Hypoxia-Inducible Factor-1 Is Central to Cardioprotection
A New Paradigm for Ischemic Preconditioning

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Background—Ischemic preconditioning provides strong cardioprotection from ischemia, but its molecular mechanisms remain unknown. Convincing evidence confirms a central role of hypoxia-inducible factor (HIF)-1 in mammalian oxygen homeostasis. Thus, we pursued HIF-1 as a central component of cardioprotection by ischemic preconditioning.

Methods and Results—Murine studies of in situ preconditioning revealed a robust activation of cardiac HIF-1α. Moreover, in vivo small interfering RNA repression of cardiac HIF-1α resulted in abolished cardioprotection by ischemic preconditioning. In contrast, pretreatment with the HIF activator dimethyloxalylglycine was associated with cardioprotection similar to that of ischemic preconditioning itself. Finally, selective small interfering RNA repression of prolylhydroxylase 2 resulted in significant activation of HIF-1α and attenuated myocardial infarct sizes (0.44±0.09-fold). As an end point of HIF-dependent cardioprotection, we defined the role of A2B adenosine receptor (A2BAR) signaling. Although the cardiac A2BAR was induced with HIF activation, HIF-dependent cardioprotection was abolished in A2BAR−/− mice.

Conclusion—Taken together, these studies provide evidence for a critical role of HIF-1 in ischemic preconditioning via enhancing purinergic signaling pathways. (Circulation. 2008;118:166-175.)

Key Words: adenosine  ■ hypoxia-inducible factor 1  ■ ischemia  ■ ischemic preconditioning  ■ myocardial infarction

Ischemic preconditioning (IP) is defined as an experimental technique in which tissues are rendered resistant to the deleterious effects of ischemia/reperfusion injury by prior exposure to brief, repeated periods of vascular occlusion. For example, myocardial IP is associated with increased myocardial resistance to hypoxia, resulting in a profound reduction in myocardial infarct sizes.1 Multiple studies have attempted to identify molecular mechanisms involved in cardioprotection by IP. For example, polygenic responses involving mediators such as adenosine, bradykinin, opioids, erythropoietin, adrenergics, muscarinic signaling pathways, or protein kinase Cε (PKCε) activation have been proposed.2,3 However, a profound reduction in morbidity and mortality from acute myocardial infarction, as would be expected from the initial observation,1 has not been achieved in patients.

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Cardioprotection by IP involves alteration of the myocardial cellular phenotype to become more resistant to subsequent hypoxic tissue challenges. Over the past decade, convincing evidence has been shown that the transcription factor hypoxia-inducible factor (HIF)-1 has an essential role in the maintenance of oxygen homeostasis in mammalian organisms.4–13 A number of very elegant studies, exemplified by those defining induction of the erythropoietin (EPO) gene,14,15 have used multidisciplinary approaches to elucidate this pathway. HIF-1 exists as an α/β heterodimer, the activation of which depends on stabilization of an oxygen-dependent degradation domain of the subunit by the ubiquitin-proteasome pathway.6,16 HIF-1 appears to reside in the cytoplasm of normoxic cells, and like a number of other transcription factors, HIF-1 translocates to the nucleus to form a functional complex.13 Subsequently, it was determined that HIF-1 is widely expressed and that consensus HIF-1 binding sequences exist in a number of genes other than EPO and are called hypoxia-responsive enhancers.17 Thus, the discovery of HIF-1 represented a major advance in the understanding of gene regulation by hypoxia.5,18–20

