The story so far: molecular regulation of the heme oxygenase-1 gene in renal injury

Eric M. Sikorski, Thomas Hock, Nathalie Hill-Kapturczak, and Anupam Agarwal

Department of Medicine and Division of Nephrology, Hypertension, and Transplantation, Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida 32610

Sikorski, Eric M., Thomas Hock, Nathalie Hill-Kapturczak, and Anupam Agarwal. The story so far: molecular regulation of the heme oxygenase-1 gene in renal injury. Am J Physiol Renal Physiol 286: F425–F441, 2004; 10.1152/ajprenal.00297.2003.—Heme oxygenases (HOs) catalyze the rate-limiting step in heme degradation, resulting in the formation of iron, carbon monoxide, and biliverdin, the latter of which is subsequently converted to bilirubin by biliverdin reductase. Recent attention has focused on the biological effects of product(s) of this enzymatic reaction, which have important antioxidant, anti-inflammatory, and cytoprotective functions. Two major isoforms of the HO enzyme have been described: an inducible isoform, HO-1, and a constitutively expressed isoform, HO-2. A third isoform, HO-3, closely related to HO-2, has also been described. Several stimuli implicated in the pathogenesis of renal injury, such as heme, nitric oxide, growth factors, angiotensin II, cytokines, and nephrotoxins, induce HO-1. Induction of HO-1 occurs as an adaptive and beneficial response to these stimuli, as demonstrated by studies in renal and non-renal disease states. This review will focus on the molecular regulation of the HO-1 gene in renal injury and will highlight the interspecies differences, predominantly between the rodent and human HO-1 genes.

gene transcription; oxidant stress; heme proteins

HEME OXYGENASE: THE RATE-LIMITING STEP IN HEME DEGRADATION

THE HEME OXYGENASE (HO) ENZYME system catalyzes the rate-limiting step in heme degradation, producing equimolar quantities of biliverdin, iron, and carbon monoxide (CO) (Fig. 1) (131, 219, 220). Biliverdin is subsequently converted to bilirubin by biliverdin reductase. Two isoforms of heme oxygenase have been characterized: an inducible enzyme, HO-1, and a constitutive isoform, HO-2 (131). A third isoform, HO-3, that differs from HO-1 but shares ~90% amino acid identity with HO-2, has also been described (139). As products of different genes, HO-1 and HO-2 share roughly 40% amino acid identity (131) and have different regulation and tissue distribution. HO-1 (32 kDa) is localized to microsomes and HO-2 (36 kDa) to mitochondria. HO-1 is ubiquitously induced in mammalian tissues, whereas HO-2 is constitutively expressed in the brain, testes, endothelium, distal nephron segments, liver, and myenteric plexus of the gut (reviewed in Ref. 4). HO-2 may function as a physiological regulator of cellular function, whereas HO-1 plays a cytoprotective role in modulating tissue responses to injury in pathophysiological states (231). Several recent reviews and editorials have highlighted the biological effects of the reaction product(s), which possess important antioxidant, anti-inflammatory, and antiapoptotic functions as well as the importance of HO-1 as a potent cytoprotective enzyme (38, 44, 51, 55, 58, 61, 74, 92, 105, 108, 143, 152, 177, 179, 184, 191, 197, 199, 202, 224, 256). The main focus of this article is to provide a review of the current literature on the molecular regulation of HO-1 gene expression, with particular reference to the differential regulation of the human and the mouse HO-1 genes. In this review, heme oxygenase will refer to HO-1 unless otherwise specified.

FUNCTIONAL RELEVANCE OF HO-1 INDUCTION IN RENAL INJURY

Induction of HO-1 is an adaptive and beneficial response to acute renal injury secondary to ischemia-reperfusion injury (132, 200); nephrotoxins (e.g., cisplatin) (1, 201); glomerulonephritis (48, 144, 156, 229); renal transplant rejection (46, 130); and rhabdomyolysis (153, 157). The first evidence for the protective effects of HO-1 in renal injury in vivo was provided by the studies of Nath et al. (153) in a rat model of rhabdomyolysis. HO-1 mRNA was induced within 3–6 h after injury in this model, and administration of an HO-1 inhibitor, tin protoporphyrin, worsened renal damage, while prior induction of HO-1 led to a considerable decrease in mortality (153). HO-1 mRNA is also induced in the kidney as early as 3–6 h after injury in ischemia-reperfusion (132, 200) and nephrotoxic acute renal failure (1, 90, 243), models not dependent on filtered heme proteins. Renal tubular induction of HO-1 is protective in these models as well (21, 200, 201, 243). Modulation of HO-1 expression using chemical inducers, inhibitors, and HO-1 gene delivery also support a functional role for HO-1 expression in ischemia-reperfusion injury in the liver (17), brain (163, 181), and heart (45, 78). The relevance of HO-1 expression is further substantiated by the presence of HO-1 protein in renal tubules in human ischemic acute tubular necrosis (172).

The biological importance of HO-1 was underscored by the development of the HO-1 knockout mouse and the first description of a patient with HO-1 deficiency (187, 237). Poss and Tonegawa (187) first generated mice deficient in HO-1 by targeted deletion of a 3.7-kb region including exons 3 and 4 and a portion of exon 5 of the mouse HO-1 gene. Kidneys of (−/−) mice over 20 wk of age showed evidence of iron deposition in renal cortical tubules. A progressive chronic inflammation characterized by hepatosplenomegaly, lymphadenopathy, leukocytosis, hepatic periportal inflammation, and occasionally glomerulonephritis was reported in addition to the iron deposition. These authors also reported that embryonic fibroblasts from the HO-1-deficient animals were more sensitive to oxidant stimuli such as heme, hydrogen peroxide, paraquat, and cadmium (186). In vivo administration of endotoxin to (−/−) mice (6–9 wk of age) resulted in significantly more liver injury and mortality. Yet et al. (242) also generated mice deficient in HO-1 and reported the occurrence of severe
right ventricular enlargement after chronic hypoxia in the (-/-) mice compared with wild-type mice. The findings of the protective effects of HO-1 in the glycerol model of acute renal injury have been confirmed in HO-1 knockout mice that demonstrate significantly worse renal function and tubular injury with 100% mortality in the HO-1 (-/-) mice compared with HO-1 (+/++) mice (157). We have also reported that HO-1 overexpression is cytoprotective in cisplatin-induced renal epithelial cell injury and demonstrated that HO-1 (-/-) mice treated with cisplatin develop more severe renal failure with increased apoptosis and necrosis, compared with cisplatin-treated wild-type or heterozygote mice (201).

The human patient with HO-1 deficiency (237) exhibited several phenotypical features similar to those in the HO-1 knockout mouse, including growth failure, anemia, increased iron binding capacity, increased ferritin, tissue iron deposition, lymphadenopathy, leukocytosis, and increased sensitivity to oxidant injury (187, 237). In HO-1 knockout mice, iron deposition was detected within renal proximal tubular epithelium (187). Kidney sections of the HO-1-deficient patient also contained multiple foci of iron deposition in the proximal tubular cells (172, 237). Clearly, comparison of the HO-1-deficient patient with the HO-1 knockout mouse has yielded valuable insights as to the role of HO-1 in renal injury. The clinical relevance and the beneficial effects of HO-1 in different settings of renal injury have been discussed in more detail elsewhere (86).

