

HIF Hydroxylase Pathways in Cardiovascular Physiology and Medicine

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Abstract: Hypoxia inducible factors (HIFs) are α/β heterodimeric transcription factors that direct multiple cellular and systemic responses in response to changes in oxygen availability. The oxygen sensitive signal is generated by a series of iron and 2-oxoglutarate-dependent dioxygenases that catalyze post-translational hydroxylation of specific prolyl and asparaginyl residues in HIF α subunits and thereby promote their destruction and inactivation in the presence of oxygen. In hypoxia, these processes are suppressed allowing HIF to activate a massive transcriptional cascade. Elucidation of these pathways has opened several new fields of cardiovascular research. Here, we review the role of HIF hydroxylase pathways in cardiac development and in cardiovascular control. We also consider the current status, opportunities, and challenges of therapeutic modulation of HIF hydroxylases in the therapy of cardiovascular disease. (*Circ Res.* 2015;117:65-79. DOI: 10.1161/CIRCRESAHA.117.305109.)

Key Words: dioxygenases ■ hypoxia ■ hypoxia inducible factor ■ prolyl hydroxylases

One of the principal functions of the cardiovascular system is the delivery of oxygen to respiring tissues. The existence of a wide range of adaptive cardiovascular responses to hypoxia has accordingly long been recognized by physiologists. Historically, most research emphasized the importance of metabolism, as opposed to direct regulation by oxygen. This perspective was, however, changed by the recognition that direct transcriptional regulation by the availability of oxygen (first identified in the context of erythropoietin [Epo] production) was in fact widespread in mammalian cells,¹ by the molecular elucidation of the transcription factors (hypoxia inducible factors [HIFs])^{2,3} and by the definition of the oxygen-sensing mechanism (post-translational hydroxylation of HIF α by a set of 2-oxoglutarate dependent dioxygenases).⁴⁻⁷

HIF complexes bind DNA as α - β heterodimers, each subunit being represented in higher animals by a series of isoforms that are the products of gene duplications at the base of vertebrate evolution.⁸ In humans, there are 3 isoforms of the regulatory dimerization partner HIF α , each of which is a target for the oxygen-sensing dioxygenases. The best characterized HIF α isoforms, HIF-1 α and HIF-2 α bind to an identical core consensus (RCGTG) in hypoxia response elements, but transactivate distinct, although partially overlapping, sets of genes.^{9,10} Both HIF α isoforms are regulated by oxygen levels, through a dual system of prolyl and asparaginyl hydroxylation (Figure 1). Prolyl hydroxylation promotes association with the von Hippel-Lindau ubiquitin E3 ligase and destruction by the ubiquitin-proteasome pathway, whereas asparaginyl

hydroxylation impairs the recruitment of coactivators to the transcriptional complex. HIF prolyl hydroxylation is catalyzed by 3 closely related enzymes termed prolyl hydroxylase domain (PHD) 1, 2, and 3; otherwise known as EglN2, 1, and 3.^{6,7} HIF asparaginyl hydroxylation is catalyzed by a single enzyme, factor inhibiting HIF (FIH).¹¹⁻¹⁴

Both types of HIF hydroxylase are members of the Fe(II) and 2-oxoglutarate-dependent dioxygenase superfamily. Catalysis couples the oxidation (hydroxylation) of HIF α to the oxidative decarboxylation of 2-oxoglutarate to succinate and carbon dioxide.¹⁵ This process is inhibited by hypoxia allowing HIF α subunits to escape destruction and form a transcriptionally active DNA-binding complex when oxygen levels are low. The system is conserved throughout the animal kingdom, the primitive PHD2/HIF-1 couple being observed in every species and the most widely expressed in mammalian cells.¹⁶ All PHD enzymes operate on both HIF-1 α and HIF-2 α , although relative isoform selectivity is observed. PHD2 is the most important enzyme in setting general levels of HIF-1 α , whereas the more tissue restricted isoforms PHD1 and PHD3 seem to be somewhat more active against HIF-2 α .^{17,18}

A large number of processes act to modulate this basic oxygen-sensing pathway, including transcriptional and translational controls affecting synthesis of HIF, alternative (non-oxygen-dependent) degradation systems, non-oxygen-dependent controls of activity, and signal pathway crosstalk. For more detailed descriptions of these processes, the reader is referred to other reviews.^{19,20} Here, we will focus on the role of the HIF

Original received April 8, 2015; revision received May 1, 2015; accepted May 6, 2015. In April 2015, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 13.84 days.

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DOI: 10.1161/CIRCRESAHA.117.305109

Nonstandard Abbreviations and Acronyms

Epo	erythropoietin
FIH	factor inhibiting HIF
HIF	hypoxia inducible factor
MI	myocardial ischemia
PHD	prolyl hydroxylase domain
PHI	PHD inhibitor

hydroxylase system in cardiovascular biology, including cardiovascular development, cardiovascular physiology, and the potential for therapeutic manipulation in cardiovascular disease.

Development

Extensive research has revealed the existence of heterogeneous regions of profound hypoxia in the developing embryo.²¹ These regions overlap, at least partially, with spatially and time-restricted patterns of HIF activation. Markers of profound hypoxia and activation of the HIF system are both observed in the developing heart, during the period in which cardiac chambers are formed.²² A range of cardiac anomalies have been observed in mouse strains bearing inactivating alleles of components of the HIF system. Taken together, these findings raise important questions as to the role played by the

HIF system in cardiovascular development, including the possibility that activation of the HIF system by intercurrent ischemia/hypoxic stresses during embryogenesis might contribute to the burden of human congenital heart disease. Below, we review recent experimental data bearing on this question.

Tissue Hypoxia and HIF Activation During Cardiac Development

Fetal development occurs in a hypoxic environment that is highly heterogeneous. For instance, studies of hypoxia markers, such as pimonidazole, have revealed that hypoxia affects different regions within the embryo at different times during organogenesis.²³ In the mouse heart, these studies reveal that cardiac development (occurring between E7.5 and E15) coincides with such a period of gestational hypoxia. Mouse cardiac progenitor cells adopt a crescent structure at E7.75, fusing into a linear heart tube at E8.25, undergoing looping morphogenesis and chamber formation at E8.5–E12, with division of the chambers by septation at E12.5–E15.^{21,24} Hypoxia is widespread in the developing heart tube at day E9.5, when delivery of oxygen is limited by diffusion, but becomes restricted to the outflow tract, interventricular septum, and atrioventricular cushions when the myocardial cells become perfused with blood.^{25,26} That happens as the coronary vasculature connects to the aorta at E14.5. Patterns of HIF activation conform broadly to this pattern.^{23,27,28} For instance, HIF-1 α is widely