Recent studies have more clearly defined the mechanisms that regulate HIF-1 activity. HIF expression depends predominantly on the degradation of the α subunit in normoxia, which occurs through the ubiquitin-proteasomal pathway.21,22
Oxygen-dependent degradation of HIF-1α is triggered by binding of the von Hippel–Lindau tumor-suppressor protein (VHL), which interacts with the protein elongin C, resulting in proteasomal degradation. VHL binding depends on the hydroxylation of proline residue 402, 564, or both by the prolylhydroxylase 2 (PHD2). PHD1 and PHD3 also hydroxylate HIF-1α when overexpressed, but their physiological functions have not been established. PHD2 activity is reduced under hypoxic conditions as a result of either substrate limitation or inhibition of the catalytic center, resulting in stabilization of HIF-1α. On the basis of the central role of HIF-1 in mammalian adaptation to hypoxia, we hypothesized a critical role of HIF-1α in coordinating cardioadaptive responses elicited by IP to render the heart more resistant to subsequent hypoxic episodes. To pursue this hypothesis, we used a recently developed model of murine in situ IP using a hanging-weight system for intermittent coronary occlusion that essentially avoids any surgical tissue trauma and results in highly reproducible infarct sizes.

Methods

Murine Model of Myocardial Ischemia and IP

Murine myocardial ischemia and IP were performed as described previously (for details, see the online-only Data Supplement). Because previous studies in this model have revealed optimal cardioprotection with 4 cycles of IP treatment (5 minutes of ischemia, 5 minutes of reperfusion), we used this regimen throughout the present study.

In Vivo Small Interfering RNA Repression

For in vivo small interfering RNA (siRNA) repression, a model of intraventricular siRNA infusion was developed (for details, see the online-only Data Supplement; siRNA sequences are summarized in supplemental Table I).

Transcriptional Analysis, Western Blot Analysis, and Immunohistochemistry

Transcriptional analysis, Western blot analysis, and immunohistochemistry were performed as described previously (for details, see the online-only Data Supplement; primer sequences are summarized in supplemental Table II).

Adenosine Measurements

Tissue adenosine levels were determined via high-performance liquid chromatography as described previously.

Pharmacological Agents

As described previously, the PKC inhibitor GF 109203X (Tocris Bioscience, Ellisville, Mo) was given intraperitoneally at a dose of 4.2 μg 30 minutes before IP treatment.

Data Analysis

Data were compared by 2-factor ANOVA with Bonferroni posttest or by Student t test when appropriate. Values are expressed as mean±SD from 6 animals per condition. For analysis of changes in transcript, a 1-way ANOVA was carried out, and multiple comparisons between control and treatment groups were made with the Dunnett posttest. Data are expressed as mean±SEM. Values of P<0.05 were considered statistically significant. For all statistical analyses, GraphPad Prism 5.0 software for Windows XP (GraphPad Software, San Diego, Calif) was used.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

In Situ IP Treatment Leads to Stabilization of Cardiac HIF-1α In Vivo

Transcriptional adaptation to hypoxia is currently an area of intense investigation, and convincing evidence has shown HIF to be a key transcriptional regulator of mammalian oxygen homeostasis and adaptation to limited oxygen availability. Because cardiac IP results in profound increases in myocardial resistance to ischemic tissue injury, we hypothesized a critical role of HIF-1 in transcriptional coordination of cardiac IP. To study this hypothesis, we used a previously described model of murine in situ IP via a hanging-weight system for intermittent coronary occlusion. We first gained insight from studies of...
murine in situ IP followed by excision of preconditioned myocardial tissues at different time points after IP treatment (Figure 1A). We used these tissues to study HIF-1 activation by performing Western blot analysis for HIF-1α/H9251. In fact, we found a robust and persistent stabilization of HIF-1α/H9251 protein after IP treatment (Figure 1B). Consistent with previous studies showing PKC in cardioprotection by IP and HIF stabilization,26 IP-associated HIF stabilization was attenuated after treatment with a PKC inhibitor (Figure 1C). Immunohistochemical staining for HIF-1α in preconditioned myocardium localized HIF-1α to the cytosole and the nucleus of preconditioned myocardium (Figure 1D).

