Hypoxic Preconditioning Protects against Ischemic Brain Injury

Frank R. Sharp,*†‡§ Ruiqiong Ran,*†‡§ Aigang Lu,*†‡§ Yang Tang,*†‡§ Kenneth I. Strauss,‡ Todd Glass,† Tim Ardizzone,*†‡§ and Myriam Bernaudin¶

*Departments of Neurology, †Pediatrics, ‡Neurosurgery, §Neuroscience Program, University of Cincinnati, Cincinnati, Ohio 45267; and ¶UMR 6551 Centre National de la Recherche Scientifique,¶Université de Caen, IFR 47, Caen, France

Summary: Animals exposed to brief periods of moderate hypoxia (8% to 10% oxygen for 3 hours) are protected against cerebral and cardiac ischemia between 1 and 2 days later. This hypoxia preconditioning requires new RNA and protein synthesis. The mechanism of this hypoxia-induced tolerance correlates with the induction of the hypoxia-inducible factor (HIF), a transcription factor heterodimeric complex composed of inducible HIF-1α and constitutive HIF-1β proteins that bind to the hypoxia response elements in a number of HIF target genes. Our recent studies show that HIF-1α correlates with hypoxia induced tolerance in neonatal rat brain. HIF target genes, also induced following hypoxia-induced tolerance, include vascular endothelial growth factor, erythropoietin, glucose transporters, glycolytic enzymes, and many other genes. Some or all of these genes may contribute to hypoxia-induced protection against ischemia. HIF induction of the glycolytic enzymes accounts in part for the Pasteur effect in brain and other tissues. Hypoxia-induced tolerance is not likely to be equivalent to treatment with a single HIF target gene protein since other transcription factors including Egr-1 (NGFI-A) have been implicated in hypoxia regulation of gene expression. Understanding the mechanisms and genes involved in hypoxic tolerance may provide new therapeutic targets to treat ischemic injury and enhance recovery. **Key Words:** Hypoxia, preconditioning, hypoxia-inducible factor, HIF, VEGF, erythropoietin, EPO, ischemia, stroke, oxygen, stress proteins.

INTRODUCTION

Definitions

Hypoxia preconditioning refers to a period of hypoxia followed by a period of time, often between 1 and 2 days later, during which there is protection from an otherwise lethal insult. Hypoxia is defined as a decrease in tissue or ambient tissue oxygen concentration below normal. Ischemia is defined as a decrease in blood flow to tissue that prevents adequate delivery of oxygen and glucose and other nutrients. Stroke or infarction are defined as the death of most or all cellular elements in the tissue, i.e., in brain this is death of neurons, glia, and other cells, often including the vascular cells themselves.

Address correspondence and reprint requests to Frank R. Sharp, Departments of Neurology and Pediatrics, University of Cincinnati, Vontz Center for Molecular Studies Room 2327, 3125 Eden Avenue, Cincinnati, OH 45267-0536. E-mail: frank.sharp@uc.edu.

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Different types of preconditioning in different organs

There are a number of types of preconditioning that are being studied in the brain, heart, retina, liver, kidney, and other organs.[1–3] Some of these studies use ischemic preconditioning where blood flow is temporarily decreased before an ischemic insult that would ordinarily produce infarction.[4–16] Hypoxia preconditioning has been described in the brain, heart, retina, and other tissues.[10,14,15,17–35] Other types of preconditioning have been described *in vivo* and *in vitro* including hyperthermia,[7,8,36] hypothermia,[37,38] chemical preconditioning by blocking the Krebs cycle or respiratory chain,[39] glutamate and seizures,[5,29] linoleic acid,[5] erythropoietin,[40] tumor necrosis factor (TNF),[41] ceramide,[41,42] desferrioxamine and cobalt,[43,44] isoflurane,[45] thrombin,[46,47] and others.[3]

Why study preconditioning and its mechanisms?