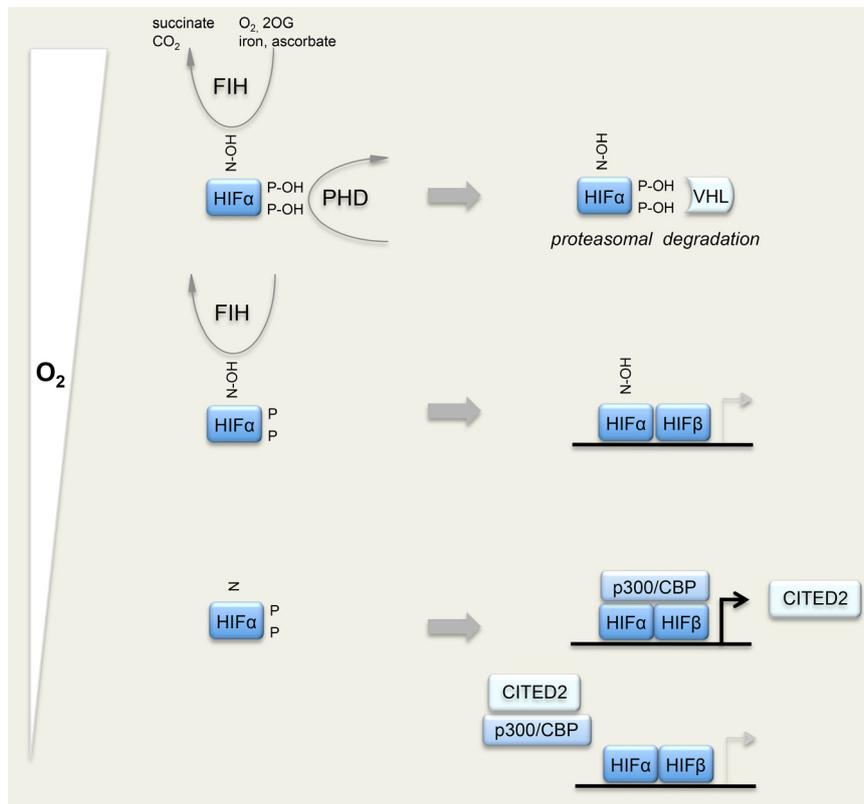


Figure 1. Oxygen-dependent regulation of hypoxia inducible factor α (HIF α) by prolyl and asparaginyl hydroxylation. In the presence of oxygen, both HIF prolyl hydroxylases (PHDs) and factor inhibiting HIF (FIH) are active. PHDs hydroxylate 2 proline residues on HIF α , targeting HIF α for VHL-mediated proteasomal degradation. Under hypoxia, PHDs are inactive and HIF α escapes proteolytic degradation. FIH hydroxylates 1 asparaginyl residue on HIF α to prevent binding of the transcriptional coactivator p300/CREB binding protein (CBP), thus reducing the transcriptional potential of HIF. Under more severe hypoxia, FIH is also inactivated, allowing for p300/CBP binding to HIF α and resulting in transcriptional activation. CBP/p300-interacting transactivator, with glu/asp-rich carboxy-terminal domain, 2 (CITED2), a HIF target gene, acts as a negative regulator of HIF activation by competing with HIF α for binding to p300/CBP.

expressed in the developing heart tube, but becomes more restricted after coronary perfusion.^{21,22} Interestingly, multiple HIF α isoforms are expressed in the developing heart, although patterns are distinct at the cellular level.²⁹ For instance, expression of HIF-1 α is mainly myocardial, whereas HIF-2 α is mainly endothelial, suggestive of differential requirements for HIF-1 and 2 in cardiomyocyte function (eg, proliferation, contractility) versus vascular function (eg, angiogenesis) during development.³⁰

These associations suggest that activation of HIF by developmental hypoxia might contribute directly to cardiac morphogenesis. In keeping with this, abnormalities of cardiac development are common in mice in which key components of the HIF system have been targeted by homologous recombination. Inactivation of HIF-1 α (HIF-1 $\alpha^{-/-}$ mice) results in major and extensive vascular and heart defects (principally, arrested morphogenesis at various stages from the cardiac crescent stage) and consistently leads to embryonic lethality at \approx E10.^{31–33} In contrast, inactivation of HIF-2 α (HIF-2 $\alpha^{-/-}$ mice) results in a highly variable set of outcomes and, despite expression of HIF-2 α in the developing heart, HIF-2 $\alpha^{-/-}$ mice do not manifest major structural abnormalities in cardiac development. Rather, the different outcomes range from late embryonic death (after the period of cardiac morphogenesis)^{34,35} to survival into adulthood³⁶ and encompass failure of sympathoadrenal development,³⁵ vascular defects,³⁴ lung defects,³⁷ and metabolic dysregulation.³⁶ Increased activation of HIF may also result in cardiac abnormalities. For instance, inactivation of CREB-binding protein (CBP)/p300-interacting transactivator, with glu/asp-rich carboxy-terminal domain, 2 (CITED2), a negative regulator of HIF-1 α (Figure 1), is associated with a high prevalence of congenital heart defects.³⁸ Although CITED2 has a range of transcriptional functions, including left–right determination, the abnormalities can be ameliorated by combined heterozygosity for HIF-1 α .³⁹ This suggests that, at least in part, they reflect overactivation of HIF-1. In keeping with this, upregulation of HIF after disruption of the major oxygen sensor, PHD2, also results in cardiac abnormalities, including underdevelopment of the ventricular myocardium, septal defects, and cardiac chamber enlargement.⁴⁰

Although these studies are all consistent with the hypothesis that precise control of HIF signaling within the developing heart is important for its proper development, it is also possible that they reflect secondary effects from other embryonic or placental defects created by general inactivation of the relevant gene. For instance, in PHD2 $^{-/-}$ mice, the expected upregulation of HIF-1 α was observed in many tissues, but surprisingly not in the abnormal heart.⁴⁰ To address this, several investigators have used cardiac tissue expression of Cre recombinase to inactivate the relevant gene specifically in cardiac cells (Table 1). Taken together, these studies support the direct importance of HIF activity. Thus, cardiac-specific inactivation of HIF-1 α and CITED2 are both associated with developmental heart defects.^{27,41} Interestingly, however, 2 similar studies of cardiac-specific inactivation of HIF-1 α have generated somewhat different results. One study (using MLC2vcre) observed defective apoptosis, myocardial hyperplasia, and arrested heart development with embryonic lethality at \approx E11,

that is, similar to the phenotypes described in the nontissue selective HIF-1 $\alpha^{-/-}$ mice.²⁷ In contrast, another study reported a high prevalence of cardiac abnormalities after nonselective inactivation of HIF-1 α in the mesoderm posterior 1 (MesP1^{Cre}) but only a small and nonsignificant excess of cardiac abnormalities after either cardiac-specific deletion (NK2 homeobox 5^{IRESCre}) or vascular-specific (tyrosine kinase, endothelial [Tek]-Cre) deletion of HIF-1 α .⁴² This led the authors to hypothesize that secondary hypoxia generated by placental or other extracardiac abnormalities might interact with myocardial HIF-1 α deficiency to generate the cardiac phenotypes associated with general HIF-1 α deficiency. However, the importance of hypoxic activation of HIF-1 α in the developing myocardium for normal cardiac development is also supported by recent work describing the effects of timed inactivation of HIF-1 α . Kenchegowda et al²⁸ observed that inactivation of HIF-1 α (tamoxifen-inducible β -actinCre) from E10.5 was associated with cardiac abnormalities, whereas inactivation from E13.5 was not. The same study also reported that inactivation of HIF-1 α (Wnt1Cre) in the neural crest cells (from which cardiac progenitors are derived) was associated with cardiac abnormalities. Both these recent studies showed that appropriately timed intercurrent maternal hypoxia increased the severity of hypoxia and the activity of HIF-1 in the developing heart. However, reports of effects on cardiac developmental abnormalities were different. Kenchegowda observed cardiac anomalies in association with maternal hypoxia during the developmental window (E10.5–13.5) but not later (E13.5–17.5). In contrast, using a shorter (8 hours) period of hypoxia at E9.5, O'Reilly et al⁴² found only small and nonsignificant increases in cardiac developmental abnormalities after cardiac-specific inactivation of HIF-1 α (NK2 homeobox 5^{IRESCre}), although severe maternal hypoxia clearly reduced embryo survival.

