Urea and hypertonicity increase expression of heme oxygenase-1 in murine renal medullary cells

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Tian, Wei, Herbert L. Bonkovsky, Shigeki Shibahara, and David M. Cohen. Urea and hypertonicity increase expression of heme oxygenase-1 in murine renal medullary cells. Am J Physiol Renal Physiol 281: F983–F991, 2001. First Published August 15, 2001; 10.1152/ajprenal.00358.2001.—Epithelial cells derived from the mammalian kidney medulla are responsive to urea at the levels of signal transduction and gene regulation. Hybridization of RNA harvested from control- and urea-treated murine inner medullary collecting duct (mIMCD3) cells with a cDNA expression array encoding stress-responsive genes suggested that heme oxygenase (HO)-1 mRNA was upregulated by urea. RNase protection assay confirmed this upregulation; hypertonicity also increased HO-1 mRNA expression but neither hypertonic NaCl nor urea were effective in the nonrenal 3T3 cell line. The effect on HO-1 expression appeared to be transcriptionally mediated on the basis of mRNA half-life studies and reporter gene analyses using the promoters of both human and chicken HO-1. Although urea signaling resembles that of heavy metal signaling in other contexts, the effect of urea on HO-1 transcription was independent of the cadmium response element in this promoter. Urea-inducible HO-1 expression was sensitive to antioxidants but not to scavengers of nitric oxide. Urea also upregulated HO-1 protein expression and pharmacological inhibition of HO-1 action with zinc protoporphyrin-sensitized mIMCD3 cells to the adverse effects of hypertonicity but not to urea. Coupled with the prior observation of others that HO-1 expression increases along the renal corticomedullary gradient, these data suggest that HO-1 expression may comprise an element of the adaptive response to hypertonicity and/or urea in renal epithelial cells.

THE MOLECULAR MECHANISM THROUGH which cells respond to exogenous urea, although incompletely understood, is relevant both to resident cells of the mammalian kidney medulla under physiological conditions, and to cells throughout the body in the setting of chronic renal failure, characterized by the pathological accumulation of urea and other metabolic end-products. In cells of the murine inner medullary collecting duct (mIMCD3), urea applied at concentrations within the medullary physiological range (e.g., 200 mM) activates several signaling pathways including the mitogen-activated protein kinase kinase (MEK)-extracellular signal-regulated kinase (ERK)-Elk-1-Egr-1 (8, 9, 42) and the phospholipase C-γ-inositol-1,4,5-trisphosphate/protein kinase C (10), and the phosphatidylinositol-3 kinase (PI3-K)-Akt-p70 S6 kinase pathways (48). A subset of these molecular events appears to confer protection from urea stress (46, 48).

Urea increases transcription and expression of the stress-responsive transcription factor, growth arrest- and DNA damage-inducible gene-153 (GADD153), and does so in an oxidative stress-dependent fashion (47). Urea treatment induces oxidative stress in diverse cell types, as evidenced by a decrease in the intracellular content of reduced glutathione (47). In contrast to urea stress, hypertonic stress has a modest (20) or even negligible (47) effect on GADD153 expression. Hypertonic stress is associated with marked upregulation of expression of a different GADD gene, GADD45 (20), an event that is potentially mediated via the stress-activated protein kinase/jun N-terminal kinase (JNK) family of mitogen-activated protein kinases (MAPKs) (20).

In addition to GADD153, other gene products are reputed to confer cytoprotection in the context of oxidative stress. Heme oxygenase (HO) catalyzes the rate-limiting step in the degradation of heme to bilirubin and in so doing cleaves heme into biliverdin while liberating iron and CO (14). Of the three isozymes described (HO-1 through HO-3), only HO-1 is inducible and in so doing plays a protective role against one of the major end-products of heme degradation, bilirubin. Although the pathway by which HO-1 expression is induced is not yet fully elucidated, the adaptive response of HO to oxidative stress is consistently observed as described in the present study. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
attendant cytoprotective properties (1). Antiapoptotic and anti-inflammatory properties have also been attributed to HO, perhaps related to the protection it affords from oxidative stress (1). HO-1 is ubiquitously expressed; however, particularly high levels are observed in the kidney (14).

With the use of an expression array strategy, HO-1 was identified prospectively as a gene product upregulated in response to urea treatment in renal medullary epithelial cells. Upregulation, which was demonstrated at the mRNA and protein level in mIMCD3 cells but not in 3T3 cells, appeared to be transcriptionally mediated and occurred independently of the cadmium-response element. Similar to urea-inducible expression of GADD153, urea-inducible HO-1 expression was antioxidant sensitive, underscoring the prooxidant effect of urea treatment.