Gidday et al.[20,21] first showed that exposure of neonatal rat pups to hypoxia alone (8% oxygen for 3 hours) protected these animals 1 day later from a stroke produced by combined hypoxia/ischemia using a carotid occlusion and exposure to 8% oxygen for 3 hours.[31] In addition, exposure to hypoxia also protected adult ro-

dents against stroke.[22,48] Although the mechanisms of hypoxic preconditioning and ischemic preconditioning are still being elucidated, both of these types of preconditioning appear to require synthesis of new RNA and protein.[4,9,10,18,20,33] Thus, studies designed to look at induction of RNA or protein should identify the molecules associated with the preconditioning. These results also suggest that down-regulation of RNA or protein synthesis is not likely to be the primary mechanism mediating either hypoxia or ischemic tolerance.

Though this review is directed mainly at hypoxia preconditioning, there has been a tendency to confuse hypoxia with ischemia pre-conditioning and even other types of conditioning. Some of the confusion over mechanisms of hypoxia-induced tolerance could be related to the degree of hypoxia. If animals are exposed to oxygen levels below about 8% to 9% they develop cardiac arrhythmias and systemic hypotension and ischemia that may induce genes related to the systemic ischemia and not due to the hypoxia itself.[49,50] Therefore, in any hypoxia preconditioning paradigm it is essential to ensure that the cardiac rhythm is maintained along with blood pressure[20,22,48] to not to confuse ischemic and hypoxic preconditioning.

A number of mechanisms and molecules have been linked with various types of preconditioning. These include adenosine A1 receptors, ATP-sensitive potassium channels, nuclear factor κB (NFκB), vascular endothelial growth factor (VEGF), erythropoietin (EPO), NOS, hypoxia-inducible factor (HIF), *N*-methyl-D-aspartate (NMDA) receptors, MnSOD, TNF, glycogen, lactate, and others.[5,6,10,13,17,21,23,24,29,32,35,41,43,51–54] Unfortunately, this array of different molecules has not yielded a clear systematic approach to understanding mechanisms of preconditioning.

One method for making sense of the preconditioning paradigms is to examine the similarities between the genes induced by these different paradigms. We propose that a given stimulus is likely to produce characteristic changes in gene expression that has similarities in every organ, including the brain, and following various types of pre-conditioning. For example, following ischemia, hypoglycemia, hypoxia, and kainate-induced seizures there is a subset of genes induced by all of these conditions.[55–57] Examples include certain sets of genes induced by low levels of glucose, called glucose-regulated proteins (GRPs) and endoplasmic-regulated proteins (ERPs).[36,58–64] HIF-regulated proteins are specific gene products that are induced by hypoxia, also known as oxygen-regulated proteins (ORPs) or hypoxia-regulated proteins (HRPs).[49,65–67] In addition, unique sets of genes are regulated by hemorrhage in the brain, which may relate to the presence of free heme and hemoglobin.[36,55,56] We suggest that following transient exposure to each of the above types of insults, including ischemia, hypoglycemia, and seizures, glutamate, hemorrhage, and thrombin could precondition brain to resist subsequent injuries. A rational approach for searching for mechanisms of preconditioning would be to search for those molecules and pathways that are common to many or all of these injuries, or to concentrate on the mechanisms that might be most fundamental for cell survival from all of these injuries.[56] This approach is supported by our recent findings that a number of genes are regulated in common in brain by ischemia, hypoglycemia, status epilepticus, and hemorrhage.[56] We propose that transcription factors, regulated in parallel by multiple injury modalities, would affect many common pathways, and thus would be the most promising candidates in the search for the molecules responsible for protection or repair.

HYPOXIA PRECONDITIONING IN BRAIN AND OTHER ORGANS

We have chosen to pursue the mechanisms of hypoxia preconditioning for several different reasons. First, hypoxia preconditioning has been shown to protect the brain and the heart against ischemia, as well as protect the brain from several types of injury including ischemia, seizures, and edema.[10,14,18–25,27–32,35,49,54,66,68–76] Hypoxia protects many types of ischemia including focal and global ischemia in adult and neonatal brain with and without reperfusion.[10,20,22,31,33,48] Hypoxia-induced tolerance in brain is not blocked by glutamate receptor antagonists, but is blocked by inhibitors of RNA and protein synthesis.[10,19,20,33] This suggests that increased transcription and translation are necessary for hypoxia-induced tolerance or protection, and that studies designed to demonstrate increases of RNA or protein should be able to detect the molecules associated with this tolerance and protection. Finally, many of the molecules implicated in various other types of preconditioning are also induced by hypoxia or hypoxia-induced tolerance.[43,51,52,66,77,78]