The concept of developmental windows in which the developing fetus might be specifically sensitive to environmental stresses, such as maternal hypoxia, is further supported by findings in noncardiac tissues. Thus, in classical studies reported in 1952, Ingalls et al⁴³ observed an increase in hemivertebra anomalies in association with a short (5 hours) exposure to severe hypoxia specifically at E8.5 to 9.5 (although no increase in ventral septal defects). More recently, interaction between clinically associated genetic predisposition and experimental gestational exposure to hypoxia has been reported for Notch signaling defects and congenital scoliosis.⁴⁴ Other studies have reported limb defects and myocardial thinning in association with maternal hypoxia in a range of species,^{22,45} but have not related these studies to the developmental windows of HIF activation.

Taken together, these findings indicate that activation of HIF by developmental cardiac hypoxia does play a role in cardiac morphogenesis and that inappropriately timed maternal hypoxia has the potential to disrupt this process and impact adversely on fetal outcome (Figure 2). The exact conditions associated with that risk, however, and the mechanisms involved remain unclear. That interplay is important from a basic science perspective because it offers an insight into the general principles that govern genetic–environmental interactions during development, as well as their impact on postnatal

Table 1. HIF in Cardiac Development

Genetic Intervention	Cre Recombinase Transgene	Outcome	Potential Mechanisms	References
Inactivation of HIF-1 α in cardiomyocytes	<i>MLC2vcre*</i>	Myocardial hyperplasia and arrested heart development with embryonic lethality at \approx E11	Reduced expression of the cardiac transcription factors Mef2C, Tbx5, and titin; defective apoptosis	27
Inactivation of HIF-1 α in cardiac precursor cells	<i>Nkx2-5^{RESCre*}</i>	Small, nonsignificant excess of cardiac abnormalities	Reduced myocardial proliferation	42
Inactivation of CITED2 in cardiac precursor cells	<i>Nkx2.5Cre*</i>	High prevalence of congenital heart defects	Reduced VEGFA	41
Inactivation of HIF-1 α in vascular endothelial cells	<i>Tek-Cre*</i>	Small, nonsignificant excess of cardiac abnormalities	Not tested	42
Inactivation of HIF-1 α in neural crest cells	<i>Wnt1Cre</i>	High prevalence of cardiac abnormalities	Not tested	28
Inactivation of HIF-1 α in mesoderm	<i>MesP1^{Cre*}</i>	High prevalence of cardiac abnormalities and of embryonic lethality (<E17.5)	Not tested	42
Global, inducible inactivation of HIF-1 α	<i>Tamoxifen-inducible β-actinCre</i>	Cardiac abnormalities (and incompletely penetrant embryonic lethality at >E16.5) when tamoxifen treated from E10.5 (but not from E13.5)	Not tested	28

HIF-1 α indicates hypoxia inducible factor-1 α ; Mef2C, myocyte enhancer factor 2C; and Tbx5, T-box 5.

*Used (HIF-1 α or CITED2) flox/- alleles to generate phenotype.

life. From the clinical perspective, congenital heart disease is common (\approx 1% of live births and \approx 20% of still births⁴⁶) and knowledge of familial history, or the presence of associated parental mutations, has the potential to allow for the identification of high-risk pregnancies. That would allow for precautionary measures to be taken in targeted cases to avoid maternal hypoxia or other stresses.

Currently, however, certain questions will need to be answered before such a principle could be applied from a rational perspective. First, it is unclear how hypoxia and HIF activation drive cardiac morphogenesis (Figure 2). One study identified the direct activation of cardiac-specific transcription factors (titin, T-box 5, myocyte enhancer factor 2C).²⁷ More general effects of HIF on apoptosis/survival decisions or metabolic regulation might also be important. In keeping

with this, myocardial proliferation was observed to be reduced by HIF-1 α inactivation (NK2 homeobox 5^{RESCre}) in embryonic hearts.⁴² In the same study, however, maternal hypoxia did not alter proliferation or apoptosis in the developing heart. This was despite activating HIF-1 α . Second, a major uncertainty is the difficulty in relating activation of HIF to clear positive or negative effects on oxygen homeostasis. Although proper cardiac development is clearly required for oxygen delivery, the direct effects of increased or decreased HIF activation during cardiac development are difficult to predict, and their relationship to overall physiological oxygen homeostasis is difficult to define. It is therefore unclear whether (even within the heart) abnormalities are being driven directly by abnormal HIF activity or indirectly by the effect of dysregulated HIF activity on hypoxia itself (Figure 2).

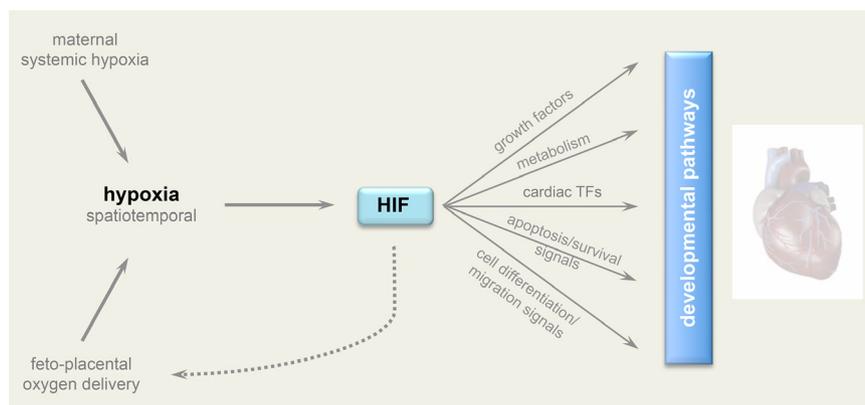


Figure 2. Hypoxia inducible factor (HIF) and cardiovascular development. The developing heart is hypoxic in a spatiotemporal-restricted manner. Physiological hypoxia activates HIF α and HIF-dependent processes (such as cardiac transcription factors, TFs) which interface with developmental pathways to direct cardiogenesis. Disturbances to these spatiotemporal variations in hypoxia (eg, through maternal systemic hypoxia or insufficient feto-placental oxygen delivery) alter HIF expression. This may interfere with cardiac development either directly by disturbing activation of HIF-dependent processes or indirectly by disturbing placentation and therefore feto-placental oxygen delivery to exacerbate hypoxia.

Thus, although the issue is of medical importance, these uncertainties are substantial impediments to predicting clinical effects from experimental studies. Furthermore, clinical data are difficult to interpret. That said, difficulties associated with reproduction at altitude among nonadapted populations have long been appreciated. Spanish settlers of the former Inca empire in Peru in the 16th Century—as an example—knew about the risks.⁴⁷ Offspring from high-altitude pregnancies are smaller, with a figure of 100 g reduction in weight per 1000 m altitude gain being reported.⁴⁸ However, only a small number of studies have reported on the incidence of specific congenital anomalies. In reviewing the literature on obstetrics at high altitude, Gonzales⁴⁷ estimates a 4-fold increase in congenital abnormalities on the Andean plateau, but exact figures for congenital heart disease are difficult to define.