MECHANISMS MEDIATING THE PROTECTIVE EFFECTS OF HO-1 INDUCTION

The protective effects of HO-1 are mediated through one or more of several potential mechanisms. Increased HO-1 activity results in degradation of the heme moiety, a toxic prooxidant (23, 24). The reaction also results in the generation of bilirubin, an antioxidant that is capable of scavenging peroxo radicals, inhibits lipid peroxidation, and has recently been shown to protect cells from a 10,000-fold excess of hydrogen peroxide (27, 56, 126, 207). In essence, the induction of HO-1 results in a shift of cellular redox toward a more antioxidant state rather than a prooxidant milieu. HO-1 induction has been associated with increased iron efflux, and the latter has been suggested as a mechanism for the cytoprotective effects of HO-1 (66). In addition, ferritin is coinduced with HO-1, allowing safe sequestration of unbound iron liberated from heme degradation (22, 25). CO, the gaseous product, has vasodilatory effects similar to those of nitric oxide (NO) (103, 114, 134), as well as antiapoptotic and cytoprotective functions (18, 34, 71, 176, 178, 193, 225). Recent studies have also demonstrated an important role for the cell cycle regulatory protein p21 in mediating the protective effects of HO-1 expression in cell injury (57, 94).

It should be noted that HO-1 may have a dual role in tissue pathology and is not “therapeutic” in all instances (51, 184, 210, 249, 250). Each of the products of the reaction can be potentially injurious as well. CO stimulates mitochondrial generation of free radicals and can poison heme proteins (252). The iron liberated during heme degradation can catalyze free radical reactions, and increased accumulation of bilirubin is associated with kernicterus in neonates (52). It has been suggested that an appropriate level of HO-1 induction is beneficial, whereas too much HO-1 may, in fact, be a perpetrator of tissue injury (184, 210). Taken together, the data suggest that optimal levels of HO-1 are critical to determine whether the ultimate effect is one of protection or worsening of tissue injury. By deciphering the underlying molecular mechanism that controls the level of HO-1 enzyme activity, it will be possible to fine-tune HO-1 gene expression in disease states and exploit its use as a therapeutic strategy in the pathophysiology of renal injury.

MOLECULAR REGULATION OF HO-1

The human HO-1 gene is located on chromosome 22q12 (116) and consists of 5 exons spanning ~14 kb. The cDNAs for three mammalian HO-1 genes, including rat (148), mouse (8), and human (244), have been cloned and sequenced, as well as the HO-1 gene in the chicken (128). The mechanisms underlying HO-1 induction by its multiple inducers are complex, cell and tissue specific, and tightly regulated at the
transcriptional level. However, one common denominator for most of the stimuli that upregulate HO-1 is a significant shift in cellular redox (19, 227). The induction of HO-1 in response to most stimuli tested, including heme, heavy metals, growth factors, NO, oxidized lipids, and cytokines, has been demonstrated to be a consequence of de novo transcription (5, 12, 26, 36, 37, 68, 81, 91, 111, 112, 189). Consensus binding sites for nuclear factor-κB (NF-κB), activator protein-1 (AP-1), AP-2, Sp1, upstream stimulatory factor (USF), c-myc/max and interleukin-6 (IL-6) response elements, as well as other transcription factors have been reported in the promoter region of the human HO-1 gene (54, 118, 194, 228), suggesting a potential role for these factors in modulating HO-1 induction. Both positive and negative regulatory elements have been discovered in the human HO-1 promoter. Positive regulatory regions containing consensus binding sites for AP-1 (−1872), STATx (−1751), c-Rel (−1723), hepatocyte nuclear factor-1 (HNF-1) (−1709), HNF-4 (−1787), and GATA-X (−1803, −1672) have been identified between −1976 and −1655 bp of the human HO-1 promoter (214, 215). Interestingly, these regions are functional in HepG2 cells but not in HeLa cells in transient transfection studies (215). A potential cadmium response element (CdRE) (TGCTAGATTT) has been identified at approximately −4 kb 5’ relative to the transcriptional initiation site of the human HO-1 gene (218). Negative regulatory elements (NRE) containing consensus binding sites for NRE boxes [sequences similar to the silencer elements of the chicken lysozyme gene (228)] have been identified between −981 and −412 bp of the human HO-1 promoter (215). Another negative regulatory region consisting of a polymorphic GT repeat region is also present in the proximal promoter of the human HO-1 gene (238). A genomic map of the human HO-1 gene with potential regulatory sites is shown in Fig. 2.

The work of Alam and colleagues (7–10) has identified multiple inducer-specific elements, localized within 10 kb of the 5’-flanking region of the mouse HO-1 gene. Specifically, Alam et al. (7–9, 14, 15) have described two distal promoter regions, named E1 and E2 (previously referred to as SX2 and AB1, respectively) at −4.0 and −10 kb, that are required for induction of the mouse HO-1 gene in response to most of the inducers including heme, NO, heavy metals (cadmium), hydrogen peroxide, hyperoxia, LPS, phorbol ester, sodium arsenite, and various electrophiles (7–9, 14, 15, 75, 76). These investigators have proposed that all of these stimuli have a commonality in their activation mechanism, mediated exclusively via E1 and/or E2. Both these regions contain three repeats of a 10-bp sequence [(T/C)GCTGAGTCA] referred to as stress response elements (SRE) with potential similarity to binding sites for the AP-1 family. Overlapping with this sequence is a putative antioxidant and heme-response element, GCnnnGTCA, which resembles binding sites for the v-Maf oncoprotein and the transcription factor NF-E2 (93). NF-E2-related transcription factor 2 (Nrf2) binds to this sequence (14).