Many of the molecules induced by hypoxia are known to be protective, e.g., EPO, VEGF, and others.[47] In addition, ischemia, thrombin, hemorrhage, and possibly hypoglycemia all induce HIF and HIF target genes.[43,44,51,52,56,79] that have been implicated in hypoxia-induced tolerance, as described below.[10,43]

Finally, mild to moderate hypoxia (>8% oxygen) does not produce cell death in brain[50,57] if hypoxia does not produce cardiac arrhythmias and hypotension.[50] However, recent studies do show that hypoxia can damage both nuclear and mitochondrial DNA in brain cells and that this stimulates DNA repair.[80–83] This hypoxia-induced DNA damage is obviously of great interest related to neural protection and degenerative neurological diseases. Whether this DNA repair response to hypoxia

plays an important role in cellular protection afforded by hypoxia preconditioning is not known.

HIF AND HYPOXIA PRECONDITIONING

As noted, hypoxia preconditioning requires the synthesis of new RNA and new protein for protection against brain ischemia and is not dependent upon activation of glutamate receptors.[20] One potential candidate for this protection was heme oxygenase, a stress protein that protects against ischemia and is induced by hypoxia in some systems.[63,84] However, hypoxia of the duration necessary to induce hypoxic tolerance does not induce heme oxygenase in brain.[84]

We next turned our attention to a transcription factor recently implicated in oxygen sensing and gene responses to hypoxia, HIF. HIF is composed of two proteins, HIF-1α and HIF-1β. HIF-1α (120 kDa) and HIF-1β (91 to 94 kDa) are basic helix-loop-helix (bHLH) proteins of the PAS family (named for Per, ARNT, and Sim, the first members recognized). The bHLH domain, near the N terminus in both proteins, is essential for DNA binding. There are two transcriptional activation domains in HIF-1α referred to as the N-terminal activation domain (NAD) and the C-terminal activation domain (CAD). Between these two domains is an oxygen-dependent degradation domain (ODD), which, when deleted, confers stability of the protein in the presence of oxygen.[77,85,86]

HIF-1α can be substituted by recently described homologous proteins including HIF-2α (also called EPAS1, HOP2, HLF), HIF-3α, and perhaps others.[77] HIF-1β, also called ARNT, is expressed constitutively in all cells, does not respond to changes of oxygen tension, but is essential for hypoxia-induced changes of transcription mediated by the HIF heterodimer.[66] Knockout of either HIF-1α or HIF-2α is embryonic-lethal, showing that each has unique functions, and the absence of HIF-1β prevents HIF activation of gene transcription.[65,66,77] These data suggest that although HIF-1α/HIB-1β dimers and HIF-2α/HIF-1β dimers bind the same DNA sequences, they either bind slightly different DNA sequences and therefore different target genes, or are differentially expressed in different tissues, resulting in non-overlapping functions.[77]

HIF-1α protein is absent or nearly absent in most normoxic cells. With the onset of hypoxia, oxygen-sensing mechanisms immediately stabilize the HIF-1α protein, which is continuously being synthesized from HIF-1α mRNA. HIF-1α protein dimerizes with HIF-1β protein, and along with other transcription factors (e.g., p300/CRB) bind to hypoxia response elements (HREs) in the regulatory regions of target gene's DNA. This transcriptional complex induces the transcription of HIF target genes including VEGF, EPO and others, as described below.[65,66,78,86–89] In addition, hypoxia activates HIF-1α transcription, as described below, resulting in more HIF-1α protein and HIF target gene induction.[66]

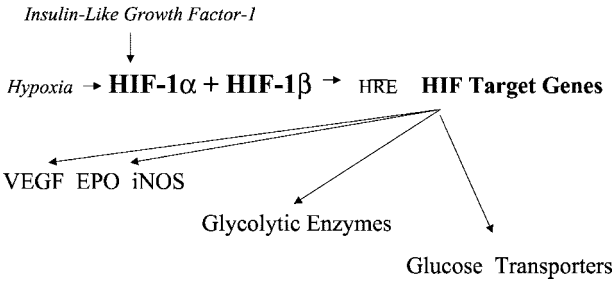

FIG. 1. Hypoxia and growth factor stabilization of HIF-1α leads to binding to HIF-1β and binding to HREs in promoters of HIF target genes to activate transcription of HIF target genes.