Despite current uncertainties, more detailed analysis of hypoxia signaling in cardiac development, together with clinical studies of genetic predisposition to, and epidemiology of, cardiac anomalies, should provide better understanding and prevention. New molecular insights into these interactions could also inform the more general (and more difficult) question of the mechanistic basis of the fetal effects on more complex later-life cardiovascular phenotypes, such as body mass, blood pressure, and vascular disease. In particular, the recognition that a range of other human 2-oxoglutarate oxygenases are involved in epigenetic regulation of DNA and histone methylation, lipid metabolism and protein synthesis,¹⁵ raises the possibility that other oxygen-dependent processes might mediate the effects of hypoxia on development, and merits exploration.

Cardiovascular Control

In keeping with the fundamental role of the cardiovascular and pulmonary systems in oxygen delivery, control of these organs is exquisitely responsive to oxygen. These controls encompass both rapid and delayed responses. The first reports recognizing this were the studies by Douglas et al⁴⁹ >100 years ago that revealed an immediate increase in ventilation at altitude, followed by a further progressive increase, occurring over a period of days. Classical studies of the pulmonary vascular response to hypoxia also revealed both acute increases in resistance (s/min), and delayed responses occurring over days or weeks.^{50,51}

To date, there is little evidence to support the involvement of the HIF hydroxylase system in mediating more rapid responses to hypoxia that occur over the time-scale of seconds or minutes. Many such rapid responses involve regulated ion channel activity. A large number of oxygen-sensitive ion channels have been described in a range of excitable cells,^{51–53} the first of which being oxygen sensitive K⁺ channels in type I cells of the carotid body,⁵⁴ where hypoxia closes the channel leading to depolarization. The exact origin of the oxygen-sensitive signal operating on most of these channels is unclear despite intense investigation. The speed of the response precludes HIF-mediated transcription. In theory, HIF hydroxylases could signal rapid responses to hypoxia through reduced hydroxylation of

channels or channel-associated proteins. To date, however, no mechanism for reversing such hydroxylation has been described, and without reversal it is difficult to envision how rapid bidirectional changes in channel conductance could be transduced. Nevertheless, in addition to HIF, the asparaginyl hydroxylase FIH hydroxylates a large range of ankyrin repeat domain containing proteins,⁵⁵ including ion channels. For instance, the ankyrin repeat domain in the transient receptor potential cation channel, subfamily V, member 3 (TRPV3) is efficiently hydroxylated by FIH.⁵⁶ Both hypoxia and FIH inhibitors potentiate TRPV3 channel activity, although it remains unclear as to whether rapid responses to oxygen are transduced through FIH activity. PHD2 has also been reported to hydroxylate a prolyl residue in transient receptor potential cation channel, subfamily A, member 1 (TRPA1),⁵⁷ although there have not been any follow-up studies investigating the effect of the proposed site of hydroxylation on physiological responses to hypoxia. A small number of studies have tested the effect of HIF hydroxylase inhibitors on acute responses to hypoxia in integrated systems. Ortega-Sáenz et al⁵⁸ found no effect of the general 2-oxoglutarate oxygenase inhibitor dimethylallylglycine on hypoxia-induced catecholamine release from carotid bodies, whereas in our own laboratory, we observed no immediate effect of a more specific HIF PHD inhibitor (PHI) on ventilation.⁵⁹ One caveat to these studies is that it is difficult to prove that the drug reached the relevant cell population in sufficient concentration.

Overall, it is not possible to exclude the involvement of oxygen-sensing HIF hydroxylases in rapid responses to hypoxia, even though there is little experimental support for that being the case. In contrast, there is abundant evidence that the HIF hydroxylase system operates over longer periods of time to modulate the sensitivity of acute cardiovascular and ventilatory sensitivity to hypoxia. Effects of the system on ventilatory sensitivity have been well reviewed elsewhere.⁶⁰ Here, we focus on cardiovascular control.

Pulmonary Circulation

Responses of the pulmonary circulation to hypoxia differ from those of the systemic circulation in being dominated by vasoconstrictor responses that operate to maintain ventilation-perfusion matching, but become maladaptive in systemic hypoxia associated with altitude or cardiopulmonary disease. The central involvement of the HIF hydroxylase system is supported by both experimental and human studies.

Experimental Studies of Pulmonary Vascular Responses

In contrast with the apparently more central role of HIF-1 in development, both HIF-1 and HIF-2 are critically important for the development of pulmonary hypertension in mouse models. Animals with heterozygous inactivation of either HIF-1 α or HIF-2 α reach adult life and are largely normal in unstressed conditions. HIF-1 α ^{-/-} mice manifest blunted rises in right ventricular pressure and right ventricular hypertrophy in response to chronic hypoxia (10% oxygen for 3 weeks),⁶¹ whereas in a different study HIF-2 α ^{-/-} mice exposed to a similar stress (10% oxygen for 4 weeks) manifest essentially total loss of the pulmonary hypertensive response.⁶² Further, mice

with activating mutations in HIF-2 α spontaneously develop pulmonary hypertension and right ventricular hypertrophy.⁶³ Other evidence for the importance of HIF-2 α in this response has been provided by studies of a mouse model of the human disease Chuvash polycythaemia.⁶⁴ In this condition, biallelic inheritance of a hypomorphic *VHL* allele (R200W) impairs degradation of HIF α isoforms and upregulates HIF.⁶⁵ The development of pulmonary hypertension in this model is partially compensated by heterozygous inactivation HIF-2 α .⁶⁴

The molecular mechanisms underlying these effects seem to be highly complex. Altered expression of ion channels,⁶⁶ transporters (Na⁺/H⁺ exchange⁶⁷), and vasoconstrictors (endothelin-1) have been observed in HIF-1 α defective pulmonary vascular smooth muscle cells.⁶⁸ Moreover, endothelin-1 itself can act to increase expression of HIF-1 α , potentially creating a positive feedback loop.⁶⁹ Mechanisms underlying the action of HIF-2 α have not been clearly defined, although may involve crosstalk with the endothelium where this isoform is strongly expressed.⁷⁰

Studies of cell type-specific inactivation of HIFs have not yielded a coherent picture in respect of the pathogenesis of pulmonary hypertension. Unexpectedly, 1 study of inactivation of HIF-2 α in endothelial cells (vascular endothelial-cadherin-Cre) reported the development of pulmonary hypertension, but this appeared to arise from vascular leakage into the lung parenchyma.⁷¹ Studies of the inactivation of HIF-1 α using timed or developmentally active Cre drivers have revealed apparently conflicting results. In 1 study (using tamoxifen-inducible smooth muscle-specific-Cre, SMMHCCreERT2), conditional loss of HIF-1 α in smooth muscle cells in the adult reduced both pulmonary artery pressure and thickening of the pulmonary arterial wall in response to chronic hypoxia.⁷² Another study (using the smooth muscle-specific conditional, but not inducible smooth muscle protein 22 α -Cre) observed exacerbation of pulmonary hypertension under apparently similar conditions.⁷³ Differences in the extent and timing of the intervention, non-cell autonomous effects and mouse strain background, may all have contributed to differences in outcome.

Taken together, these studies reveal a highly complex interface between HIF activation and the development of pulmonary hypertensive and related phenotypes. Although they clearly demonstrate the potential for interventions targeted to the HIF hydroxylase system to affect these responses, the complexity of interactions makes extrapolation to human clinical intervention difficult to predict.

Human Genetics

The importance of the HIF hydroxylase system in modulating pulmonary vascular function is clearly reflected in human genetics. Altered pulmonary vascular resistance is observed both in single gene disorders affecting HIF hydroxylase system and in high-altitude populations that have been subject to selection of variants at these loci.