METHODS

General methods, immunoblot analysis, and RNase protection assay. Cell culture and solute treatment were performed as previously described (48). The following inhibitors and stimuli were used: 200 mM urea; 100 mM NaCl; 200 mM mannitol; and 100 μM CdCl₂ for 30 min, followed by washout [5-h recovery (47)]; 30 mM N-acetylcysteine, 30-min pretreatment; 25 mM dimethylthiourea (DMTU), 30-min pretreatment; 1 mM 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide potassium (cPTIO), 30-min pretreatment; 50 μM PD-98059, 30-min pretreatment; 50 μM SB-203580, 30-min pretreatment; 30 μM LY-294002, 30-min pretreatment; and 300 μM zinc deuteroporphyrin 2,4 bis-ethylene glycol (ZnDPBG), 1-h pretreatment. All pretreatment compounds were left in place for the duration of the subsequent treatment interval. Treatment with urea, NaCl, mannitol, or CdCl₂ was for 6 h unless otherwise indicated. Immunoblot analysis was performed as previously described (9), using polyclonal anti-HO-1 antibody (StressGen) and horseradish peroxidase-conjugated secondary antibody; not all lots of this polyclonal antibody were found to be functionally equivalent. Visualization was via enhanced chemiluminescence (NEN products). LIVE/DEAD Viability/Cytotoxicity assay (Molecular Probes, Eugene, OR), based on ethidium homodimer-1 exclusion from viable cells and intracellular cleavage of the cell-permeant nonfluorescent calcine-acetoxyethyl ester to fluorescent calcine in viable cells, was performed in microtiter plates in accordance with the manufacturer’s directions. Data were quantitated using a fluorescence microplate reader (Cytofluor II) with excitation at 530 nm and emission at 585 nm for ethidium homodimer-1 exclusion and at 485 nm and excitation at 530 nm for calcine (optimal: 494/517 nm).

RESULTS

Expression array analysis was performed using the Atlas mouse stress array (Clontech) containing 140 duplicate-spotted cDNAs on a pair of nylon membranes. mRNA prepared from control-treated mIMCD3 cells was compared with that of urea-stressed (200 mM for 6 h) cells. Fewer than half of the gene products were detected under either condition (data not shown); the majority remained unchanged in abundance (e.g., the extremely abundantly expressed ubiquitin and phospholipase A₂; Fig. 1), as quantitated densitometrically. HO-1 was upregulated several-fold (Fig. 1) and was selected for further analysis. HO-1 is known to be oxidative stress responsive (1), and urea has been implicated in the genesis of oxidative stress in multiple cell types (47), suggesting functional significance.

The ability of urea to upregulate HO-1 mRNA abundance was corroborated via an RNase protection assay using a murine HO-1 EST in the public domain (see METHODS). In mIMCD3 cells, urea (200 mM for 6 h)
increased HO-1 mRNA abundance several-fold (Fig. 2). The hypertonic stressors NaCl and mannitol also up-regulated HO-1 mRNA. In contrast, in the nonrenal fibroblastic 3T3 cell line, only mannitol significantly increased HO-1 mRNA abundance.

Because cell stressors may influence mRNA stability, the effect of urea on HO-1 mRNA half-life was examined in mIMCD3 cells. Cells received control treatment or treatment with urea (200 mM for 6 h) before transcriptional arrest with actinomycin D. Under control conditions, HO-1 mRNA was quite stable with only a modest decrease in abundance after a 4-h incubation (Fig. 3). Urea treatment increased HO-1 mRNA abundance and decreased HO-1 mRNA stability. It was concluded that the ability of urea to increase HO-1 mRNA was not likely a consequence of enhanced mRNA stability.