To have relevance to preconditioning in the brain, HIF should be induced in brain following ischemia and hypoxia. We found that focal cerebral ischemia (produced using the suture model in adult rats) induced mRNAs encoding HIF-1α, glucose transporter-1, lactate dehydrogenase, and several other glycolytic enzymes in the peri-infarct penumbra.[51] This was observed by 8 hours after the onset of ischemia and increased further at 19 and 24 hours. In areas of HIF-1 and HIF-1 target gene induction regional cerebral blood flow was moderately decreased at 1 and 24 hours after ischemia. Because hypoxia induces HIF-1 in other tissues, systemic hypoxia (6% O_2 for 4.5 hours) was also shown to increase HIF-1α protein expression in the adult rat brain.[51] It was proposed that decreased blood flow to the penumbra decreases the supply of oxygen and that this induces HIF-1α mRNA, stabilizes HIF-1α protein, which leads to induction of HIF-1 target genes. This was the first study to show induction of HIF-1 after focal ischemia in brain and suggest that durations of hypoxia sufficient to produce tolerance or hypoxia-induced conditioning also induced HIF-1 in brain.

OXYGEN-SENSING SYSTEM

In the past few years the mechanisms of oxygen sensing and oxygen induction of HIF and HIF target genes has been the subject of intense investigation and discovery. Though some of the mechanisms are still unclear, the following pathways are believed to mediate hypoxia sensing and HIF induction of HIF target genes.

Hypoxia stabilizes HIF-1α protein leading to induction of HIF target genes

With a marked decrease of oxygen (hypoxia) the von Hippel-Lindau protein (pVHL) dissociates from the HIF-1α protein. This results in decreased degradation of HIF-1α protein via the ubiquitin-proteasome system and the immediate accumulation of HIF-1α protein[85,90–101];

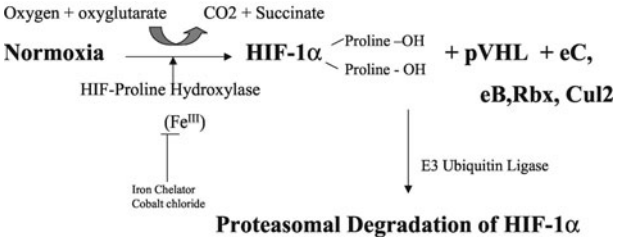

FIG. 2. Normoxia leads to activation of HIF proline hydroxylase, which hydroxylates two prolines on HIF-1α (prolines 402 and 564) that leads to binding of pVHL and elongin-C,-B, Rbx, and Cul2 and degradation of HIF-1α in the proteasome.

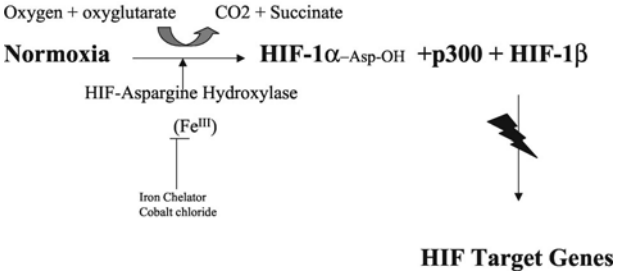

FIG. 3. Normoxia leads to activation of HIF asparagine hydroxylase, which hydroxylates asparagine 803 on HIF-1α that prevents binding of p300 to the HIF-1α and HIF-1β complex and impairs activation of HIF target genes.

the HIF-1α protein then dimerizes with HIF-1β protein. This complex, including transcription factor p300/CRB, binds at HREs on HIF target genes to induce transcription of those genes (see FIG. 1).[65,88,89,102–106] In addition, hypoxia induces the transcription of HIF1α mRNA that increases HIF-1α protein in the presence of continued hypoxia that also promotes transcription of HIF target genes.[51,52,107]