Based on these studies, we first evaluated a −9.1-kb human HO-1 promoter fragment that contains regions analogous to the SRE sequences in the mouse E1 and E2 sequences (regions A and B, respectively) (Fig. 3A) (87). The human HO-1 sequence corresponding to the E1 and E2 regions is also shown in Fig. 3B. We observed that these regions were only partially responsive for heme- and cadmium-mediated HO-1 gene induction when tested in human renal proximal tubular epithelial and aortic endothelial cells (87). Unlike the mouse HO-1 gene, our results have demonstrated that regions A and B in the human HO-1 promoter do not respond to other stimuli, such as oxidized lipids, hyperoxia, iron/hyperoxia, hydrogen peroxide, or transforming growth factor-β (TGF-β) (89). In our efforts to mimic steady-state Northern blot induction, we have identified an enhancer region internal to the human HO-1 gene, which, together with the 4.5-kb promoter, recapitulates levels of induction with heme and cadmium (87). This enhancer region...
TGF-β/H9252 such as hyperoxia, oxidized lipids, hydrogen peroxide, and moter (87). The enhancer is not responsive to other stimuli, mouse HO-1 gene, is involved in activation of the human HO-1 the transcription factor Nrf2, implicated in the regulation of the elements in response to heme or cadmium. Further studies to regions by Alam and Den (10) did not reveal any regulatory MOUSE HO-1 GENE ROLE OF NRF2 IN THE REGULATION OF THE functions in an orientation-independent manner and requires a region between −3.5 and −4.5 kb of the human HO-1 promoter (87). The enhancer is not responsive to other stimuli, such as hyperoxia, oxidized lipids, hydrogen peroxide, and TGF-β (87, unpublished observations). In contrast, an analysis of the entire protein coding region of the mouse HO-1 gene in conjunction with portions of the mouse 5′- and 3′-flanking regions by Alam and Den (10) did not reveal any regulatory elements in response to heme or cadmium. Further studies to characterize this enhancer region that regulates human HO-1 gene expression as well as its function in the context of the larger −9.1-kb human HO-1 promoter fragment, containing the StRE sequences, are in progress in our laboratory. Whether the transcription factor Nrf2, implicated in the regulation of the mouse HO-1 gene, is involved in activation of the human HO-1 enhancer region would be of significant interest.

**ROLE OF NRF2 IN THE REGULATION OF THE MOUSE HO-1 GENE**

Nrf2 belongs to the cap ‘n’ collar (CNC) transcription factor family and is important for the regulation of several oxidant-responsive genes, including mouse HO-1 and γ-glutamylcysteine synthetase subunit genes (14, 161, 235, 248). Nrf2 is involved in mouse HO-1 induction by a variety of stimuli, including oxidants, hyperoxia, heme, and cadmium (14, 15, 26, 43, 75). It has two other members, Nrf1 and Nrf3, both of which are more widely and abundantly expressed (39). Originally found in erythroid cells (142), Nrf2 mRNA is ubiquitously expressed in several different murine tissues (101, 142). Nrf transcription factors typically exist as heterodimers with a smaller family of proteins known as Mafs (for musculoaponeurotic fibrosarcoma) (30, 133). The regulatory network involving Maf and CNC transcription factors have been recently reviewed (145). These small proteins share two common structures, a basic leucine zipper (bZIP) and a CNC region, but they lack a transactivation domain. Activation of Nrf2/Mafk DNA binding activity occurs through several different pathways, including transcription, degradation, and activation (30, 133, 145, 205). Using yeast two-hybrid and coimmunoprecipitation studies, Nrf2 has been shown to dimerize with ATF-4 and bind to the StRE in the mouse HO-1 promoter and activate transcription after stimulation with cadmium (85).

Another mechanism for Nrf2 activation is through interaction with CBP [CREB (cAMP-responsive element binding protein) binding protein] (107). The interaction was shown to take place using two different transactivation domains termed Neh4 and Neh5. While Neh4 was shown to be a common motif found in CNC proteins, Neh4 was discovered to be a novel motif recently identified in the transcription factors p53 and E2F. Nrf2 interacts with Keap1 (Kelch-like erythroid-derived CNC homology-associating protein 1). Overexpression of Nrf2 results in the direct localization of Nrf2 in the nucleus and potent transactivation (106). Concomitant expression of Keap1 sequesters Nrf2 from the nucleus and represses Nrf2 activity (101). In summary, Nrf2 is a potent positive regulator of the mouse HO-1 gene and mediates inducer-dependent gene expression.

**REPRESSION OF HO-1 GENE EXPRESSION**

**Role of Bach1**

One antagonist to Nrf2 activation of HO-1 is the transcription factor Bach1 (31, 171, 180). Bach1 is a bZIP protein that interacts with the small oncoproteins of the Maf family (180). Bach1 maps to human chromosome 21q22.1 and is expressed as a 5.8-kb transcript in several tissues including the kidney (31). Bach2 is restricted to B lymphocytes and the brain (31). Both Bach1 and Bach2 act as transcriptional repressors, but Bach1 function as a transcriptional activator in erythroid cells (180). Bach1 heterodimerizes with MafK and binds to multiple Maf recognition sites (MARES) in the mouse HO-1 promoter, repressing gene expression (209). Recent studies have demonstrated that Bach1 also participates in hypoxia-inducible repression of the human HO-1 gene (113). Sun et al. (209) have shown that HO-1 is constitutively expressed at higher levels in many tissues of Bach1-deficient mice, suggesting that Bach1 acts as a negative regulator of transcription of the mouse HO-1 gene. It has been proposed that Bach1 prevents accessibility of enhancers to Nrf2 by binding to them. On heme stimulation, Bach1 is displaced, allowing binding of Nrf2 to its DNA binding sites and consequent transcriptional activation to increase HO-1 expression for heme degradation (209). The roles played by Bach1 and Nrf2 in mouse HO-1 gene regulation are very similar to the roles of the lac repressor and activator with the lac operon (209). Bach1 DNA binding
activity is negatively affected by the binding of heme to cysteine-proline motifs located in the COOH-terminal region, thereby releasing repression on transcription by Bach1 (169).

Length Polymorphisms in the GT Repeat Region

A (GT)n repeat region that functions as a negative regulatory region is located between −198 and −258 of the human HO-1 promoter and is absent in the mouse HO-1 gene. Length polymorphisms of this region vary between subjects and correlate with disease activity in patients with emphysema (238), coronary artery disease (42, 104), and vascular restenosis after balloon angioplasty (63). Individuals with shorter repeats (<25) demonstrate higher levels of HO-1 and milder disease, whereas individuals with longer repeats (>25) have lower levels of HO-1 and more severe disease (238). In preliminary studies, we have deleted the (GT) repeat region in the human HO-1 promoter and observed higher levels of basal reporter gene activity, similar to results reported by Yamada et al. (238), who used HO-1 promoter constructs with varying lengths of GT repeats. Although the molecular basis for this phenomenon is not known, it is postulated that the longer nucleotide repeats, including (GT)n and (CA)n, are capable of forming an alternate DNA structure known as zDNA (160). zDNA formation has been discovered in several other eukaryotic genes, such as the rat prolactin gene (160) and immunoglobulins (165) and has been shown to inhibit transcription in these genes. Whether zDNA formation in the (GT)n repeat region of the human HO-1 gene plays a role in regulating its expression remains to be determined.

It is interesting to note that a protective gene such as HO-1 has developed inherent genetic mechanisms to remain repressed in normal conditions. Kitamuro et al. (113) have suggested several possible explanations for the physiological implications of HO-1 repression. HO-1 repression reduces energy expenditure because the HO-1 reaction consumes oxygen and NADPH during heme degradation. In addition, as discussed before, the excessive generation of byproducts of the HO-1 reaction, namely, CO, iron, biliverdin, and bilirubin, can have potential harmful effects. It is therefore possible that the Bach1 and the (GT)n repeat region function as repressors of HO-1 expression to prevent undesired excess release of these products in normal physiological states.