Studies of individuals and families ascertained through congenital or familial erythrocytosis have revealed mutations in genes encoding *VHL*, *PHD2*, and *HIF-2 α* .^{74,75} These monogenic forms of hereditary erythrocytosis reflect generalized activation of the HIF hydroxylase pathway and are associated

with exaggerated cellular and systemic responses to hypoxia, including pulmonary vascular responses. Thus, individuals with Chuvash polycythaemia manifest modestly elevated resting pulmonary artery pressures but a greatly exaggerated rise in response to hypoxia in comparison with both normal individuals and those with acquired erythrocytosis.^{76,77} Resting elevation and exaggerated rises in pulmonary artery pressures have also been reported in individuals with mutations in HIF-2 α that are adjacent to 1 of the residues that is targeted for prolyl hydroxylation.^{78,79}

At the population level, genome-wide association studies of altitude-adapted populations living on the Tibetan plateau have identified strongly selected haplotypes at both the *PHD2* and *HIF-2 α* loci.^{80,81} Tibetans manifest reductions in several responses to hypoxia at altitude, including erythropoiesis and pulmonary hypertension, and at sea level they have somewhat reduced pulmonary artery pressure responses to hypoxia.⁸² Although the precise mechanistic basis is not fully understood, it seems likely that the selected alleles are responsible for reduced HIF activation under hypoxia. In keeping with this, kinetic studies of a coding sequence polymorphism in *PHD2* have been reported to manifest a reduction in KmO₂.⁸³

Thus, both monogenic and polygenic human studies strongly implicate the HIF hydroxylase pathway—particularly *PHD2* and *HIF-2 α* —in the regulation of pulmonary vascular responses to hypoxia. Somewhat surprisingly HIF-1 α has not been implicated in these human studies. This may suggest a greater role for HIF-2 α in human pulmonary vascular control. However, it might also indicate that HIF-1 α is more important in other processes whose disruption would preclude a viable adult phenotype, or in the case of the monogenic cases, ascertainment bias through erythrocytosis, where HIF-2 α is the most important isoform.^{84,85}

Role of Iron

Taken together, these studies indicate that the HIF hydroxylase pathway modulates pulmonary vascular responses to hypoxia in a way that may be important clinically. Of interest in this respect is the role of iron status in the regulation of pulmonary vascular responses. The oxygen-sensing HIF hydroxylases are nonhaem iron enzymes in which association of the catalytic iron with the apo-enzyme is labile. In keeping with this, they are strongly inhibited by iron chelators,⁸⁶ which mimic the effect of hypoxia in cultured cells.⁸⁷ This raises the question as to whether physiological or pathophysiological alterations in iron balance might alter cardiovascular responses to hypoxia. In support of this, infusion of desferrioxamine, like sustained hypoxia, results in a delayed increase in pulmonary vascular resistance.⁸⁸ Interestingly, infusion of iron—even to individuals with apparently normal iron balance—greatly reduced the enhanced pulmonary vascular sensitivity to hypoxia that is observed after sustained hypoxic exposure, whereas phlebotomy of patients with erythrocytosis resulted in an increase in pulmonary artery pressure.^{89,90} Although in these human studies, it is not possible to be certain that the effects are because of modulation of HIF, the delayed time course, and reduction of effects on chronic, but not acute hypoxia, support such a mechanism. It is also interesting that a high incidence of iron deficiency has been reported in cohorts of

patients with primary pulmonary hypertension.⁹¹ The findings suggest that the practice of controlling hematocrit in individuals with erythrocytosis secondary to cardiopulmonary disease through phlebotomy may lead to an exacerbation of pulmonary hypertension by iron deficiency. It also suggests that the general pathophysiology of disordered iron metabolism should be reviewed in the light of a potential interface with oxygen-sensing pathways.

Systemic Circulation

In contrast with the pulmonary circulation, hypoxia causes rapid systemic vasodilation. As argued above, it is unlikely that such rapid responses are controlled by the HIF hydroxylase system. However, the extensive interfaces of HIF pathways with processes, such as cardiovascular development, angiogenesis, endothelial function, catecholamine metabolism, energy metabolism, and vasomotor regulators, would suggest modulation of systemic vascular responses at multiple levels. Somewhat surprisingly, this has not been extensively studied, especially when experimental studies have indeed revealed a range of effects on the systemic circulation. For instance, PHD3 is required for physiological, developmental apoptosis of sympathoadrenal cells and PHD3^{-/-} mice manifest hypotension, most probably because of defective sympathoadrenal function.⁹²

Interestingly, several studies suggest that HIF-1 and HIF-2 may have opposing actions on the systemic circulation. Thus, inactivation of either HIF-1 α or HIF-2 α in keratinocytes (using K14cre) results in divergent effects on nitric oxide metabolism (reduced expression of nitric oxide synthase 2 after inactivation of HIF-1 α , and reduced expression of arginases 1 and 2 after inactivation of HIF-2 α), which is associated with increased or decreased systemic blood pressure, respectively.⁹³ However, more general modulation of HIF could have different effects. For instance, HIF-2 α ^{+/-} mice have been found to develop hypertension as a result of unstable ventilatory control.⁹⁴ The complexity and context specificity of these effects therefore makes it difficult to predict the overall effects of modulating the HIF hydroxylase system on the systemic circulation and blood pressure.

Despite these multiple interfaces of HIF pathways on the systemic circulation, general modulation of the HIF system, either genetically or pharmacologically, has not been reported to have major effects on blood pressure. In Chuvash polycythaemia, modest reduction in systemic blood pressure has been reported.⁹⁵ Measurements of blood pressure in animals exposed to HIF PHIs (see below) have generally not revealed significant changes. However, given ongoing trials of these agents in anemia associated with kidney disease, it is of interest that reduced blood pressure has been reported in a rat model of chronic kidney injury after exposure to the compound, BAY85-3934.⁹⁶

Therapeutic Modulation of HIF in Ischemia

Hypoxia is a major component of ischemia disease. In keeping with this, HIF is induced to a variable extent in ischemic tissues⁹⁷⁻⁹⁹ and activates a range of responses that protect cells from hypoxic damage or promote reoxygenation and repair.^{100,101} The principle behind therapeutic modulation of the

HIF pathway is that pharmacological activation could either enhance protective responses during ischemia or, if applied before the event, could moderate ischemic injury by preconditioning the tissue to better withstand the stress.

A range of methods for augmenting the HIF response have been described, including expression of activated HIF genes and genetic or pharmacological targeting of the HIF prolyl hydroxylases. The most advanced of these clinically are small molecule HIF PHIs, which are in late stage trials for therapy of anemia. Nevertheless other types of intervention on the HIF system have been applied in experimental ischemia models, yielding data that could be relevant to clinical application. Here, we will review this work focusing on myocardial ischemia (MI); for reviews of other settings, see Selvaraju et al¹⁰² and Singh et al.¹⁰³

In theory, activation of HIF could improve ischemia outcomes by multiple mechanisms. Some activities, such as reprogramming of metabolism and induction of angiogenesis, have the potential to improve oxygen homeostasis. Other effects of HIF activation (such as on apoptosis, autophagy, cell survival, cell migration, and stem cell behavior) might also assist protection. These effects vary greatly in terms of predicted time course. For instance, metabolic changes will likely be rapid, whereas structural changes to the vasculature will likely take time to develop. Therefore, the most appropriate mode of application (ie, duration and timing of intervention) is not straightforward to predict. Nevertheless, 2 types of empirical observation support the value of HIF activation in ischemia protection. First, negative intervention on the HIF pathway impairs different types of ischemic preconditioning. Second, positive intervention on the HIF pathway improves ischemia outcomes, at least under some circumstances.