siRNA Repression of HIF-1α Abolishes Cardioprotection of IP Treatment
To study a functional contribution of HIF-1α to cardioprotection by IP, we next developed an in vivo siRNA technique to repress cardiac HIF-1α. For this purpose, we infused a commercially available mixture of siRNA molecules specifically designed to target HIF-1α combined with a transfection agent (siPORT Amine, Ambion, Austin, Tex) into the left ventricle. After advanced correct position of the catheter within the left ventricle. After correct placement of the catheter, an infusion of target or csIR, together with transfection agent (siPORT Amine), was given over the indicated time periods (1.5 μg siRNA/g body weight). We used these tissues to study HIF-1 activation by performing Western blot analysis for HIF-1α/H9251. In fact, we found a robust and persistent stabilization of HIF-1α/H9251 protein after IP treatment (Figure 1B). Consistent with previous studies showing PKC in cardioprotection by IP and HIF stabilization,26 IP-associated HIF stabilization was attenuated after treatment with a PKC inhibitor (Figure 1C). Immunohistochemical staining for HIF-1α/H9251 in preconditioned myocardium localized HIF-1α to the cytosole and the nucleus of preconditioned myocardium (Figure 1D).

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contrast, transcript levels of HIF-1α were unchanged with infusion of the control siRNA (csiR). To determine whether other HIF isoforms are affected by this treatment, we also measured HIF-2α transcript levels. In fact, HIF-2α transcript levels were unchanged in HIF-1α–siRNA– or control-siRNA–treated mice (data not shown). Similarly, increases in HIF-1α activity as measured from preconditioned myocardium by Western blot analysis confirmed HIF-1α activation with preconditioning in csiR-treated mice, whereas HIF-1α activation was abolished in mice treated with siRNA specific for HIF-1α (Figure 2C). Measurements of hemodynamic parameters (blood pressure and heart rate; supplemental Tables III and IV) revealed no off-target effects of siRNA treatment. To study functional consequences of DMOG treatment, mice were subjected to 60 minutes of ischemia followed by 120 minutes of reperfusion. Infarct sizes are expressed as percentage of the area at risk (mean±SD; n=6). Note that although untreated myocardium showed only weak immunoreactivity, staining for HIF-1α was significantly increased after DMOG treatment (arrow marks cytosolic; star shows nuclear HIF-1α staining). C, Functional consequences of DMOG treatment on cardiac infarct size. C57BL/6 mice were anesthetized and, after left ventricular catheter placement, were treated with infusion of csiR or HIF-1α–specific siRNA transfection agent; they then received 1 mg DMOG or vehicle control. Two hours after DMOG treatment, mice were subjected to 60 minutes of ischemia followed by 120 minutes of reperfusion. Infarct sizes are expressed as percentage of the area at risk (mean±SD; n=6). D, Representative images of infarcts from the experiment are displayed in D. E, Troponin I plasma levels were measured by ELISA (mean±SD; n=6). Note the attenuated infarct size after DMOG in mice with csiR treatment and abolished cardioprotection by DMOG after HIF-1α siRNA. WT indicates wild type.

HIF Activator Dimethylxaloylglycine Attenuates Myocardial Infarction

To study HIF activation in myocardial ischemia, we first pursued a pharmacological approach using the pro-hydroxylase inhibitor dimethylxaloylglycine (DMOG), which is known to stabilize HIF-1α protein via nonspecific inhibition of PHD1, PHD2, and PHD3.27 As a first step, we used different dosing regimens (0.01 to 1 mg/mouse) and timing intervals (1 to 12 hours) (data not shown), which revealed maximal HIF-1α activation >2 hours after 1 mg DMOG was given intraperitoneally (Figure 3A and 3B). Next, we used this pharmacological approach in functional studies. These studies revealed significant cardioprotection with DMOG (0.34±0.06-fold reduction in infarct size; Figure 3C). This also was obvious from representative infarct staining (Figure 3D) or plasma troponin I levels (Figure 3E) but was abolished after HIF-1α–siRNA repression. Taken together, these results suggest HIF activation by DMOG in attenuation of myocardial ischemia/reperfusion injury.