DIFFERENCES BETWEEN HUMAN AND MOUSE HO-1

Studies from our laboratory as well as others demonstrate significant differences in the regulation of the human vs. the mouse HO-1 gene and are summarized in Table 1. For instance, the (GT)n repeat identified in the human HO-1 promoter is not present in the mouse HO-1 gene (238). A potential CdRE has been identified in region A of the 5′-flanking region of the human HO-1 gene; however, the CdRE in the mouse HO-1 gene is immediately downstream of this region and is a StRE, which is a binding site for Nrf2 and its partners (15, 218). Our recent studies have identified an internal enhancer in the human HO-1 gene that regulates induction by heme and cadmium (87), while the E1 and E2 regions of the mouse HO-1 promoter exclusively mediate gene expression (14, 15). The E1 region in the mouse HO-1 promoter regulates responsiveness to hyperoxia, hydrogen peroxide, and oxidized lipids (44, 119, 122). However, the analogous region in the human HO-1 promoter is not responsive to these stimuli (68, 89). We have identified a regulatory region between −9.1 and −11.6 kb of the human HO-1 promoter by DNase I hypersensitivity studies and promoter deletion analysis that, at least in part, mediates oxidized lipid-inducible HO-1 gene expression (89). Hypoxia is a potent inducer of HO-1 in rat, bovine, mouse, and monkey cells but is a repressor in human cells (113, 120). In addition, HO-1 has also been referred to as heat shock protein 32 based on its inducibility by heat shock in rodent cells (198). However, heat shock does not induce HO-1 in human cells (174, 194, 197). Cytokines such as IFN-γ induce HO-1 in rodent cells but not in human cells. On the contrary, IFN-γ represses human HO-1 gene expression (197). The interspecies differences in the regulation of the human and mouse HO-1 genes will be important considerations for the ultimate use and development of molecular therapies from the “bench to the bedside.”

<table>
<thead>
<tr>
<th>FACTORS RESPONSIBLE FOR HO-1 INDUCTION</th>
<th>IN RENAL INJURY</th>
</tr>
</thead>
</table>
| The protective effects of HO-1 activity were first recognized due to its dramatic induction after exposure to a wide variety of injurious stimuli (19, 111, 206). Inducers of HO-1 include heme; ultraviolet A radiation; hydrogen peroxide; cytokines (IL-1, IL-6, IL-10, TNF-α, IFN-γ); endotoxin; growth factors (PDGF, TGF-β); heavy metals; oxidized lipids; shear stress; hyperoxia; NO; NO donors; angiotensin II; glucose deprivation; and others (2, 5, 6, 12, 13, 15, 16, 27, 35, 37, 40, 41, 49, 59, 60, 68, 69, 79, 81, 83, 88, 95, 100, 115, 119, 121, 135, 156, 221, 232). Several of these stimuli, such as heme, nephrotoxins (e.g., cadmium), NO, growth factors, cytokines, and angiotensin II play an important role in the pathophysiology of acute renal failure, as shown in Fig. 4. It has been proposed that these factors activate the HO-1 gene, leading to increased HO-1 expression.

Table 1. Differences between the regulation of mouse and human HO-1 genes

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mouse HO-1 Gene</th>
<th>Human HO-1 Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Promoter elements</td>
<td>E1 and E2 regions (all stimuli)</td>
<td>A and B regions (limited stimuli)</td>
</tr>
<tr>
<td>GT repeat region in promoter</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Additional regulatory elements</td>
<td>None detected</td>
<td>Internal enhancer</td>
</tr>
<tr>
<td>Heme-response element</td>
<td>E1 and E2 regions</td>
<td>Internal enhancer + A and B regions</td>
</tr>
<tr>
<td>Cadmium-response element</td>
<td>(T/C)GCTGAGTCA (E1 region)</td>
<td>TGCTAGATT (A region)</td>
</tr>
<tr>
<td>Response to hyperoxia</td>
<td>E1 region</td>
<td>A and B regions are nonfunctional</td>
</tr>
<tr>
<td>Response to hypoxia</td>
<td>Gene activation</td>
<td>Gene repression</td>
</tr>
<tr>
<td>Response to interferon-γ</td>
<td>Gene activation</td>
<td>Gene repression</td>
</tr>
<tr>
<td>Response to heat shock</td>
<td>Gene activation</td>
<td>Nonresponsive</td>
</tr>
<tr>
<td>Response to oxidized lipids</td>
<td>E1 region</td>
<td>Region between −9.1 and −11.6 kb</td>
</tr>
<tr>
<td>Response to hyperosmolarity</td>
<td>Gene activation</td>
<td>Gene repression</td>
</tr>
</tbody>
</table>

HO-1, heme oxygenase-1.
Heme serves as the prosthetic moiety for heme proteins, such as hemoglobin, myoglobin, cytochromes, prostaglandin endoperoxide synthase, NO synthase (NOS), catalase, peroxidas, respiratory burst oxidase, and pyrrolases, which play an important role in the maintenance of critical functions that include oxygen delivery, mitochondrial respiration, and signal transduction (86, 155, 184). Interestingly, heme serves not only as a substrate for HO-1 but also stimulates HO-1 gene transcription both in vivo and in cultured cells (12, 13, 153, 183). In models of renal injury, increased levels of heme are observed in the kidney before the induction of HO-1 (1, 132, 200). The source of this heme is attributed to either excessive generation of heme or the destabilization of intracellular heme proteins (e.g., cytochromes) in settings of rhabdomyolysis (153, 157) or from the destabilization of intracellular heme proteins as would occur in the setting of ischemia-reperfusion. The heme lead to the induction of HO-1 and serve as a cytoprotective response.

Heme binds specifically to Bach1 and regulates its DNA-binding activity (169). Ogawa et al. (169) have proposed the following model for transcriptional regulation by heme through Bach1. In the presence of low heme concentrations, genes are repressed through the interaction of Bach1 with MARE sequences, whereas higher concentrations of heme inactivate the binding of Bach1, allowing access of transcription factors such as Nrf2 to interact with the MARE sequences (169), which in turn allows gene activation. Whether a similar mechanism operates in the transcriptional regulation of the human HO-1 gene by heme remains to be elucidated.

In the mouse HO-1 gene, the E1 and E2 regions are required for heme-mediated HO-1 induction (7–9). Recent studies have identified Nrf2, MafG, ATF3, as well as Jun and Fos family members as StRe binding proteins in nuclear extracts from immortalized rat proximal tubular cells exposed to heme (11). In these studies, heme did not directly increase Nrf2 transcription but rather decreased the rate of Nrf2 degradation (11). It was proposed that the expression of Nrf2 in unstimulated cells was curtailed by rapid degradation and HO-1 gene expression is maintained at a low level by the binding of repressor proteins (e.g., Bach1) to the StRE sites in the E1 and/or E2 regions (11, 209). Heme stimulation leads to decreased Nrf2 degradation, allowing for accumulation of Nrf2 in the nucleus, where it heterodimerizes with partners such as MafG. These heterodimers can lead to the displacement of the repressor proteins bound to the HO-1 regulatory regions and increase HO-1 gene transcription (11).