Upon establishing normoxic conditions, HIF1α is rapidly degraded via the following recently described pathway. In the presence of dioxygen, iron, and oxoglutarate, one or several HIF protein hydroxylases (PH) (also called oxoglutarate dehydrogenases) are activated and proceed to hydroxylate proline residue 564 and one other proline residue on the HIF-1α protein.[85,92,96,108–115] Hydroxylation of these proline residues changes the HIF protein conformation, which allows it to be recognized by pVHL. Once pVHL binds diproline-hyroxylated HIF-1α, other factors bind to the complex including elongin C, elongin B, Rbx 1, and Cul-2 (a member of the Cullin family).[77,85,86,99,108] This complex acts as an E3 ubiquitin ligase for HIF-1α polyubiquitination by targeting the ODD and degradation of HIF-1α via the proteasome (FIG. 2). Mutations in wild-type pVHL lead to von Hippel-Lindau disease with failure to degrade HIF-1α, overexpression of various HIF-1 target genes including VEGF, and formation of various types of vascular tumors.[77,86,98,99] Therefore, the oxygen sensor appears to be the protein hydroxylase that requires dioxygen and oxyglutarate as co-factors to hydroxylate at least two proline residues on the hypoxic HIF-1α protein.[85,92] These protein hydroxylases contain an iron that can be bound by chelators like desferrioxamine and displaced by heavy metal ions like cobalt, and account for HIF induction by these compounds.[77,85,96] Of interest is the finding that p53 can bind HIF-1α protein and mediate its degradation via MDM2 (another E3 ubiquitin ligase) in the proteasome independent of oxygen.[77]

Hypoxia leads to increased transcriptional activity of HIF-1α

In the presence of oxygen, an asparagine protein hydroxylase hydroxylates an asparagine on HIF-1α protein that prevents the binding of p300 to HIF-1α and prevents the transcriptional activation of HIF target genes.[110,111] Therefore, during hypoxia the asparagine hydroxylase is inactivated, leading to active HIF-1α protein, which dimerizes with HIF-1b that binds p300/CBP and activates HIF target genes (see FIG. 3).[77]

In addition, hypoxia leads to increased transcription of HIF-1α mRNA and increased HIF-1α protein in brain and in other organs.[43,51,52,77] The mechanisms controlling the transcription of HIF-1α mRNA and the translation of HIF-1α mRNA into protein are still being defined. Heregulin and insulin-like growth factor 1 (IGF-1) induced phosphorylation of the translational regulatory proteins 4EBP-1, p70 S6 kinase, and EIF4E.[77] These proteins are substrates for the phosphatidyl 3-kinase (PI3K)/AKT-FRAP/mTOR (FKBP12-rapamycin-associated protein/mammalian target of rapamycin cascade). This cascade can be activated by a variety of factors, including growth factors and cytokines including EGF, heregulin, insulin, IGF-like growth factors, interleukin (IL)-1β and others to induce HIF-1α accumulation and target gene expression.[66,77,116,117] IGF in brain also plays a role in increasing HIF target gene transcription.[118] A number of studies show that inhibitors targeting PI3K or FRAP/mTOR prevent growth factor- and cytokine-induced HIF-1α accumulation.[77] PTEN overexpression, which dephosphorylates PI3K targets, also decreases HIF-1α expression in glial cell lines.[119] This pathway may explain how thrombin induces HIF in brain[47] and possibly the various interactions of NFκB, early growth response factor-1 (Egr-1), Etx, glucose, and HIF.[28,60,74,120–122] In addition, many HIF target genes likely have promoter elements that respond to many factors in addition to HIF, including Egr-1, NFκB, glucose-regulated proteins, Ets/Elk-1, PKC, Raf, mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), and others.[28,47,60,74,118,120–127]

HIF TARGET GENES

HIF target genes include those related to vasomotor control (NOS2), angiogenesis (VEGF, FLT-1), blood

and iron metabolism (EPO, transferrin, transferrin receptor, ceruloplasmin), cell proliferation [IGF-1, IGFBP-1, -3, transforming growth factor (TGF)β], and energy metabolism (glucose transporter-1, -2, and -3, phosphoenolpyruvate carboxylase, lactate dehydrogenase A, aldolase, phosphoglucokinase-1, -L and -C, pyruvate kinase, enolase, and many others, including prolyl 4-hydroxylase and adenomedullin).[65,88,89,104,105] Many additional genes are likely HIF targets.[52,104] Tyrosine hydroxylase is an important HIF target gene with several HREs in its promoter that may well use EPAS1 rather than HIF-1α.[128,129] Plasminogen activator inhibitor-1, DEC1, a basic helix-loop-helix transcription factor that is induced by hypoxia in glioma cells, the endothelin-1 gene and others appear to have functional HREs and respond to hypoxia.[130–134]