PHD2 Is Critical for HIF-1-Dependent Cardioprotection

We next pursued studies to gain more specific insights into the mechanisms of HIF-1 stabilization during cardiac IP. In fact,
Figure 4. In situ IP after in vivo siRNA repression of PHD1, PHD2, or PHD3. A–C, In vivo siRNA repression of PHD1, PHD2, or PHD3. C57BL/6 mice were treated with a left ventricular infusion of siRNA specific for PHD1, PHD2, or PHD3 or csiR. After the indicated time periods, hearts were excised; total RNA was isolated; and PHD1 (A), PHD2 (B), and PHD3 (C) transcript levels were determined by real-time reverse-transcription polymerase chain reaction. Data were calculated relative to an internal housekeeping gene (β-actin) and are expressed as fold change vs control (0 hour of siRNA infusion ± SEM at each indicated time; n = 6). D through F, PHD Western blot analysis of cardiac tissue after siRNA repression of individual PHDs. C57BL/6 mice were treated with control or PHD1-, PHD2-, or PHD3-specific siRNA (see above); cardiac tissues were excised at indicated time points, flash-frozen, and lysed; proteins were resolved by SDS-PAGE; and the tissues were transferred to nitrocellulose. Membranes were probed with an anti-PHD1 (D), anti-PHD2 (E), or anti-PHD3 (F) antibody. The same blots were probed for β-actin expression as a control for protein loading. One representative experiment of 3 is shown. G through I, HIF-1α Western blot analysis of cardiac tissue after siRNA repression of individual PHDs. C57BL/6 mice were treated with control or PHD1-, PHD2-, and PHD3-specific siRNA (see above); cardiac tissues were excised at indicated time points, flash-frozen, and lysed; proteins were resolved by SDS-PAGE; and tissues were transferred to nitrocellulose. Membranes were probed with an anti-HIF-1α antibody. The blot probed for β-actin expression was the control for protein loading. One representative experiment of 3 is shown. J–L, Myocardial infarct size after PHD1, PHD2, or PHD3 repression. C57BL/6 mice were treated with a left ventricular infusion of siRNA specific for PHD1, PHD2, or PHD3 or csiR combined with a transfection agent (see above). After 2 hours of siRNA infusion, mice were subjected to 60 minutes of ischemia. Mice were killed after 120 minutes of reperfusion, and infarct sizes were measured. Infarct sizes are expressed as percentage of the area at risk (mean ± SD; n = 6). Troponin I plasma levels were measured by ELISA (mean ± SD; n = 6). Note the attenuated myocardial infarct sizes after PHD2 repression.
previous studies have shown that oxygen-dependent transcription pathways (eg, for HIF-1 or nuclear factor-kB) are controlled by PHD1, PHD2, and PHD3.5,6,9,28 Thus, we hypothesized that during IP intermittent oxygen deprivation may inhibit PHD activity, resulting in cardiac HIF-1α stabilization. To pursue this hypothesis and to study the contribution of individual PHDs (PHD1, PHD2, and PHD3) to HIF-1 stabilization and cardioprotection by IP, we used specific siRNAs to individually target PHD1, PHD2, and PHD3. We applied siRNA for PHD1, PHD2, and PHD3 via intraventricular infusion together with a transfection agent (siPORT Amine) over 2 hours (1.5 μg/g body weight). In fact, this treatment was associated with significant repression of PHD1 (0.1±0.2-fold; Figure 4A), PHD2 (0.11±0.13-fold; Figure 4B), and PHD3 (0.15±0.14-fold; Figure 4C) transcript levels compared with treatment with csiR. Similarly, PHD1, PHD2, and PHD3 protein levels as assessed by Western blot analysis were repressed after specific siRNA treatment (Figure 4D through 4F). In contrast, treatment with a nonspecific csiR did not affect PHD1, PHD2, and PHD3 siRNA transcript or protein levels (Figure 4A through 4F). Studies of HIF-1α protein revealed that HIF-1α levels are elevated after PHD2 repression, whereas PHD1 or PHD3 repression was not associated with changes of HIF-1α protein (Figure 4G through 4I). As a next step, we used this siRNA regimen in functional studies (Figure 4J through 4L). In fact, infarct sizes after 60 minutes of coronary artery ligation and 2 hours of reperfusion were not attenuated with prior siRNA repression of PHD1 (Figure 4J) or PHD3 (Figure 4L). In contrast, siRNA repression of PHD2 was associated with a degree of reduction in myocardial infarct size that was similar to IP treatment (Figure 4K). Infarct sizes were reduced from 43.6±3.7% of the area at risk in csiR-treated mice to 19.5±5.1% in PHD2 siRNA-treated mice (P<0.001; Figure 4K). Cardioprotection after PHD2 repression also was obvious from measurements of plasma troponin I levels (Figure 4K), whereas plasma troponin I levels were not attenuated after siRNA repression of PHD1 or PHD3. Taken together, these data reveal a critical role of PHD2 inhibition as a mechanism of cardiac HIF-1α stabilization and attenuation of infarct sizes.