Our laboratory has evaluated the regulation of the human HO-1 gene by heme in human renal proximal tubular and aortic endothelial cells (87). Heme stimulation (5 μM) causes a ~20- to 30-fold induction of HO-1 mRNA at 4-h incubation. Such induction occurs via direct increases in de novo gene transcription and is not dependent on increased mRNA stability, findings consistent with previous studies in other cell types (5, 12). Based on the identification of heme-responsive elements in the
mouse HO-1 gene, we first evaluated multiple human HO-1 promoter constructs up to \(-9.1\) kb from the transcriptional start site. These constructs contained the StRE sequences similar to those described in the E1 and E2 regions of the mouse HO-1 gene (Fig. 3). We observed only a partial response of the reporter gene after heme stimulation. The levels of the reporter gene did not correlate with steady-state Northern blot analysis levels of HO-1 induction with heme, suggesting that additional regulatory sequences are required. In an effort to mimic endogenous stimulus-dependent levels of HO-1 induction, we evaluated the entire 12.5-kb of the human HO-1 gene, including introns and exons, in conjunction with a \(-4.5\)-kb human HO-1 promoter and observed significant heme- and cadmium-mediated induction, suggesting the presence of an internal enhancer (87). Similar results were observed with two different reporter genes, human growth hormone and luciferase. The internal enhancer functioned as a true enhancer because it functioned in both orientations (5' and 3') (87). The enhancer did not function in the context of a heterologous thymidine kinase promoter and required a region between \(-3.5\) and \(-4.5\) kb of the human HO-1 promoter for transcriptional activation (87). Further studies to delineate the important regulatory sequences within this internal enhancer are in progress.

**Cadmium**

Occupational exposure, such as working with cadmium-containing pigments, plastics, glass, metal alloys, and electrode material in nickel-cadmium batteries, and nonoccupational exposure, such as food, water, and cigarette smoke, contribute to the buildup of cadmium in the body. Cadmium, absorbed either through the lungs or the gastrointestinal tract, accumulates mainly in the kidneys and liver and has a long biological half-life of 15 yr. Chronic long-term exposure leads to renal dysfunction with slow progression to stage 5 chronic kidney disease. In the kidney, the proximal tubular cells are the predominant site for cadmium accumulation and cell injury. After absorption, cadmium is bound to apoprotein, metallothionein, and the cadmium-protein complex is filtered through the glomerulus into the urinary space where it becomes endocytosed by the proximal tubule cells and degraded by the lysosomes, resulting in the release of the cadmium and consequent cellular damage. Cadmium-induced cell injury is mediated via the generation of reactive oxygen species, lipid peroxidation, protein crosslinking, DNA damage, and alteration of intracellular calcium.

Cadmium is a potent inducer of HO-1 gene expression (12, 15, 217, 218). Such induction may represent a cellular defense against cadmium-mediated injury (218). HO-1 induction by cadmium occurs via transcriptional activation (12). Takeda et al. (218) have identified a 10-bp sequence (CdRE), TGCTA-GATT, at approximately \(-4.0\) kb of the human HO-1 promoter that confers cadmium-mediated induction in HeLa cells. The GC dinucleotides and the G residue in the CdRE are essential for cadmium-mediated gene activation (218). Immediately downstream of this CdRE sequence is a StRE sequence that is also present in the E1 region of the mouse HO-1 gene (Fig. 3B). Mutational analysis of the StRE sequence in the human HO-1 promoter did not affect cadmium-mediated induction (218). The CdRE of the human HO-1 gene was not responsive to other HO-1 inducers, including heme, sodium arsenite, and cobalt protoporphyrin, and metals such as zinc (218). The CdRE is distinct from the metal-responsive element identified in the human metallothionein gene, which is responsive to both cadmium and zinc (217). The CdRE is also involved in the induction of human HO-1 by tobacco smoke and hydrogen peroxide in human monocytic cells (64). The proteins binding to the human CdRE have not as yet been identified. It should be noted that only a threefold increase in reporter activity was observed in HeLa cells, levels consistent with our studies in human renal proximal tubular cells with the \(-4.5\)-kb construct (5). Similar to heme, we have reported that cadmium-mediated HO-1 induction also requires the internal enhancer to recapitulate steady-state Northern blot analysis level induction of the endogenous HO-1 gene in human cultured cells (87).

In the mouse HO-1 gene, the E1 region is necessary for HO-1 gene activation by cadmium and involves the p38 MAPK pathway and Nrf2 (15). However, significant activation of a 15-kb mouse HO-1 promoter containing the E1 and E2 regions is seen only in MCF7 cells, a human mammary epithelial cell line (15). A mechanism involving Nrf2 has been proposed for cadmium-mediated activation of the mouse HO-1 gene in mouse hepatoma cells (205). Cadmium increased the half-life of Nrf2, and this increase in Nrf2 was via the ubiquitin-proteasome pathway, because proteasomal inhibitors enhanced Nrf2 expression (205). Studies in the rat HO-1 gene have demonstrated a role for USF in cadmium-mediated HO-1 induction in rat gliona cells (129).

Recent studies by Zhang et al. (253) have generated a transgenic mouse using a 15-kb mouse HO-1 promoter linked to the luciferase gene to allow for in vivo spatiotemporal transcription patterns of HO-1 gene expression in living animals. Injection of cadmium into these mice led to a significant, dose-dependent increase in luciferase activity in the liver and kidney (253). A modest increase in luciferase gene expression was observed after heme was administered intraperitoneally, but not when heme was given intravenously to these mice, possibly due to the formation of heme-serum protein complexes (253). The ability to monitor changes in gene expression in vivo in living animals offers a novel approach to test and identify potential therapeutic agents previously determined by in vitro molecular studies.

Other nephrotoxins such as cisplatin and mercury also induce HO-1 in renal tubular cells (1, 154). In cisplatin-induced renal injury, the induction of HO-1 is preceded by increases in renal heme content, suggesting that heme may be the stimulus responsible for mediating HO-1 induction (1). Recent studies have demonstrated that cytokines such as TNF-α and IL-1β and growth factors such as TGF-β are increased in a mouse model of cisplatin-induced renal injury (188). It is possible that these mediators also contribute to HO-1 induction in this model. The molecular mechanism(s) and the regulatory sequences controlling HO-1 induction by cisplatin have not been delineated.