It was therefore anticipated that the effect of urea was mediated at the transcriptional level, as has been observed with other genes in the context of urea signaling (11, 47) and is the generally, but not universally (32), observed mechanism regulating HO-1 expression. To confirm this hypothesis, the effect of urea on a luciferase reporter gene driven by the HO-1 promoter was examined. The HO-1 promoter has been well studied; functional consensus elements have been mapped but are not completely uniform across species. For this reason, we examined both the available chick and human promoters. When normalized to β-galactosidase expression from a cotransfected lacZ-encoding expression plasmid under the control of the cytomegalovirus long-terminal repeat promoter (see below), urea modestly increased reporter gene activity driven by both the chick and the human HO-1 proximal 5′-flanking sequence and promoter (Fig. 4A). Interestingly, the oxidative stressor cadmium failed to activate the chick promoter, whereas it exerted a pronounced effect on the human promoter; interpretation of the cadmium data was complicated by the significant effect of this stressor on β-galactosidase activity (Fig. 4C). Specifically, this heavy metal increased activity by ~80% in the mIMCD3 cell model. When only HO-1 promoter-driven luciferase activity is considered in the absence of normalization (Fig. 4B), a substantial effect of cadmium on the human promoter is now noted and the
“suppressive” effect on the chick promoter is no longer evident. The effect of NaCl on both promoters appeared to exceed that of urea; however, this was a manifestation of the slightly greater activating effect of urea on β-galactosidase activity (Fig. 4C).

The portion of the human HO-1 5′-flanking sequence that confers cadmium-responsiveness has previously been mapped to a 10-bp element between 4.0 and 4.5 kb upstream of the transcriptional start site (Ref. 39; Fig. 5), although others have recently attributed this property to an immediately adjacent element also included in this 0.5-kb region (19). A series of promoter deletion mutants linked to the luciferase reporter gene permitted elucidation of the role of this element and adjacent sequences in mediating the cadmium response (39). Because some urea effects are mediated through oxidative stress (47) and some effects of cadmium are oxidative stress dependent, the ability of the cadmium-responsive element to confer urea responsiveness was investigated. With the use of the reporter gene construct containing the full 4.5 kb of the human HO-1 5′-flanking sequence (pHHOSVL15; hHO-1 in Fig. 4), cadmium increased luciferase activity 125% (Fig. 5); the effect of urea was more modest (~60% increase). As anticipated, removal of the cadmium-responsive element (plasmid pHHOSVL14) abolished cadmium responsiveness. Interestingly, the relative effect of urea in the absence of this putative consensus element remained unchanged. These data, which achieved statistical significance, suggested that whereas both urea and cadmium are oxidative stressors, urea was not acting primarily through the cadmium response element. When the promoterless luciferase reporter plasmid pL1 was used, no activity was detectable under any condition (Fig. 5). Of note, the basal (control) level of luciferase expression from the shorter (pHHOSVL14) construct was significantly greater (by ~90%) than from the longer (pHHOSVL15) plasmid; hence, data were normalized to control values for the respective plasmid in Fig. 5.

Immunoblot analysis was next used to examine the effect of urea on HO-1 protein expression. Urea (200 mM) increased HO-1 abundance at 6 and 24 h of treatment; the effect of 400 mM urea was only evident at 6 h (Fig. 6). Urea (800 mM) downregulated HO-1 protein abundance at both time points, an effect potentially attributable to the previously observed toxicity of this degree of urea exposure. Hypertonic NaCl (100 and 200 mM) also increased HO-1 protein abundance to a comparable degree. Although the upregulation in response to solute treatment was modest, even the potent positive control, hydrogen peroxide, failed to increase HO-1 expression to a significantly greater extent (data not shown).

An effort was made to examine the effect of perturbation of known urea-regulated signaling events on urea-inducible HO-1 expression. A subset of these events has previously been implicated in signaling to HO-1 expression (15, 37, 44). Pretreatment with the inhibitor of ERK function, PD-98059, failed to appreciably influence HO-1 expression in response to urea.

Fig. 4. Urea increases transcription driven by the HO-1 promoter. Effect of urea (200 mM for 6 h), NaCl (100 mM for 6 h), and cadmium chloride (100 μM for 1 h, followed by 5-h recovery) on normalized luciferase reporter gene activity (A), unnormalized luciferase (Luc) reporter gene activity (B), and control β-galactosidase (Gal) reporter gene activity (C; see text) in mIMCD3 cells transfected with luciferase reporter gene constructs driven by the gallus (chicken; cHO-1) or human (hHO-1) HO-1 promoter is shown. cHO-1 (15) includes 7.1 kb of the gallus HO-1 promoter whereas hHO-1 (39) includes 4.5 kb of the human HO-1 promoter (see METHODS). Data are representative of 3 separate experiments, each with determinations performed in triplicate.
Results were less clear cut with the inhibitors of p38 and PI3K function, where a modest basal increment in HO-1 expression precluded rigorous evaluation of inhibition (Fig. 7A).