Cardiac Adenosine Levels Are Elevated With HIF Activation

On the basis of previous studies showing a critical role of extracellular adenosine generation and signaling during cardiac IP,24,25,29–31 we next studied the contribution of purinergic signaling pathways to HIF-dependent cardioprotection by IP. First, we measured cardiac adenosine levels with HIF activation. Consistent with previous studies,24,25 IP treatment was associated with elevations of cardiac adenosine levels (Figure 5A and 5B). However, IP-associated increases in cardiac adenosine levels were attenuated after siRNA repression of HIF-1α, and baseline adenosine levels were elevated after HIF activation with DMOG.

HIF-1α–Dependent Induction of Cardiac CD73 and the A2B Adenosine Receptor During IP

Next, we screened transcriptional responses of CD73, the key enzyme for extracellular adenosine generation, and of all 4 adenosine receptors (ARs) to cardiac IP in mice treated with specific HIF-1α or csiR. Consistent with previous work showing HIF-1α in hypoxia induction of CD7332 or the A2B adenosine receptor (A2BAR),33 the present studies revealed induction of CD73 and selective induction of the A2BAR in csiR-treated mice with IP. In contrast, CD73 induction and A2BAR induction with IP were abolished after siRNA repression of HIF-1α (Figure 6A through 6E). Similarly, immunohistochemical staining for the A2BAR confirmed A2BAR induction on the myocardium and on small coronary vessels after IP in csiR-treated mice, whereas HIF-1α siRNA treatment abolished this response (Figure 6F). Moreover, A2BAR transcript levels (Figure 6G) and protein (Figure 6H) were significantly elevated after treatment with the HIF activator DMOG (1 mg intraperitoneally 2 hours before cardiac biopsy). In additional studies, we measured A2BAR transcript (Figure 6I through 6K) after in vivo siRNA repression of cardiac PHD1, PHD2, and PHD3, showing selective induction of the cardiac A2BAR after siRNA repression of PHD2. Increased A2BAR expression also was confirmed on a protein level by Western blot analysis from cardiac biopsies at the indicated time points after siRNA repression of PHD2 (Figure 6L). These studies reveal selective and robust induction of A2BAR during in situ IP via HIF-dependent transcriptional pathways.
HIF-1–Dependent Cardioprotection Involves CD73-Dependent Adenosine Generation and Signaling Through the A2BAR

We next pursued functional studies of in situ IP to investigate the role of the CD73-A2BAR pathway as an end point of HIF-dependent cardioprotection. We performed studies of DMOG-dependent cardioprotection in previously described cd73−/− mice or corresponding littermate controls matched in sex and weight. As shown in Figure 7A, DMOG-associated increases in cardiac adenosine levels were abolished in cd73−/− mice (1 mg intraperitoneally, cardiac biopsy after 4 hours). Whereas DMOG-dependent cardioprotection (1 mg intraperitoneally) was maximal at 4 hours after DMOG treatment, baseline myocardial infarctions were increased in cd73−/− mice, and cardioprotection by DMOG was completely abolished (Figure 7B). Similarly, A2BAR−/− mice showed abolished cardioprotection by DMOG (Figure 8A and 8B) or siRNA PHD2 treatment (Figure 8C and 8D), whereas cardioprotection in matched littermate controls was maximal 4 hours after DMOG or after 4 hours of PHD2-siRNA treatment.

