HYPOXIA-INDUCIBLE FACTOR (HIF)-1 is a redox-responsive transcription factor that regulates the expression of numerous genes under physiological and pathological conditions (19). HIF-1 has been implicated in the regulation of angiogenesis, cellular survival and proliferation, cell motility and adhesion, glucose metabolism, vascular tone, and pH regulation (19). Unstable under normoxic conditions, the HIF-1 heterodimer consists of the HIF-1α and HIF-1β subunits. HIF-1α protein synthesis is regulated by the activation of the phosphatidylinositol 3-kinase and the MAPK pathways by tyrosine kinases as well as by G protein-coupled receptors (19). Degradation of HIF-1α is controlled by hydroxylation of two proline residues by oxygen-dependent prolyl-4-hydroxylases. These hydroxylation events serve as the signal for interaction with the E3 ubiquitin ligase, polyubiquitination, and degradation of HIF-1α by the proteasome pathway (4, 19). HIF-1 signaling is critical for embryonic cardiovascular development (21) as well as for the adaptation of adult myocardium to hypoxia (10). Importantly, HIF-1 signaling has been implicated in the development of hypoxia-induced delayed protection (5).

Heme oxygenase (HO)-1, the inducible isoform of HO, catalyzes the rate-limiting step of heme oxidation to biliverdin, CO, and free ferrous iron (15, 18). Biliverdin is then rapidly converted by biliverdin reductase to bilirubin, a molecule with antioxidant properties, and free iron is sequestered by ferritin (15, 18). HO-1 is expressed in response to a panoply of stimuli that are associated with oxidative stress and inflammation, including heme, hypoxia, ischemia, nitric oxide (NO), ultraviolet radiation, heavy metals, shear stress, endotoxin, and proinflammatory cytokines (15, 18), making HO-1 one of the most (if not the most) ubiquitously induced genes. This may not be coincidental, because emerging evidence points to HO-1 as one of the most important cardioprotective proteins in a wide variety of tissues and conditions (15). All of the three byproducts of HO-1 (bilirubin, CO, and ferritin) exert salubrious actions that protect the cell from oxidative damage and death (15). In the setting of myocardial ischemia-reperfusion injury, cardiac-specific overexpression of HO-1 has been shown to be protective in vivo (23). Supplementation of bilirubin attenuates postischemic dysfunction and enzyme leakage in isolated hearts (6), and administration of a CO donor during reperfusion after a 30-min coronary occlusion reduces infarct size in vivo (8). In addition to these acute cardioprotective effects of CO, administration of CO donors 24 h before a 30-min coronary occlusion-reperfusion has also been shown to induce a delayed preconditioned-like state (20). Thus HO-1 provides both immediate and delayed protection against ischemia-reperfusion injury. The relative roles of bilirubin, CO, and ferritin in alleviating ischemic myocardial injury, however, have not been fully elucidated.

Although the HO system was described more than 40 years ago, the precise mechanism that enables recruitment of HO-1 remains to be defined. In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Ockaili and colleagues (14) report a novel pathway whereby stabilization of the HIF-1 dimer via prolyl hydroxylase inhibition ameliorates posts ischemic myocardial injury. Using in vitro setting of HIF-1 activation by dimethyloxalylglycine (DMOG) in HMEC-1 cells and an in vivo rabbit model of ischemia-reperfusion injury, these authors have identified a link between HIF-1-induced HO-1 expression, suppression of IL-8 production, reduction of neutrophil infiltration, limitation of infarct size, and improvement in left ventricular function after ischemia-reperfusion injury. Because expression of IL-8 has been associated with complications of myocardial infarction (17), with myocardial reperfusion after coronary angioplasty (13), and with angioplasty-related complications (16), and because inhibition of IL-8 by monoclonal antibodies has been shown to reduce infarct size (3), the authors propose that induction of HO-1 via HIF-1 activity attenuates ischemia-reperfusion injury via inhibition of IL-8 production.