Because HO-1 is responsive to oxidative stressors and because urea has been implicated in the genesis of oxidative stress, the ability of antioxidants to abrogate the effect of urea on HO-1 expression was examined. Pretreatment with the nonspecific sulfhydryl-containing antioxidant N-acetylcysteine completely abolished the effect of urea on HO-1 expression (Fig. 7B). It has recently been shown that HO-1 expression may be augmented by nitrosative stress, which may also be alleviated by N-acetylcysteine but that is not influenced by another thiol-containing antioxidant, dimethylthiourea (27). To discriminate the effect of urea from that of nitrosative stress, DMTU was used. Similar to the effect of N-acetylcysteine and inconsistent with a role for nitrosative stress, DMTU blocked the effect of urea on HO-1 expression (Fig. 7B). To further underscore the independence of the present phenomenon from nitric oxide (NO)-mediated stress, cPTIO pretreatment was used. Preincubation with this potent NO scavenger failed to influence urea signaling to HO-1 expression (Fig. 7C). We were unable to convincingly demonstrate oxidative stress dependence of HO-1 expression in response to hypertonic NaCl or mannitol treatment.

Attention was also directed toward the functional significance of osmotically inducible and urea-inducible HO-1 expression. It was reasoned that expression of this protein product should confer resistance to stress-inducible apoptosis in one or more of these experimental contexts. Pharmacological interruption of HO-1 enzymatic activity was achieved through cell treatment with a porphyrin-based HO inhibitor, ZnDPBG. The effect of this compound on urea-inducible stress was difficult to assess because relatively high concentrations of urea are required for cytotoxicity. In addition, the fluorescent nature of the inhibitor excluded the use of fluorescence-based assays for determination of metabolic consequences in this model [e.g., activated caspase-3 assay (46)]. For this reason, a

![Fig. 5. Urea is an effective activator even in the absence of the cadmium-response element (CdRE). Effect of control, urea, and cadmium treatment on normalized luciferase reporter gene activity in mLMD3 cells transfected with a luciferase reporter gene driven by the proximal portion of the hHO-1 5' flanking sequence, including (pHHOSVL15) or excluding (pHHOSVL14) the CdRE is shown. Plasmid pL1 is the empty (control) vector. Data are means ± SE of 3 separate experiments, each with determinations performed in triplicate.](image)

![Fig. 6. Urea increases HO-1 protein expression in mLMD3 cells. Effect of urea at the indicated concentrations and treatment intervals on HO-1 immunoreactivity by immunoblot analysis is shown. A: representative immunoblot. B: means ± SE of 4 separate experiments with data determined densitometrically. †P < 0.05.](image)
crystal violet-based assay previously validated in the context of osmotic stress-resistance was employed (34). This assay provides an index of DNA content and hence, cell number. Cells received ZnDPBG in the presence or absence of solute for 24 h. ZnDPBG decreased A₆₃₀ (for crystal violet) by 6% under control conditions, whereas the inhibitor decreased A₆₃₀ by 14% with NaCl treatment (Fig. 8A). Interestingly, ZnDPBG had no effect on crystal violet staining in response to urea. Of note, urea and NaCl treatments in the absence of ZnDPBG decreased crystal violet staining by 9 and 22%, respectively, relative to control treatment. ZnDPBG did not appear to exert a nonspecific adverse effect that might merely disproportionately compound the adverse effect of elevated solute concentration (NaCl or urea) because it failed to influence A₆₃₀ in the setting of urea treatment (Fig. 8A). In corroboratory studies, a second index of cytotoxicity was examined. Viable cells are capable of incorporating the cell-permeant AM ester of the fluorescent dye calcein. Cleavage of the AM ester by intracellular esterases in viable cells liberates calcein resulting in an intense fluorescence at 517 nm after excitation at 494 nm. By this index, there was no effect of ZnDPBG pre-treatment on cell survival under control or urea-stressed conditions, but the HO-1 inhibitor decreased cell viability by 16% after NaCl treatment (Fig. 8B); the effect of ZnDPBG achieved statistical significance only in the presence of NaCl treatment. Of note, urea and NaCl treatment decreased cell viability by 7 and 12%, respectively, relative to control treatment using this index.