Discussion

In the present study, we pursued the contribution of HIF-1 to cardiac IP using loss- and gain-of-function studies of HIF-1α. For this purpose, we used a technique of cardiac in vivo siRNA repression of selective genes via intraventricular infusion of specific siRNA followed by studies of murine in situ preconditioning and myocardial infarction. We first gained insight from Western blot analysis for HIF-1α in CD73 and AR expression during IP. A through E, CD73 and AR transcript levels after IP with siRNA repression of HIF-1α. C57BL/6 mice were treated with a left ventricular infusion of siRNA specific for HIF-1α or csIR. After 2 hours, in situ preconditioning consisting of 4 cycles of ischemia/reperfusion (5 minutes each) followed by indicated times of reperfusion (0 to 90 minutes) was performed. Hearts were excised; total RNA was isolated; and transcript levels of CD73 (A), A1AR (B), A2AAR (C), A2BAR (D), or A3AR (E) were determined by real-time reverse-transcription polymerase chain reaction. Data were calculated relative to an internal housekeeping gene (β-actin) and are expressed as fold change vs control (0 hour of siRNA infusion ± SEM at each indicated time; n=6). F, Preconditioned myocardial tissues from hearts treated with siRNA specific for HIF-1α or csIR were excised; sectioned, and stained with A2BAR antibody (magnification ×600). G, DMOG treatment. To study the effects of DMOG on cardiac A2BAR expression, C57BL/6 mice were treated intraperitoneally with 1 mg. Hearts were excised after the indicated time periods (0 to 2 hours) and flash-frozen; total RNA was isolated; and transcript levels of A2BAR were determined by real-time reverse-transcription polymerase chain reaction. Data were calculated relative to an internal housekeeping gene (β-actin) and are expressed as fold change vs control (n=6). H, C57BL/6 mice were treated intraperitoneally with DMOG (1 mg). After 2 hours, hearts were harvested, flash-frozen, and lysed; proteins were resolved by SDS-PAGE; and tissues were transferred to nitrocellulose. Membranes were probed with an anti-A2BAR antibody. The same blot was probed for β-actin expression as a control for protein loading. Control mice were treated with csIR for 2 hours. One representative experiment of 3 is shown.
the cardioprotective effects of IP were abolished with siRNA repression of HIF-1α, pharmacological or genetic activation of HIF-1α was associated with a degree of cardioprotection similar to IP treatment itself. Additional studies of end-point signaling after HIF activation suggested a critical role of HIF-dependent activation of purinergic signaling pathways involving CD73 and A2BAR. Taken together, these studies reveal a central role of HIF-1 in myocardial IP via transcriptional activation of purinergic signaling pathways to increase myocardial resistance to subsequent ischemic tissue injury and suggest pharmacological strategies of HIF-1 activation in the treatment of acute myocardial ischemia.