Ischemia Preconditioning

It has long been established that application of an ischemic stress can provide protection against damage suffered during a subsequent episode of ischemia, a phenomenon known as preconditioning.^{101,104} The preconditioning stimulus can be direct (ie, applied to the organ itself) or remote. Different processes are thought to underlie different types and timing of preconditioning effects. Substantial evidence supports a role for HIF activation in both direct and remote effects (Table 2).

Thus, a range of preconditioning stimuli that potentially operate directly (eg, intermittent coronary occlusion,¹⁰⁷ ischemia-reperfusion of the isolated heart¹⁰⁶), remotely (eg, intermittent femoral artery ligation^{105,109}), or by both routes (eg, intermittent systemic hypoxia¹⁰⁸) have been used to assess the role of the HIF in preconditioning the myocardium.¹¹⁰ Studies that have considered these stimuli have examined effects of both long-term impairment of the HIF pathway (eg, heterozygosity for HIF-1 α ^{105,106,108,109}) and acute knockdown in HIF-1 α (eg, intracardiac infusion of small interfering RNAs targeting HIF-1 α ¹⁰⁷). Overall, the data support involvement of HIF in both direct and remote preconditioning, and on both early (\approx 3–5 hours)¹⁰⁵⁻¹⁰⁷ and longer term (\approx 1 day)^{108,109} cardioprotective outcome in response to ischemia (Table 2). A wide range of associated activities have been highlighted (Table 2), although given the complexity of HIF pathways the importance of a single defined mechanism is often difficult to

prove. Although these studies indicate that activation of HIF can make important contributions to ischemia preconditioning, not all studies have been positive. For instance, using an intermittent femoral artery occlusion as a model of remote preconditioning, Kalakech et al¹⁰⁵ did not observe loss of protection against MI in HIF-1 α heterozygous mice.

Effects on Acute MI

Improved outcomes from experimental MI have been reported following several different genetic strategies that aim to augment activation of HIF in the ischemic tissue (Table 3). These include transgenic cardiomyocyte-specific overexpression of HIF-1 α ,¹¹¹ small interfering RNAs/small hairpin RNAs targeting the HIF hydroxylase PHD2 delivered into the left ventricular cavity¹⁰⁷ or myocardium,¹¹² cardiomyocyte-targeted inactivation of PHD2 by Cre-recombinase,¹¹³ and a PHD2 hypomorphic mouse line expressing variably reduced levels of PHD2 in the heart and other tissues.^{114,115} In combination, these studies strongly suggest that there are potential benefits from activation of HIF in the ischemic myocardium. However, there are several caveats, particular in respect of clinical application. First, the intervention precedes^{107,111,113–120} or is applied immediately at time of the ischemic challenge,¹¹² which may be difficult to achieve clinically. Second, most assessments have been made in the short-term and long-term measurements have not always mirrored short-term benefits. For instance, in a mouse model of myocardial infarction small hairpin RNA targeting PHD2 was reported to improve left ventricular fractional shortening at 4 weeks but not 8 weeks postinfarction.¹¹² Finally, some studies report negative outcomes from HIF activation, with overexpression of either HIF-1 α or HIF-2 α resulting in the spontaneous development of cardiomyopathy.^{111,121–123}

Other studies have directly assessed small molecule PHIs in mouse or rat models of MI and have also reported beneficial effects. These studies reveal benefit when animals are exposed to PHIs either before^{107,119,124–128,130} or after^{125,129,131} induction of ischemia and when PHIs are used just at the time of ischemia^{107,119,124–128} or for ≤ 4 weeks after the event (Table 3).^{129–131} Importantly, 1 study (of the compound GSK360A) also provided functional data at 3 months (2 months after cessation of treatment) and demonstrated a persistent, albeit smaller, improvement of left ventricular ejection fraction.¹³¹

Taken together with genetic models, these studies indicate that inhibition of HIF prolyl hydroxylases can improve outcome in MI. However, they do not exclude at least some of the reported effects arising from actions on other targets. Dimethylxalylglycine has been extensively used as an activator of HIF pathways in MI.^{107,127,128} Although it is a powerful inhibitor of both HIF prolyl and asparaginyl hydroxylases, it is nonselective with variable activity against most 2-oxoglutarate oxygenases which may have other relevant actions. Interestingly, early PHIs were developed as procollagen PHIs and the initial report of action on experimental MI assigned beneficial effects of 1 compound FG0041 to inhibition of collagen synthesis.¹²⁹ Subsequent work revealing beneficial actions of more specific HIF PHIs (FG2216, GSK360A) suggests that these effects are likely to be due mainly to actions on the HIF system.^{119,125,131,133} GSK360A is reported to possess ≈ 10 -fold selectivity for PHD2 over procollagen prolyl hydroxylase (Ki 100 nmol/L versus 1 μ mol/L).¹³¹ However, there is little data on other reportedly selective compounds.

Interestingly, studies of small interfering RNA-based intervention have unexpectedly suggested that activity against procollagen hydroxylases might be beneficial. In a series of articles, Natarajan et al¹¹⁷ describe activation of HIF and

Table 2. Effect of HIF on Cardiac Preconditioning

Intervention	Preconditioning	Ischemia Model	Outcome	Onset of Protection	Potential Mechanisms	References
HIF-1 α ^{+/-} mice	Intermittent limb ischemia (4 cycles of: 5-min femoral artery ligation, 5-min reperfusion) immediately followed by MI	30-min LAD ligation	No effect on infarct size 2 h after MI	< ≈ 3 h	Not tested	105
HIF-1 α ^{+/-} mice	Global ischemia (10 min) in isolated heart 5 min before MI	30-min ischemia in isolated heart	Reversal of conditioning induced reduction in infarct size 2 h after MI	< ≈ 3 h	Increased apoptosis; reduced ROS production/PTEN oxidation/AKT phosphorylation	106
Two-hour intracardiac infusion of HIF-1 α siRNA before conditioning	Intermittent myocardial ischemia (4 cycles of: 5-min ischemia, 5-min reperfusion) 0–4 h before MI	60-min LAD ligation	Reversal of conditioning induced reduction in infarct size 2 h after MI	< ≈ 5 h	Loss of CD73 and A2BAR induction	107
HIF-1 α ^{+/-} mice	Intermittent hypoxia in whole mouse (5 cycles of: 6-min 6% oxygen, 6-min normoxia) 24 h before MI	30-min global ischemia in isolated heart	Reversal of conditioning induced reduction in infarct size 2 h after MI	< ≈ 1 d	Loss of Epo induction (proposed remote preconditioning)	108
HIF-1 α ^{+/-} mice	Intermittent limb ischemia (3 cycles of: 5-min femoral artery ligation, 5-min reperfusion) 24 h before MI	30-min LAD ligation	Reversal of conditioning induced reduction in infarct size 2 h after MI	< ≈ 1 d	Loss of IL-10 induction	109

A2BAR indicates alpha 2b adrenergic receptor; Epo, erythropoietin; HIF-1 α , hypoxia inducible factor-1 α ; IL-10, interleukin-10; MI, myocardial ischemia; LAD, left anterior descending coronary artery; PTEN, phosphatase and tensin; and ROS, reactive oxygen species.

