9 cells in response to either azide or cobalt was well below that which coincides with any significant induction of GLUT-1 mRNA by these agents (unpublished findings). Thus the possibility remains that novel signaling molecules play an important role in coupling the signals generated in response to inhibition of oxidative phosphorylation to the stimulation of GLUT-1 gene expression.

In addition to transcriptional mechanisms, posttranscriptional regulation of mRNA levels can also influence overall gene expression in response to hypoxia. Indeed, GLUT-1 mRNA half-life is increased in cells exposed to hypoxia as well as to inhibitors of oxidative phosphorylation (10, 13). In contrast to the transcriptional response of the GLUT-1 gene to cobalt chloride,
overexpression of HuR increases the stability of VEGF mRNA.

Conclusion

Results of studies over the past decade demonstrate that the stimulation of GLUT-1 gene expression and GLUT-1 transport function by hypoxia is achieved at several levels and by multiple signaling systems. Such complexity of control underscores the importance of the regulation of glucose transport during conditions of high demand for glucose. It also reflects the rate-limiting nature of glucose uptake in a variety of cells and tissues. Although much has been understood regarding the general nature of the adaptive response to hypoxia, the identification of specific intermediaries has, for the most part, remained elusive. Future insight into this area will require more intensive efforts aimed at understanding the mechanisms and pathways underlying the regulation of specific genes that are responsive to inhibition of oxidative phosphorylation.

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References