Previous studies have shown that the human A2BAR promoter has a functional HIF-1 binding site.33 In fact, studies using constructs of the human A2BAR promoter, site-directed mutagenesis of the HIF binding site, and HIF loss and gain of function strongly suggest HIF-1 in hypoxia inducibility of the human A2BAR.34 Moreover, transcription factor binding studies revealed direct binding of HIF-1 to the putative promoter region. Similar to the human A2BAR promoter, the murine A2BAR promoter has 2 putative HIF binding sites. Previous studies have not directly addressed HIF binding to the murine A2BAR promoter. However, other studies showed a robust induction of the murine A2BAR with ambient hypoxia in vivo34 or during IP treatment.35 In addition, the present studies show loss of A2BAR inducibility after HIF-1 repression during IP (Figure 6) and suggest a central role of HIF-1 in A2BAR induction during murine in situ IP. Moreover, and consistent with the present studies, previous work has suggested a critical role of HIF-1 in cardioprotection. As such, a study of cardiac ischemia/reperfusion injury found cardioprotective effects with siRNA-mediated repression of PHD2 and activation of HIF-1α.35 Similarly, a very elegant study of gene-targeted mice for HIF-1α found complete loss of cardioprotection by IP in heterozygote mice (HIF-1α+/− mice).36 Other excellent studies have examined preconditioning phenomena by hypoxic preexposure in association with HIF-1. Exposure of wild-type mice to intermittent hypoxia resulted in protection of isolated hearts against ischemia/reperfusion injury 24 hours later. However, cardiac protection induced by intermittent hypoxia was lost in HIF-1α−/− mice.37

It is important to point out that the use of DMOG as an HIF-1 inducer serves mainly as a positive control in the present study, although this reagent obviously is not specific for HIF-1α activation. For example, a recent study revealed that the stability of the transcription factor nuclear factor-κB is regulated during hypoxia by PHDs in a manner somewhat similar to HIF-1α.38 Thus, it is conceivable that PHD inhibition with DMOG also could lead to changes in nuclear factor-κB activity. Moreover, noncardiospecific effects of DMOG (eg, increases in hematocrit or red cell volume, direct effects of erythropoietin)37–39 may have contributed to the observed cardioprotective effects. It is also important to note that intermittent ischemia/reperfusion appears to be a very robust inducer of HIF-1α expression and activity (possibly even more than hypoxia alone), as well as the expression of other HIF isoforms, which is consistent with results over the past several years from a number laboratories.40,41 Functional studies using a tissue-specific approach for selectively targeting different HIF isoforms will elucidate their individual contributions to cardioprotection in the near future.

Taken together, the present studies reveal a critical role of HIF-1 in the transcriptional coordination of adaptive responses elicited by IP of the murine heart. In fact, stabilization of HIF-1 is an essential component of myocardial ischemia/reperfusion protective pathways.
protection conferred by IP. HIF-1 end-point signaling involves enhancement of purinergic signaling pathways through the A2BAR. Extension of these findings will reveal whether activation of HIF-1 signaling pathways will serve as an approach to acute myocardial ischemia.

Acknowledgments
We gratefully acknowledge Stephanie Zug and Marion Faigle for technical assistance.

Sources of Funding
This work was supported by Fortune grant 1416-0-0, Interdisciplinary Center for Clinical Research Verbundprojekt 1597-0-0 from the University of Tübingen, German Research Foundation grant EL274/2-2, and Foundation for Anesthesia Research and Education grant to Dr Eltzschig and IZKF Nachwuchsgruppe 1605-0-0 to Dr Eckle.

Disclosures
None.

References
During the past decade, very exciting research efforts have revealed a central role of hypoxia-inducible factor (HIF) in mammalian oxygen homeostasis. In addition to its role in increasing erythropoietin production in response to limited oxygen availability, many other adaptive responses to hypoxia were found to be regulated by HIF, including metabolic and angiogenic responses. The use of prolylhydroxylase inhibitors appears to be a particularly promising pharmacological approach. However, many questions remain about the use of HIF activators. For example, the potential consequences of HIF activation on the risk of de novo thromboembolic complications. Nevertheless, HIF activators, particularly the prolylhydroxylase inhibitors, represent a group of novel therapeutic agents that have great therapeutic potential in the treatment of conditions characterized by the acute need of tissues to adapt to hypoxia such as that which occurs during inflammatory bowel disease, stroke, renal ischemia, or myocardial infarction. Thus, these compounds could cover a very wide range of clinical applications.
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Circulation. 2008;118:166-175; originally published online June 30, 2008;
doi: 10.1161/CIRCULATIONAHA.107.758516
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/118/2/166

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