**NO**

The role of NO in ischemia- and nephrotoxin-induced renal injury has been recently reviewed (158, 168, 245). NO is generated from L-arginine by a group of heme-containing enzymes.
enzymes, NOS. NO plays a dual role in acute renal injury because it can both attenuate or exacerbate renal injury, depending on a balance between beneficial hemodynamic effects and cytotoxicity as well as the site and rate of NO production and the chemical fate of the NO produced (167, 168, 245, 246). The inducible isof orm of NOS (iNOS) generates significant amounts of NO in response to cytokines and endotoxin and is critical to NO-dependent toxicity in both in vitro and in vivo models of renal ischemia-reperfusion injury (167, 168). Inhibition of iNOS, using iNOS antisense oligonucleotides, attenuates renal ischemia-reperfusion injury (167), which is further corroborated by studies in iNOS knockout mice (124, 125). NO induces cell injury through multiple mechanisms including generation of peroxynitrite, nitrosylation of thiols, and impairment of iron-sulfur clusters of proteins (28). Numerous studies have reported the capacity of NO and NO donors to dramatically induce HO-1 in diverse cell types including endothelial, smooth muscle, renal tubular, and mesangial cells (16, 33, 41, 49, 59, 69, 79, 81, 123, 135, 212). Studies have also demonstrated that NO-mediated HO-1 induction attenuates cytokine- and oxidant stress-induced cytotoxicity (185). Most studies have implied that induction of HO-1 by distinct NO donors occurs predominantly via transcriptional mechanisms (59, 81) and, in part, through increased HO-1 mRNA stability (33, 81), and represents an adaptive response to the harmful effects of NO.

Nuclear run-on studies have confirmed that de novo transcription is responsible for the NO-mediated activation of the HO-1 gene in rat vascular smooth muscle cells (59). However, the regulatory regions that are responsible for NO-mediated HO-1 gene induction have not been identified. Marquis and Demple (135) evaluated a 4.7-kb HO-1 promoter construct in IMR-90 cells (a human embryonic lung fibroblast cell line) and HeLa cells and observed a ≈2.5-fold increase in luciferase activity after NO stimulation. These levels were far lower than the maximal induction of HO-1 mRNA by NO, suggesting that sequences beyond the 4.7-kb promoter are required. Takahashi et al. (212) also evaluated a 4.5-kb human HO-1 promoter (containing the CdRE) in HeLa cells and found no significant reporter activity in response to NO donors. Our studies have observed that the heme- and cadmium-responsive internal enhancer also regulates NO (spermine NONOate)-mediated HO-1 induction in human aortic endothelial and proximal tubular epithelial cells (Hill-Kapturczak N and Agarwal A, unpublished observations).

Studies have also demonstrated that HO-1 mRNA is dramatically stabilized after NO exposure in IMR-90 cells (33). Iron chelation with deferoxamine and the antioxidant N-acetylcysteine (NAC) block NO donor-mediated HO-1 induction in LLC-PK1 (porcine renal proximal tubular cells) and in rat aortic smooth muscle cells (81, 123). The NO scavenger carboxy-2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide completely blocks NO-mediated HO-1 induction (123), whereas cGMP is not involved in NO-mediated HO-1 induction (123, 147). The reactive nitrogen compound peroxynitrite, formed by the interaction of NO with superoxide anion, induces HO-1 in bovine vascular endothelial cells but not in LLC-PK1 cells (70, 123). Incubation of rat smooth muscle cells with a mixture of cytokines (IL-1β and TNF-α) increases nitrite production as well as induced HO-1 expression (59). Inhibition of iNOS using NOS-methyl-L-arginine attenuated both the production of nitrite as well as the induction of HO-1, suggesting that endogenously released NO can also stimulate HO-1 gene expression (59).

Most previous studies have used NO donors such as sodium nitroprusside (SNP), S-nitroso-N-acetylpenicillamine (SNAP), and 3-morpholinosydnonimine (SIN-1) to evaluate effects of NO on gene expression. However, these donors have significant drawbacks. For example, SNP releases cyanide and iron and SIN-1 releases NO and superoxide simultaneously. Levels of nitrite production with these donors also do not correlate with the levels of HO-1 induction. For instance, SNP releases the lowest amount of nitrite (~10 μM) compared with SIN-1 (~40 μM), but SNP is the most potent inducer of HO-1 mRNA (79). Differential effects of NO donors have been observed in transient transfection studies as well. Harra et al. (79) tested the 4.5-kb human HO-1 promoter and observed that treatment with SNP, but not with S-nitroso-L-glutathione (GSNO) or SIN-1, increased the expression of the reporter gene through the CdRE of the human HO-1 gene, suggesting that SNP induces HO-1 mRNA expression through a mechanism different from that for GSNO or SIN-1. To more directly study the effects of NO on HO-1 gene expression, NO gas or NO-releasing compounds belonging to the diazeniumdiolate family (called NONOates) have been used as sources of bioactive NO (67, 135). The commonly used NONOates include diethyltriamine/NO and spermine NONOate. Diethyltriamine/NO is a slow NO generator, whereas spermine NONOate mimics a burst release of NO. The by-products of these NO generators do not affect HO-1 mRNA expression (33). Consistent with the observations in other cell types, we have observed significant HO-1 mRNA induction by spermine NONOate in human renal proximal tubular cells (Fig. 5).

**Growth Factors**

TGF-β, a member of the TGF-β superfamily, is a regulatory cytokine implicated in a variety of kidney diseases where, on the one hand, it promotes scarring and proinflammatory events and, on the other hand, stabilizes and attenuates tissue injury (204) through the activation of cytoprotective proteins, including HO-1 (109, 136). Increased TGF-β is the final common pathway in the pathogenesis of kidney disease by several factors including angiotensin II, hypoxya, high glucose, auto-antibodies, immune complexes, advanced glycosylation end products, and PDGF (32, 166). In response to injury, TGF-β and other growth factors are released via autocrine and/or paracrine mechanisms to maintain cellular homeostasis. While chronic elevation of TGF-β plays an important role in the progression of renal diseases (195), TGF-β also has beneficial effects. TGF-β has been shown to increase the expression of HO-1 in human retinal pigment epithelial cells (117), human renal proximal tubular epithelial cells (88), and human pulmonary epithelial cells derived from a lung cell carcinoma (A549 cells) (164), as well as in the bovine choroid fibroblasts (117). However, TGF-β does not induce HO-1 in all cell types, including HeLa, HEL, or bovine corneal fibroblasts (117). Interestingly, in an LPS-induced rat model of endotoxemia, as well as in IL-1β-treated cultured rat vascular smooth muscle cells (i.e., HO-1 is preinduced), TGF-β conferred a negative effect on HO-1 and contributed to the beneficial effects of TGF-β in a model of endotoxic shock (182). It is speculated
that TGF-β-mediated HO-1 induction may counteract the negative effects of TGF-β by affecting cell proliferation, apoptosis, and deposition of extracellular matrix.

The molecular regulation and signaling pathway(s) involved in TGF-β-mediated HO-1 induction are incompletely understood. TGF-β initiates signaling through interactions with TGF-β type I and type II receptors, which can subsequently activate a group of structurally related proteins called Smads (136). Thus far, Smad proteins are the only known TGF-β receptor substrates and signal transducers involved in many signaling responses induced by TGF-β (136). The molecular mechanism of TGF-β signal transduction through Smads has been studied extensively in cancer models. Smad6 and Smad7, the anti-Smads, inhibit activation of TGF-β-responsive genes such as human plasminogen activator inhibitor-I (PAI-1) and collagen (20, 62, 84, 151, 254). Our previous studies have demonstrated that TGF-β induced not only HO-1 but Smad7 as well in human renal epithelial cells (88). Furthermore, overexpression of Smad7, but not Smad6, inhibited the induction of the endogenous HO-1 gene (88).