**DISCUSSION**

Using a blinded approach, we have prospectively identified HO-1 as a urea-inducible and osmotically responsive gene product in cells derived from the renal
medulla. Expression is upregulated at the mRNA and protein level and is likely a consequence of enhanced transcription. Thiol-containing antioxidants block HO-1 expression in these contexts, although solute-inducible expression does not require the cadmium-responsive portion of the HO-1 promoter. Furthermore, pharmacological inhibition of this enzyme may sensitize cells to the adverse effects of hypertonicity, although likely not to urea stress per se.

Although HO-1 is primarily an inducible HO isoform, constitutive expression has been observed in the renal inner medulla (49). Interestingly, there appears to be a graded increase in HO-1 expression (at both the mRNA and protein levels) with descent from renal cortex, through outer medulla, to inner medulla (49). On the basis of in vivo data using the HO inhibitor, ZnPBP, Zou et al. (49) concluded that constitutive medullary HO expression may play a role in maintaining renal medullary, but not cortical, circulation. In light of the present data, we speculate that constitutive medullary HO-1 expression may be a direct consequence of urea or hypertonic stress in this physiologically challenging milieu.

Other in vivo experimental models relevant to renal physiology are associated with increased HO-1 expression. Inhibitor-based studies have demonstrated that HO-1 expression is protective in several models of in vivo renal tubular injury (1). In addition, targeted disruption of the HO-1 gene rendered mice substantially more sensitive to the renal consequences of experimental rhabdomyolysis (28). A human mutation in this region resulting in absent basal and stress-responsive HO-1 expression was associated with hematuria and proteinuria, mesangial proliferation, and endothelial cell injury (30, 41). These findings, however, occurred in the setting of persistent hemolysis with proximal tubular iron deposition. No data regarding renal tubular function or salt and water balance were reported. With respect to transplantation, expression of HO-1 and related genes is also protective of chronic allograft rejection (17).

HO-1 regulation has been reported in the context of hyperosmotic opening of the blood-brain barrier (33). Although direct evidence was lacking, this induction was attributable to release of denatured or heme-containing proteins from damaged cells and not to a direct effect of the hyperosmotic environment (33). Present data indicate that hypertonicity alone is sufficient for HO-1 induction, although whether the transient nature of the hyperosmotic stimulus as it was applied in the model of blood-brain barrier opening would be sufficient for this mechanism remains a matter for speculation.

Diverse stimuli have been shown to influence HO-1 expression (1). Model stressors exhibiting phenotypic features of urea and osmotic stress include heat shock, hypoxia, and heavy metals (e.g., cadmium). The osmotic stress response has long been recognized to share features of the heat shock response (7). Rat (36) and chicken (16) HO-1 genes are heat shock responsive whereas the corresponding heat shock-like element in the human gene is nonfunctional (31, 43). In addition, heat stress actually suppresses heavy metal-inducible HO-1 expression in human cells (31). Urea, unlike hypertonicity, does not activate heat shock element-dependent expression of heat shock proteins in the mIMCD3 cell line, although it may synergize with hypertonicity in upregulating 70-kDa heat shock protein abundance (29). Therefore, it is unlikely that this mechanism is operative in urea-inducible HO-1 expression.

HO-1 induction in response to hypoxia requires participation of the hypoxia-inducible factor-1 (HIF-1) binding site located ~9 kb upstream of the murine HO-1 transcriptional start site (21). Motterlini et al. (27) observed that HO-1 induction by hypoxia in endothelial cells was sensitive to the thiol-containing antioxidant N-acetylcysteine (NAC), but not by DMTU. For these and other reasons, these authors implicated the inducible form of NO synthase induction and NO signaling in this model of HO-1 upregulation. NO increases HO-1 expression in many in vitro models (5), including renal epithelial cells (22). Urea-inducible HO-1 induction in the present model, in contrast, is sensitive to both NAC and DMTU. In addition, urea-inducible HO-1 expression is not affected by the potent NO scavenger cPTIO, implying a lack of dependence on NO generation. Furthermore, NO has recently been shown to enhance HO-1 mRNA stability (5), a finding also absent from the present context.

With respect to heavy metal-like signaling, multiple CCAAT/enhancer binding protein (C/EBP) and activator protein-1 (AP-1)-like binding sites residing between 4 and 4.5 kb upstream of the transcriptional start site confer basal expression and cadmium-responsiveness (2, 39), as well as responsiveness to lipopolysaccharide (6). The cadmium-responsive element has been mapped in detailed fashion to a compound element between 4 and 4.5 kb upstream of the transcriptional start site in the human HO-1 promoter (39). An additional AP-1-containing enhancer region ~10 kb upstream in the murine model was also sufficient, in isolation, to confer heavy metal inducibility (3). An extended AP-1-like motif (19), dubbed the stress response element (or StrE) and recently shown to interact preferentially with Cap’n’Collar transcription factor family members (4), has also been implicated in signaling to HO-1 transcription by both oxidative stressors and antioxidants (4). Binding of an upstream stimulatory factor to a site immediately upstream (~51 to ~35 bp) of the HO-1 transcriptional start site has also been implicated in HO-1 gene regulation in response to cadmium (25, 35).