Table 3. Protection From Acute Ischemia

Intervention	Ischemia Model	Outcome	Onset of Protection	Potential Mechanisms	References
PHD2 hypomorphic mice	20-min global ischemia in isolated heart	Improved cardiac function (rate pressure product, dP/dt_{max}) ≤ 45 min after MI	<1 h*	Metabolic reprogramming	114
PHD1 ^{-/-} mice	30-min global ischemia in isolated heart	Reduced infarct size 2 h after MI	Less than ≈ 3 h*	Decreased apoptosis	116
Two hour left ventricular infusion of PHD2 siRNA before MI	60-min LAD ligation	Reduced infarct size 2 h after MI	Less than ≈ 5 h	Induced A2BAR	107
PHI (DMOG) 2hr before MI	60-min LAD ligation	Reduced infarct size 2 h after MI	Less than ≈ 5 h	Protection lost in A2BAR ^{-/-} and cd73 ^{-/-} mice, as well as with HIF-1 siRNA	107
PHI (DFO) 0, 2, 24, 48, 72, or 96 h before MI	30-min LAD ligation	Reduced infarct size measured 3 h after MI with intervention at 2, 24, 48, 72 h (but not at 0 and 96 h) time points	Less than ≈ 6 h	Accumulation of oxygen radicals, activation of protein kinase C	124
Cardiomyocyte-specific PHD2 ^{-/-} mice	Permanent LAD ligation	Reduced infarct size and fractional shortening 6 h after MI	Less than ≈ 6 h*	Reduced apoptosis; increased capillary surface area	113
PHI (FG2216): 1 and 6 h before MI or 1 and 5 h after MI	Permanent LAD ligation	Reduced infarct size 6 h after MI with all ICA treatments: 1 and 6 h before MI or 1 and 5 h after MI	Preconditioning: less than ≈ 12 h; postconditioning: less than ≈ 5 h	Not tested	125
PHD2 hypomorphic mice	Permanent or 30-min LAD ligation	Improved ejection fraction, fraction shortening, and improved perfusion 24 h after MI	Less than ≈ 1 d	NO-mediated vasodilation	115
PHI (cobalt chloride) 24 h before MI	20-min global ischemia in isolated heart	Reduced infarct size 30 min after MI	Less than ≈ 1 d	Protection lost in iNOS ^{-/-} mice	126
Intraperitoneal injection of P4HA2 siRNA 24 h before MI	30-min global ischemia in isolated heart	Improved left ventricular function and reduced infarct size 60 min after MI	Less than ≈ 1 d	Protection lost in iNOS ^{-/-} mice	117
Intraperitoneal injection of P4HA2 siRNA 24 h before MI	30-min LAD ligation	Reduced infarct size 120 min after MI	Less than ≈ 1 d	Reduced proinflammatory chemokine expression	118
PHI (DMOG) 24 h before MI	30-min LAD ligation	Reduced infarct size 3 h after MI	Less than ≈ 1 d	Enhanced HO-1 associated attenuated proinflammatory chemokine production	127
PHI (DMOG) 24 h before MI	30-min LAD ligation +/- subsequent intermittent ischemia (postconditioning)	DMOG reduces infarct size 3 h after MI, in particular with postconditioning treatment	Less than ≈ 1 d	Induced iNOS	128
PHI (GSK360A) 4 h before MI	30-min LAD ligation	Reduced infarct size 24 h after MI	Less than ≈ 1 d	Metabolic reprogramming and less MPTP opening	119
PHI (FG0041) twice daily starting 48 h after MI	Permanent LAD ligation	Reduced loss of ejection fraction 1–4 wk after MI	Less than ≈ 1 wk	Inhibition of collagen synthesis	129
PHI (FG2216) twice daily starting 48 h before MI	Permanent LAD ligation	Improved cardiac function (but no effect on infarct size) 7 and 30 d after MI	Less than ≈ 1 wk	Unknown	130
Heart-specific, conditional VHL ^{-/-} (tamoxifen started 5 d before MI)	Permanent LAD ligation	Reduced infarct size (unclear when harvested post MI)	Less than ≈ 1 wk	Decreased superoxide production and MPTP opening	119
Global, conditional PHD3 ^{-/-} mice (starting intracardiac tamoxifen 1 wk before MI)	40-min LAD ligation	Reduced infarct area 3 d after MI	Less than ≈ 10 d	Reduced apoptosis, reduced DNA damage response. No changes in capillary density	120
Intramyocardial PHD2 shRNA injection 10 min after MI	Permanent LAD ligation	Improved fractional shortening 2 and 4 wk after MI	Less than ≈ 2 wk	Increased capillary density	112

(Continued)

Table 3. Continued

Intervention	Ischemia Model	Outcome	Onset of Protection	Potential Mechanisms	References
PHI (GSK360A) for 4 wk starting 48 h after MI	Permanent LAD ligation	Reduced loss of ejection fraction 2 and 4 wk after MI; no effect on infarct size	Less than \approx 2 wk	Increased vessel density	131
Cardiac HIF-1 α overexpressing mice	Permanent LAD ligation	Attenuated infarct size and improved cardiac function 4 wk (but not 24 h) after MI	Greater than \approx 1 d Less than \approx 4 wk†	Increased capillary density	111

FG2216 assigned as 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido) acetic acid¹³² (also known as ICA¹³³ or IOX3⁵⁹). A2BAR indicates alpha 2b adrenergic receptor; DFO, desferrioxamine; DMOG, dimethylxylglycine; HIF-1 α , hypoxia inducible factor-1 α ; iNOS, inducible nitric oxide synthase; LAD, left anterior descending coronary artery; MI, myocardial ischemia; MPTP, mitochondrial permeability transition pore; NO, nitric oxide; P4HA2, procollagen prolyl hydroxylase α chain 2; PHD, prolyl hydroxylase domain; PHI, prolyl hydroxylase inhibitor; shRNA, small hairpin RNA; and siRNA, small interfering RNA.

*This is a constitutive knockout so it is unclear when protective effects come into place.

†Although this is a constitutive transgenic model, HIF is not stabilized and may not be expressed before MI.

beneficial effects on ischemia (including attenuation of myocardial injury and inflammatory responses and activation of endoplasmic reticulum stress pathways) of an small interfering RNA targeting a prolyl hydroxylase. Although the original description refers to this sequence as targeting mouse PHD2 (the principal HIF prolyl hydroxylase), in fact the reported sequences were those of procollagen prolyl hydroxylase alpha chain 2 (P4HA2), and referred to as such in 2 subsequent articles.^{118,134} Why inhibition of procollagen hydroxylases should induce HIF is unclear, but the work suggests that it would be premature to assign all effects of prolyl hydroxylase inhibition in MI to actions on HIF hydroxylases.