HYPOXIA-INDUCED TOLERANCE BY PHARMACOLOGICAL INDUCTION OF HIF

Hypoxic preconditioning is hypothesized to be due, at least in part, to hypoxic induction of HIF-1. This postulate was supported by the finding that a 3-hour exposure to 8% oxygen induced HIF-1α but not HIF-2α in neonatal rat brain.[43,51,52] To further support the concept that hypoxic induction of HIF-1 confers tolerance to subsequent injury, animals pretreated with cobalt or desferrioxamine (compounds known to induce HIF-1, by binding to the iron in the oxygen sensor proteins[65,78,85,87]) are protected against ischemic injury in the neonatal and in the adult rodent brain. HIF-1α and HIF-1β protein levels increase after intraperitoneal injection of cobalt chloride (60 mg/kg) and moderately increase after intraperitoneal injection of desferrioxamine (200 mg/kg) 1 to 3 hours after the injections. Preconditioning with cobalt chloride or desferrioxamine 24 hours before hypoxia-ischemia affords 75% and 56% brain protection, respectively, compared with that in vehicle-injected littermate controls. Thus, pharmacological induction of HIF-1 as well as hypoxic induction of HIF-1 appears to contribute to brain preconditioning.

It is interesting to note that the discovery of the protein hydroxylase oxygen sensor protein has made it possible to design small molecule mimics that bind the protein and also induce HIF.[96] Such molecules might be used clinically. In addition, compounds already used clinically, including desferrioxamine and isoflurane, may also induce HIF and protect the brain and all other organs in the body against ischemia.[1,44,45]

GENES INDUCED BY HYPOXIA PRECONDITIONING IN BRAIN

VEGF and EPO

Several HIF target genes have already been directly implicated in protecting the brain and other organs against ischemia. These include VEGF and EPO.[1,135–148] In addition, a recent human study suggests that early administration of EPO following stroke improves outcome in the patient population.[143] Although the mechanisms of both molecules in protecting brain are likely to be complex, several different pathways have been studied. It has been suggested that EPO acts at EPO receptors to activate Jak2, which initiates phosphorylation of IKB to activate NFκB and induce NFκB neuroprotective genes.[40] Jak2 also activates PI3K to phosphorylate Akt, which leads to phosphorylation and deactivation of the pro-apoptotic gene BAD.[79] VEGF not only promotes angiogenesis, but may also promote cell survival by acting at VEGF receptors that then lead to phosphorylation and activation of Akt that leads to cellular changes that promote cell survival.[33]

Hypoxia preconditioning not only induces VEGF and EPO but also many other genes. Exposure of neonatal rats to 3 hours of 8% oxygen induces dozens of genes in brain including HIF-1α.[52] A related hypoxia transcription factor, HIF-2α, is not induced by hypoxia preconditioning in brain. Other genes that are induced by the hypoxia preconditioning include GLUT-1, adrenomedullin, propyl 4-hydroxylase, metallothionein-1, MKP-1, CELF, 12-lipoxygenase, tissue-type plasminogen activator (tPA), CAR-1, and a number of unidentified expressed sequence tags.[52] The role of most of these molecules in mediating protection is unknown, though GLUT-1 has been shown to protect cells against ischemia and other types of injury.[149–151]

Other molecules might also have critical roles in hypoxia-induced tolerance. For example, at least one study showed that blocking the lactate transporter prevented hypoxia preconditioning *in vitro*.[29] This intriguing result was interpreted to mean that hypoxia led to up-regulation of lactate transporters on astrocytes and neurons and that the shuttle of lactate from astrocytes to neurons was enhanced by hypoxia preconditioning, which improved cell survival, whereas blocking the lactate transporter prevented this cell survival mechanism. If this were the primary mechanism by which hypoxia preconditioning occurred, then approaches for selectively up-regulating glial and neuronal lactate transporters might be worth pursuing.