The ability of several genes to respond to members of the TGF-β family requires the presence of one or more Smad binding elements (SBE) (82, 150, 230). Putative SBEs have been described in the human PAI-1 promoter, which is responsive to TGF-β (53, 208). A palindromic sequence, GTCTAGAC, has been described as the Smad3-Smad4 binding element. However, optimal Smad binding is reportedly achieved with a 5-bp sequence CAGAC (196, 251). The original palindromic sequence, which may have resulted from dimerization of recombinant Smads used in oligonucleotide selection experiments (136, 251), is not present in the human HO-1 gene. However, computer analysis reveals a consensus sequence, GTCTATACT, located at −5.7 kb in the human HO-1 promoter. To identify TGF-β-responsive cis-acting regulatory elements, we have evaluated several human HO-1 promoter fragments in transient transfection studies in human renal proximal tubular cells. An 11.6-kb HO-1 promoter construct elicited an approximately twofold increase in reporter activity, which was attenuated by cotransfection with Smad7 (Hill-Kapturczak N and Agarwal A, unpublished observations).

It has also been suggested, however, that TGF-β can signal through MAPK pathways, which may be independent of Smad proteins (47, 136, 247). It was demonstrated, using a chemical inhibitor of p38 MAPK (SB-203580) as well as transfection of a dominant-negative p38 MAPK mutant, that p38 MAPK may be responsible for transducing TGF-β signaling and stimulating HO-1 gene expression in A549 cells (164). However, SB-203580 did not prevent induction of HO-1 by TGF-β1 in human renal proximal tubular cells (88). In addition, antioxidants such as NAC did not attenuate TGF-β-mediated HO-1 induction in these cells, suggesting that the induction was potentially independent of oxidant stress (88). Further studies to examine the cellular consequences and molecular mechanisms of HO-1 gene expression in response to TGF-β will be important in the pathogenesis of renal injury.

Other growth factors, such as PDGF (60), hepatocyte growth factor (HGF) (211), VEGF (65), and nerve growth factor (NGF) (192), have also been reported to induce HO-1 gene expression in nonrenal cells. Studies with HGF, a growth factor that promotes regeneration of renal epithelial cells (162), have suggested that hypoxia-inducible factor-1α (HIF-1α) may regulate HO-1 gene expression in HepG2 cells (211). VEGF upregulates HO-1 protein expression in vivo in chick embryo choioallantoic membranes by a mechanism dependent on an increase in cytosolic calcium levels and activation of protein kinase C (65). NGF-mediated HO-1 induction by a phosphatidylinositol 3-kinase/Akt-dependent pathway has recently been reported in dopaminergic PC12 cells (192).

**Angiotensin II**

Angiotensin II is an important mediator involved in the pathophysiology of renal injury. In addition to the well-known hemodynamic effects (6), angiotensin II also has nonhemodynamic effects, including the activation of several growth factors, cytokines, and the generation of reactive oxygen species in the vasculature and the kidney (6, 32, 83, 138, 166, 173). Angiotensin II is a potent inducer of HO-1, both in vivo in the intact rat kidney and in vitro in LLC-PK1, rat renal proximal tubular epithelial, and mesangial cells (6, 29, 83, 97–99). However, the molecular regulation of HO-1 by angiotensin II has not as yet been reported. Systemic administration of angiotensin II using miniosmotic pumps resulted in significant upregulation of HO-1 in rat renal proximal tubules (83, 98). The induction of HO-1 by angiotensin II is not directly related to elevated blood pressure, because HO-1 was not induced in a rat model of hypertension after DOCA-salt (83) or norepinephrine infusion (6). HO-1 activation has also been reported in the rat heart (97) and aorta (100) after systemic angiotensin II administration.

Increased HO activity, with chemical inducers such as hemin, reverses angiotensin II-mediated decreases in glomerular filtration rate and increases in proteinuria, whereas HO inhibition leads to worsening of glomerular filtration rate and proteinuria (6), suggesting that HO-1 may provide a protective role to the potential injurious effects of angiotensin II in the kidney. Recent studies have shown that overexpression of HO-1 using a retroviral vector in human endothelial cells or hemin pretreatment in rat proximal tubular cells decreases angiotensin II-mediated cell injury (138). In our limited studies, we have not observed HO-1 induction in human proximal tubular or aortic endothelial cells using angiotensin II or angiotensin IV, its breakdown product (Hill-Kapturczak N and Agarwal A, unpublished observations). It is possible that interspecies variations may account for the differences observed in the human vs. other rodent or porcine cell lines.

**Hypoxia**

Another significant modulator of HO-1 gene expression is hypoxia. Interestingly, hypoxia induces HO-1 in rodent, bovine, and monkey cells but represses HO-1 expression in three different human cell lines (A549 human lung cancer cells, human umbilical vein endothelial cells, and human glialblasto mata cells) (102, 113, 120, 146, 216, 240), indicating species-specific HO-1 regulation. The hypoxia-mediated repression of the human HO-1 gene is associated with activation of Bach1. The MARE located immediately downstream of the CdRE at approximately −4.0 kb of the human HO-1 promoter is required for hypoxia-mediated repression of HO-1 via Bach1 (113). Hypoxia significantly increases HO-1 mRNA expression by transcriptional activation in rat renal medullary interstitial cells (240). Using inhibitors of HIF-1α degradation and cis-element...
oligonucleotide decoys to block HIF-1α. HO-1 induction was attenuated, suggesting a role for HIF-1α in hypoxia-mediated HO-1 activation (240). HIF-1α is a basic helix-loop-helix transcription factor whose expression is inducible by lower than normal levels of tissue oxygen. It acts by dimerizing and binding to a hypoxia-responsive element (HRE) in the promoter of hypoxia-responsive genes. Lee et al. (120) have demonstrated that HIF-1 mediates transcriptional activation of the mouse HO-1 gene in response to hypoxia and that the region responsible for this is located at approximately −9.5 kb upstream of the transcriptional start. Recent studies have reported the upregulation of HIF-1α in the border zone of rat renal segmental infarcts in tubular and glomerular cells, capillary endothelial cells, and infiltrating macrophages (190). HIF-1 expression colocalized with hypoxia-inducible genes, including HO-1 and VEGF. However, Wood et al. (236), using a mutant Chinese hamster ovary cell line for HIF-1α, demonstrated that HO-1 expression was largely independent of hypoxia and did not require a functional HIF-1α. Recent studies have demonstrated that the MAPK pathway, particularly p38 MAPK and MEK1, mediates the signal transduction pathway for hypoxia-mediated HO-1 induction in rodent cells (102). AP-1 DNA-binding activity is also increased and appears to be involved in HO-1 induction (191). Studies have demonstrated that HIF-1 regulates hypoxia-mediated HO-1 induction in rat aortic vascular smooth muscle cells, but not in rat pulmonary artery endothelial cells, where AP-1 is required (80). These data suggest that the regulation of the HO-1 gene in response to hypoxia in the pulmonary vasculature differs from the systemic circulation.