Induction of Cardiomyopathy

Set alongside potential benefits of HIF activation in MI are a series of reports of reduced cardiac function after sustained activation of HIF or inhibition of PHDs. Overexpression of either HIF-1 α or HIF-2 α has been associated with the spontaneous development of cardiomyopathy.^{111,121–123} So although cardiomyocyte-specific HIF-1 α overexpression improved outcome from MI,¹¹¹ further studies using the same model of transgenic (α -myosin heavy chain promoted) cardiomyocyte-specific HIF-1 α overexpression revealed the development of age-dependent cardiomyopathy and decompensation in response to aortic constriction.¹²¹ In a different study using a tetracycline-inducible stabilized HIF-1 α transgene, cardiomyopathy was observed as soon as 3 days after transgene induction, but was reversed when induction was stopped.¹²³ In another study, cardiomyocyte-specific activation of a stabilized HIF-2 α gene was associated with progressive cardiomyopathy and features of heart failure.¹²² Both general and cardiac-specific inactivation of PHD2 have been associated with the development of cardiomyopathy. This seems to be associated with the extent of HIF activation. Thus, inactivation of PHD2,¹²² combined inactivation of PHD2/PHD3,¹²² and inactivation of VHL¹³⁵ result in progressively more powerful up-regulation of HIF and progressively more severe phenotypes, whereas partial inactivation of PHD2 has not been associated with cardiomyopathy.¹¹⁴

The molecular processes underlying HIF-associated cardiomyopathy are unclear. In keeping with established functions of HIF in energy metabolism, a shift toward glycolysis

is suggested by increased expression of glycolytic genes and increased fludeoxyglucose-positron emission tomography signals.¹²¹ Together with abnormal mitochondrial morphology, this might support defective energy metabolism as the underlying cause.¹²² Counterintuitively, however, measurement of ATP levels in 1 model revealed an increase, rather than decrease, suggesting a defect in energy utilization.¹²³ This and 1 other study of HIF-1 α overexpression observed reduction in expression of the sarcoplasmic/endoplasmic reticulum calcium ATPase, but the 2 studies reported different abnormalities in calcium movements.^{121,123}

Whatever the mechanism, consistent observation of cardiomyopathy in association with HIF activation needs to be set against the benefits in acute ischemia (Figure 3). Confirmation of this dichotomy is provided by studies in which both benefits in acute ischemia and long-term cardiomyopathy has been observed in the same model.^{111,121,122}

Clinical Application

PHIs are now in late-stage clinical trials for the treatment of anemia,¹³⁶ raising important questions as to their clinical effects on ischemic heart disease. Apparent dependence of cardiomyopathy on the extent of HIF activation,^{121,122} together with evidence for reversibility¹²³ suggests that with appropriate timing and dosing in ischemia this problem could be avoided. Nevertheless, differences observed in the onset of cardiomyopathy in different models make the precise window difficult to predict. Use in clinical ischemic heart disease will also need to take account of noncardiac effects, such as excessive stimulation of erythropoiesis, and potential effects on pulmonary vascular responses, angiogenesis, and inflammation,¹³⁷ including the atheromatous process (in which immunohistochemical analysis of HIF-1 α expression has been associated with an inflammatory phenotype¹³⁸).

These considerations are also important in the use of HIF PHIs as an alternative to recombinant Epo in the treatment of anemia. Although recombinant Epo is relatively safe and protective effects in experimental ischemia have been observed,^{139,140} an increased incidence of cardiovascular events has been observed in patients receiving high doses.¹⁴¹ High plasma levels of Epo have adverse effects on the vasculature and may be responsible for this toxicity.¹⁴¹ Since

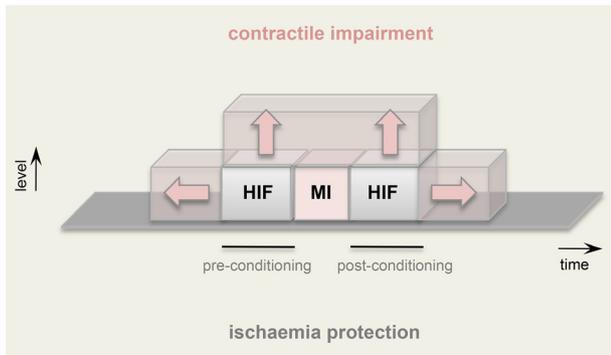


Figure 3. Therapeutic modulation of hypoxia inducible factor (HIF). Activation of HIF in the heart, either before or after myocardial ischemia (MI), confers ischemia protection (known as preconditioning or postconditioning, respectively). However, if HIF activation is either prolonged in duration or excessive in its level (pink arrows), then it may result in contractile impairment and cardiomyopathy. This suggests a temporal and dose-related therapeutic window for optimal effects of HIF activation on cardiac function.

stimulation of native Epo production by PHIs has a different time course from injected recombinant Epo and entrains other processes that support erythropoiesis.^{102,142,143} PHIs have the potential to correct anemia with only small increments in plasma Epo. They therefore offer the potential for improved cardiovascular safety. Clearly, this potential would be enhanced if they could be used at doses that offered additional cardioprotection.

There are several appealing clinical possibilities. The first is that the use of low dose PHIs would effectively correct anemia while providing modest HIF activation in the heart that would protect against ischemia, without generating cardiomyopathy or other unwanted effects. Interestingly, as with most drugs, PHIs seem to be strongly concentrated in the liver and kidneys (the Epo-producing organs),¹⁴⁴ so at low doses they may indeed only produce modest, and potentially beneficial, activation of HIF in the heart. Unfortunately, this is difficult to predict empirically. However, it is of interest that molecules being developed by different companies are structurally diverse^{142,145} and seem to show different organ-specific activation of HIF.¹⁴⁶ A key challenge is therefore to examine for differences in clinical activity in the heart that might be used to guide dosing or differentiate molecules in respect of erythropoietic versus cardioprotective efficacy. One possibility is that cardiac positron emission tomography scanning could be used to identify (and quantify) effective clinical activation of HIF in the heart and guide dosing schedules.

Other appealing clinical applications of PHIs would be for the primary treatment of ischemic heart disease, either short-term coincident with acute ischemia (eg, acute coronary syndrome or cardiac surgery) or in chronic MI that is unsuitable for revascularization. Clearly in chronic ischemia there will be a critical need to define dosing schedules, including the possibility of repeated short exposures that might effectively improve ischemia without entraining excessive erythropoiesis or adverse effects on cardiac function. Yet another possibility is nonsystemic use, whereby appropriately timed limited local delivery, for instance via an intracoronary stent, might achieve

a beneficial effect on ischemic tissue downstream of the intervention, without risk of unwanted systemic actions.

Perspectives

In summary, the elucidation of pathways that signal hypoxia, together with the development of therapeutic agents that can modulate these pathways, has opened up a new field of cardiovascular research with potentially important clinical implications. Extensive studies involving mouse and human genetics, coupled with those involving pharmacological modulation of HIF, have implicated the HIF pathway in almost all aspects of cardiovascular development and control. Many questions, however, remain unanswered. In particular, the mechanisms by which HIF exerts its effects on cardiac morphogenesis, pulmonary/systemic vascular control, and ischemic preconditioning/ischemic protection are ill-defined. The complexity of the interactions involved makes elucidation of the mechanisms, and therefore extrapolation to the clinic, difficult to predict. In our view, systematic dissection of the dose and time windows underlying adverse and beneficial effects on cardiovascular disease will be required to maximize benefit. This should include systematic, empirical studies in animal models to understand mechanisms and better define the windows of opportunity, coupled with extensive human experimental medicine studies aimed at defining the best clinical entry points and modes of application in cardiovascular diseases.

Sources of Funding

The work in the authors' laboratory was supported by the Wellcome Trust and the Ludwig Institute for Cancer Research.

Disclosures

P.J. Ratcliffe is a scientific cofounder and holds equity in ReOx Ltd, a university spin-out company that seeks to develop therapeutic inhibitors of the HIF hydroxylases. T. Bishop reports no conflicts.

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Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



HIF Hydroxylase Pathways in Cardiovascular Physiology and Medicine Tammie Bishop and Peter J. Ratcliffe

Circ Res. 2015;117:65-79

doi: 10.1161/CIRCRESAHA.117.305109

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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