Because urea is an oxidative stressor and because oxidative stress is associated with heavy metal exposure, the effect of these stimuli on transcription from constructs including or excluding the proximal cadmium response element was examined. In accordance with the observations of Takeda et al. (39), we have observed in our renal medullary cell model that cadmium responsiveness is conferred by a sequence between 4.5 and 4.0 kb upstream of the human transcrip-
tional start site; deletion of this 500-bp fragment abolishes cadmium inducibility. HO-1 induction by urea, in contrast, is preserved in the absence of this element, implying the presence of at least one other urea-responsive locus within the proximal 4.0 kb of the 5′-flanking sequence. A number of consensus elements have been detected in this region. Only the proximal 2.0 kb of the human HO-1 promoter (AF-145047) and the proximal 2.8 kb of the chicken promoter (U-95209) have been deposited in the public domain. Using the TRANSFAC database and search algorithm, it was determined that consensus elements shared by these proximal promoters include those recognized by N-myc, C-myb, HNF-3β, p53, C-rel, and C/EBP-β; family members of virtually all of these have been implicated in urea and/or hypertonic stress signaling (12, 13, 18, 26, 47). Several heat shock factor and classic AP-1 sites are also present, but we have previously found similar sites to be unresponsive to urea treatment (data not shown).

A second finding serves to discriminate between urea and cadmium signaling to HO-1: chicken HO-1 promoter is urea responsive but not cadmium responsive in the present renal epithelial cell model (Fig. 4). The relative inability of the 7-kb fragment of chicken HO-1 promoter to respond to cadmium was puzzling in this context, as heavy metal responsiveness had previously been reported to reside within the proximal 2.8 kb (23). However, because this reporter gene was transfected into murine cells and not into chick cells (which have not been shown to exhibit a signaling effect in response to urea) and because previous studies have not directly compared heavy metal inducibility of HO-1 promoters of different species in parallel, it cannot be concluded that this fragment is cadmium unresponsive. In murine cells, additional heavy metal-responsive elements have been described 10 kb upstream of the transcriptional start site (3); a corresponding site present in the chick HO-1 gene but absent from the chick promoter construct may permit synergistic induction in vivo. Alternatively, differences in the affinity or abundance of the relevant murine nuclear proteins interacting with the chick consensus elements may preclude activation in this context.

Only a limited understanding of signaling events leading to HO-1 expression in response to a given environmental stressor exists. In the model of arsenite-induced HO-1 induction (15), sequences upstream of −3,674 in the chicken HO-1 promoter were essential and sequences upstream of −4,576 further enhanced activity. The effect of arsenite was mediated via both ERK and p38 MAPKs (15); involvement of Ras and MEK1 was also implicated. Interestingly, in this model of the LMH (chicken hepatoma) cell line, cadmium and heat shock failed to substantially upregulate ERK, JNK, or p38 activity, whereas arsenite (75 μM) markedly activated all three kinases. Therefore, the relationship of arsenite signaling to urea signaling (or even to cadmium-inducible heavy metal signaling) remains unclear. Involvement of the AP-1-containing consensus element in models of HO-1 induction (14) indirectly suggested a role for MAPK isofoms in HO-1 regulation. Recently, overexpression of constituents of the ERK and JNK, but not p38, pathways resulted in enhanced transcription from the AP-1-like stress element in the HO-1 promoter (44). In this latter example, however, the extent to which these sites are occupied by classic MAPK-responsive AP-1 constituents remains to be clarified (4, 44).

In summary, the present data indicate that urea treatment (or hypertonic NaCl) upregulates HO-1 expression in renal medullary but not in 3T3 cells and that this phenomenon is likely transcriptionally mediated, and in the case of urea, oxidative stress dependent and NO independent. When coupled with the prior observation of others that HO-1 expression increases along the renal corticomedullary gradient (49), these data suggest that HO-1 expression may comprise an element of the adaptive or protective response to urea and/or hypertonicity in renal epithelial cells.

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