The observations of Ockaili and colleagues (14) have important implications for our understanding of the signaling pathways that underlie myocardial preconditioning, particularly its late phase. Late preconditioning can be elicited not only by ischemia but also by exposure to NO donors, CO donors, adenosine A1 agonists, bradykinin B2 agonists, opioid δ1 agonists, and various other compounds (2, 22). This archetypal adaptation of the heart to stress is extremely complex and its mechanism only partially known. It is widely accepted that reactive oxygen species and NO generated during brief ischemia-reperfusion activate several kinases, including the ε-isoform of PKC, the Src and Lck protein tyrosine kinases, and janus kinase (JAK)1 and JAK2, which in turn mobilize latent transcription factors, such as NF-κB, activator protein-1 (AP-1), and members of the signal transducers and activators of transcription (STAT) family (2, 22). These transcription factors are responsible for the upregulation of a battery of cardioprotective proteins, including the inducible isoform of NO synthase (iNOS), cyclooxygenase (COX)-2, and HO-1 (2, 22), which confer the preconditioned phenotype. Thus, in concert with iNOS and COX-2, myocardial HO-1 imparts an infarct-sparing effect 24–72 h after the preconditioning ischemia, possibly via the generation of CO. The present observations by Ockaili et al. (14) provide an additional important mechanism for the upregulation of myocardial HO-1 expression in response to brief ischemia or other preconditioning stimuli. HIF-1 thus joins the increasingly longer list of transcription factors that have been implicated in late preconditioning (e.g., NF-κB, AP-1, STAT1, and STAT3), consistent with the notion that a combination of multiple transcription regulatory proteins, acting in concert, is required for the activation of cardioprotective genes (2).
The results of Ockaili and colleagues (14) show that HIF-1-mediated upregulation of HO-1 is beneficial to the ischemic myocardium. As mentioned above, HO-1 can exert cardioprotective effects via multiple pathways that involve direct cytoprotective and antiapoptotic effects of CO and antioxidant effects of biliverdin/bilirubin and ferritin (18). On the other hand, the association of IL-8 with myocardial injury is largely circumstantial. Produced by monocytes and endothelial cells, IL-8 has long been known as a chemoattractant for neutrophils (12). This would suggest that the injurious role of IL-8 in myocardial damage could be secondary to accentuated neutrophil accumulation following ischemia-reperfusion. However, the role of neutrophils in myocardial ischemia-reperfusion injury has been examined repeatedly over the past three decades, and the preponderance of the evidence argues against a significant role of these blood-borne elements in causing exacerbation of tissue injury (1). It appears much more likely that neutrophil activation is a consequence, not a cause, of cell death (1). Because recruitment of neutrophils is a response to cell death, the degree of neutrophil activation is proportional to the degree of ischemia-reperfusion injury, which would explain why any intervention that limits infarct size also limits neutrophil infiltration. These considerations suggest that the deleterious effects of IL-8 on myocyte death are independent of neutrophils.

Regardless of the role of IL-8 and neutrophil accumulation, the elegant demonstration that HIF-1 stabilization induces cardioprotection via HO-1 expression is an important finding. It is likely, however, that HIF-1 stabilization activates other cardioprotective genes as well. Besides HO-1, HIF-1 is also known to regulate iNOS expression (9), and HIF-1 activation has been shown to protect cultured myocytes against simulated ischemia-reperfusion via iNOS upregulation (7). Conversely, increased NO can stabilize HIF-1 dimers (11). In vivo situations, it is plausible that NO generated by brief episodes of ischemia-reperfusion stabilizes HIF-1, enhancing HIF-1-induced HO-1 expression. Administration of NOS inhibitors before the preconditioning ischemia may illuminate the role of NO in stabilization of HIF-1 dimers in vivo. On the other hand, inhibition of iNOS 24 h after DMOG treatment (or ischemia-reperfusion) would clarify the role of iNOS in HIF-1-initiated protective signaling.

After nearly two decades of intense research, ischemic preconditioning continues to fascinate researchers with its ever-increasing signaling complexity. The authors should be congratulated on the identification of the role of HIF-1 in the induction of HO-1 during ischemia-reperfusion injury. These observations enhance our understanding of the complex molecular adaptations that are triggered by brief episodes of ischemia-reperfusion and result in delayed cardioprotection. These observations also open up possibilities that may be utilized for therapeutic purposes. For example, chronic, constitutive expression of HO-1 via gene therapy may alleviate tissue damage in patients with acute myocardial infarction. Given the role of HO-1 as a universal cytoprotectant, it is important that an effort be made to unravel the multifarious facets of HO-1 in myocardial ischemia-reperfusion.

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