Osmotic Stress

Recent studies have suggested that the HO-1 induction may represent an adaptive response to changes in tonicity in the kidney (226, 255). Tian et al. (226) have demonstrated that urea and hypertonicity induce HO-1 gene expression in murine renal medullary cells via transcriptional activation. A 4.5-kb human HO-1 promoter was modestly activated in response to urea, but deletion of the CdRE did not affect promoter activity, suggesting that urea-mediated HO-1 induction was not dependent on the CdRE in these cells (226). Antioxidants such as NAC blocked urea-mediated HO-1 induction. In human hepatoma HepG2 cells, changes in osmolality by sugar molecules during glucose deprivation does not induce HO-1, suggesting a differential regulation of the mouse and human HO-1 genes in response to osmotic stress (40). It is interesting to note that hyperosmolarity suppresses HO-1 induction in response to hemin and pretreatment with the organic osmolyte betaine restored hemin-mediated HO-1 induction in rat hepatocytes (127).

Cytokines and Endotoxin

Several inflammatory conditions such as atherosclerosis, transplant rejection, acute glomerulonephritis, and sepsis are associated with marked induction of HO-1 (3, 77, 229, 233). More importantly, the induction of HO-1 is a protective response in these disease settings (77, 96, 108, 121, 203). Proinflammatory cytokines and LPS, important mediators of these disorders, have been shown to activate HO-1, and it has been suggested that such induction occurs as an adaptive response to inflammatory stress (35, 37, 115, 175, 189, 241). However, most of these studies have been reported in rodent cells with the exception of a few in human cells (221, 222). IL-1α and TNF-α transcriptionally activate HO-1 mRNA in human umbilical vein endothelial cells (221). NAC, an antioxidant, and mepacrine, a phospholipase A2 inhibitor, blocked cytokine-mediated HO-1 induction. In addition, curcumin, an AP-1 inhibitor, decreased cytokine induction of HO-1 mRNA (222). However, curcumin by itself has been reported to induce HO-1 (26).

IL-1β has also been shown to induce HO-1 mRNA in rat mesangial cells (223). LPS also induces HO-1 via generation of hydrogen peroxide, and the transcription factor NF-kB has been implicated in such induction in mouse M1 myeloid leukemia cells (115). The combination of LPS and interferon-γ induces HO-1 in murine mesangial cells (223). Some reports have demonstrated that LPS-mediated HO-1 induction occurs through IL-1β, whereas others have shown that IL-1β knock-out mice still respond to LPS and that the cytokine TNF-α is actually responsible for HO-1 upregulation (170). IFN-γ with LPS or TNF-α induces HO-1 in human monocytic cells. IL-6, an important mediator involved in the regulation of the acute-phase response to injury, induces HO-1 in Hep3B and HepG2 cells but not in human umbilical vein endothelial cells and murine macrophages (73, 141). IL-11, a member of the IL-6 family of cytokines, has also been reported to induce HO-1 in HepG2 cells (72). We have tested the effects of cytokines alone and in combination and found no induction of HO-1 in human renal epithelial cells (Fig. 5), results consistent with the findings of Takahashi et al. (213), who reported no induction with IFN-γ and IL-1β in human glioblastoma cells, where in fact these cytokines actually repress HO-1 activation by SNI, cadmium, and hemin. The reasons for the differential responses in the human and rodent species are not entirely clear but may be similar to the interspecies differences observed in the regulation of the iNOS gene (140).

Anti-inflammatory cytokines also activate HO-1 gene expression. For instance, IL-10 induces HO-1 in murine macrophages (121). More importantly, the protective effect of IL-10 in a murine model of LPS-induced septic shock was significantly attenuated by inhibition of HO enzyme activity, suggesting that the anti-inflammatory effects of IL-10 were mediated via HO-1 induction (121). Recent findings have demonstrated the efficacy of IL-10 in animal models of acute renal injury secondary to ischemia-reperfusion and cisplatin (50), models wherein HO-1 has also been shown to be protective (1, 200, 201, 234). Given the role of HO-1 induction in mediating the protective effects of IL-10 in sepsis (121), it is tempting to speculate that the effect of IL-10 in acute renal injury also occurs via HO-1 activation. IL-13, an immunoregulatory cytokine that is a key mediator in allergic inflammation, has also been shown to induce HO-1 (110). Similar to the effects of IL-10 in sepsis, HO-1 induction has been suggested to mediate the effects of IL-13 in vivo in rat cardiac allografts and in vitro in human umbilical vein endothelial cells (110).

SUMMARY

Induction of HO-1 plays an important role in the pathophysiology of several diseases involving the kidney as well as other organ systems. The biological implications of the HO-1-catalyzed reaction have gone far beyond the initial description of

Invited Review
REGULATION OF HEME OXYGENASE-1 GENE EXPRESSION

VOL 286  MARCH 2008  www.ajprenal.org

Downloaded from: http://ajprenal.physiology.org/ by IP 10.220.33.4 on October 14, 2017
its function as the rate-limiting enzyme in heme degradation in reticuloendothelial cells, where increased levels of heme are derived from hemoglobin, released from red blood cells completing their life cycle of 120 days. The reaction products of HO-1 have both beneficial and potentially injurious effects. There is considerable heterogeneity in the tissue response to injury, and induction of HO-1 may not always be beneficial. An optimal level of HO-1 induction to provide a therapeutic level of a “reaction product” may be necessary and would depend on the setting. Several features suggest differences in the regulation of the mouse and human HO-1 gene. While the mouse HO-1 gene has been well characterized and studied, the human HO-1 gene requires further characterization. Experiments using additional promoter constructs, chromatin structure analysis, and in vivo footprinting are currently underway in our laboratory to delineate the region(s) of the human HO-1 gene that control induction. It is anticipated that the knowledge gained by studies involving the molecular regulation of human HO-1 gene expression will allow for the fine-tuning of HO-1 gene expression in disease states and hence the ability to exploit the cytoprotective effects of HO-1. Strategies to target and achieve regulated expression of HO-1 will have significant therapeutic implications in several clinical settings involving the kidney.

GRANTS

This work was supported by National Institutes of Health Grants R01-DK-59600 and R01-HL-68157 (to A. Agarwal) and K01-DK-02902 (to N. Hill-Kapturczak). E. Sikorski is supported by a National Kidney Foundation fellowship award.

REFERENCES

28. Beckman JS, Beckman TW, Chen J, Marshall PA, and Freeman BA. Hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc Natl Acad Sci USA 87: 1620–1624, 1990.
Invited Review

REGULATION OF HEME OXYGENASE-1 GENE EXPRESSION


Haugen EN, Croatt AJ, and Nath KA.


REGULATION OF HEME OXYGENASE-1 GENE EXPRESSION


139. Nath KA, Haggard JJ, Croatt AJ, Grande JP, Poss KD, and Alam J. The indispensability of heme oxygenase-1 in protecting against acute