It is important to point out that the induction of HIF or the induction of a given HIF target gene/protein cannot be thought of as equivalent, because the factors that regulate HIF expression are different from the factors that regulate expression of any given HIF target gene. For example, hypoxia, along with at least selected cytokines and perhaps selected growth factors, induce HIF-1α mRNA and/or stabilize HIF-1α protein and stimulate HIF transcriptional activation. However, different factors regulate induction of the HIF target gene, VEGF. Not only are there HIF HREs in the VEGF promoter, but

there also appear to be Egr-1, Sp1, Smad 3, Ap1, TGF, estrogen, and many other promoter sites that mediate VEGF induction by a wide variety of molecules including EGF, HGF, TGF, interferon, IL-1, IL-6, TNF, Egr-1, estrogen, calcium, STAT3, p44/p42 MAPK, IGF, Akt/PI3K, mTOR, p53, and many others.[33,87,152,153] The complex interaction of various factors on various promoter elements of VEGF and probably many other HIF target genes is highlighted by the finding that animals with a deletion of the HRE in the VEGF promoter have selective degeneration of motor neurons in the nervous system.[153] The relationship of this finding to sporadic amyotrophic lateral sclerosis is unclear, but suggests possible roles of hypoxia and factors like VEGF.

Complex role of HIF and other transcription factors regulated by hypoxia

HIF and regulation of HIF target genes has recently been shown to be important in the life and death of a variety of different cells. As discussed above, cobalt or desferrioxamine induction of HIF appears to protect the brain against stroke.[43,154] HIF has also been shown to be essential for the survival of certain cells/tissues in the body; for example, HIF-1α is essential for chondrocyte growth arrest and survival.[155] Cells that lack HIF-1 in the interior of the growth plate in bone die, and though HIF-induced VEGF is impaired, VEGF appears to be induced following hypoxia via a HIF-independent mechanism that can result in ectopic angiogenesis.[155] Other hypoxia transcription factors that would induce VEGF include NFκB, Egr-1, NGFI-A, metal transcription factors and possibly CREB.[124,156–161] The role of these other hypoxia-responsive transcription factors in hypoxia-induced tolerance is still unknown. Though HIF is clearly important, the relative roles of NFκB, Egr-1,[124] metal-responsive promoter element-binding transcription factor (MTF)[1], and CREB is still unclear. For example, tissue factor, VEGF, IL-1, PAI, TGF, 5-lipoxygenase, and other genes are regulated in part by hypoxia through Egr-1.[124,125,127,162–164]

In addition, HIF-1α is essential for myeloid cell-mediated inflammation.[165] Activation of HIF-1α is essential for myeloid cell infiltration and activation *in vivo* through a mechanism independent of VEGF. Loss of pVHL and up-regulation of HIF leads to a large increase in inflammatory responses. It appears that white blood cells obtain most of their ATP via glycolysis, and the absence of HIF-inducible glycolytic enzymes impairs the Pasteur effect in white blood cells. This impairs their function, including aggregation, motility, invasiveness, and bacterial killing.[165]

Though a number of studies suggest that HIF and HIF target gene induction appear to be protective,[40,43,154] this is not always the case. HIF-1 induction mediates the stabilization and up-regulation of p53 and p53-mediated hypoxia-induced apoptosis in some types of cells in part by down-regulation of anti-apoptotic genes like Bcl-2 and up-regulation of pro-apoptotic genes.[140,166–169]

Unanswered questions

The above discussion addresses the general question of how gene discovery can be used in the search for molecules and pathways that might be drug targets for neuroprotection for stroke and other types of injury. Is it better to target a transcription factor like HIF that likely has many modes of action? Or is it better to target a given downstream gene like EPO that is likely to have fewer modes of action but also might have fewer side effects? Will targeting individual effectors provide just as much protection as hypoxia preconditioning itself? Are some of the molecules induced by hypoxia preconditioning actually harmful during ischemia, like tPA?[170–173] Does hypoxic preconditioning protect against all types of neural injuries—ischemia, hypoglycemia, trauma, hemorrhage, neurotoxins, status epilepticus—and degenerative neuronal cell death? The answers to these questions will hopefully provide new tools and new insights for developing treatments for the ischemic brain or preventing subsequent injury should ischemia occur.

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