Inhibition of heme oxygenase augments tubular sodium reabsorption

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1Department of Basic Pharmaceutical Sciences, College of Pharmacy, 2Department of Biology, College of Arts and Sciences, University of Louisiana at Monroe, Monroe; and 3Department of Physiology and Hypertension and Renal Center, Tulane University School of Medicine, New Orleans, Louisiana

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Jackson KE, Jackson DW, Quadri S, Reitzell MJ, Navar LG. Inhibition of heme oxygenase augments tubular sodium reabsorption. Am J Physiol Renal Physiol 300: F941–F946, 2011. First published February 2, 2011; doi:10.1152/ajprenal.00024.2010.—Heme oxygenase (HO) catalyzes the degradation of heme to free iron, biliverdin, and carbon monoxide (CO). The vascular actions of CO include direct vasodilation of vascular smooth muscle and indirect vasoconstriction through inhibition of nitric oxide synthase (NOS). This study was performed to examine the effects in the kidney of inhibition of heme oxygenase alone or combined with NOS inhibition. Chromium mesoporphyrin (CrMP; 45 μmol/kg ip), a photostable HO inhibitor, was given to control rats and Nω-nitro-[l-arginine methyl ester (l-NNAME)-treated hypertensive rats (50 mg·kg−1·day−1, 12 h, 4 days). In control animals, CrMP decreased CO levels, renal HO-1 levels, urine volume, and sodium excretion, but had no effect on arterial pressure, renal blood flow (RBF), plasma renin activity (PRA), or glomerular filtration rate (GFR). In l-NNAME-treated hypertensive rats, CrMP decreased endogenous CO and renal HO-1 levels and had no effect on arterial pressure, RBF, or GFR but decreased sodium and water excretion in a similar manner to control animals. An increase in PRA was observed in untreated rats but not in l-NNAME-infused rats, indicating that this effect is associated with an absent NO system. The results suggest that inhibition of HO promotes water and sodium excretion by a direct tubular action that is independent of renal hemodynamics or the NO system.

CO have been variable ranging from a biphasic response (31) to NOS-dependent vasoconstriction (24) and direct vasodilatation (4).

The renal vasculature and tubules express HO (1, 5, 10, 18, 31), and treatment of rats with HO inhibitors has been reported to decrease renal blood flow (RBF) (2, 26). However, other investigators found that HO inhibition had no effect on basal renal hemodynamics (23). It has also been reported that HO inhibition by zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) promotes regional differences in RBF by decreasing medullary blood flow with no significant changes in cortical blood flow (37). Furthermore, NO influences renal vascular responses to HO inhibition (2, 26). Most of the studies examining the effects of HO blockade on renal hemodynamic function employed photosensitive inhibitors, such as stannous mesoporphyrin (SnMP) and ZnDPBG, that degrade in light (33). Furthermore, increasing renal HO activity by heme was shown to promote diuresis and natriuresis (25), but the effects of HO inhibition on renal excretory function have not been established. It is possible that a low dose of a HO inhibitor might reveal a direct tubular effect of CO in the absence of changes in renal hemodynamics.

The goal of the present study was to examine the effects of a photostable HO inhibitor on renal excretory function, in the presence and absence of a functional NO system. We hypothesized that HO inhibition decreases the endogenous CO levels and promotes anti-natriuresis and anti-diuresis and that these effects are enhanced by inhibition of NOS. To examine the hypothesis, the effects of a photostable inhibitor of HO, chromium mesoporphyrin (CrMP), were studied on renal excretory and hemodynamic function in untreated and Nω-nitro-l-arginine methyl ester (l-NNAME)-treated rats.

MATERIALS AND METHODS

CrMP was purchased from Frontier Scientific (Logan, UT). Inactin (thiobutabarbital sodium), bovine specific albumin (BSA), 3,3′-di-aminobenzidine, hematoxylin solution (Gills no. 3), and l-NNAME kits were purchased from Diasorin (Stillwater, MN). All other chemicals were purchased from Fisher Scientific (Houston, TX). CrMP stock solution (15 mmol/l) was prepared in 50 mmol/l Na2CO3 solution and l-NNAME (50 mmol/l) was dissolved in saline immediately before intraperitoneal injections. The composition of PBS was (in mmol/l) 12.3 NaH2PO4, 3.1 KH2PO4, 119.8 NaCl, and 0.9 CaCl2.

Animals. Male Sprague-Dawley rats (250–350 g; n = 144; Harlan, Indianapolis, IN) were used. This protocol was approved by the Tulane School of Medicine and University of Louisiana at Monroe Institutional Animal Care and Use Committees. Before experiments, rats were housed in a controlled environment and had free access to commercial rat chow and tap water. Subsets of animals were chronically treated every 12 h for 4 days with an inhibitor of NOS, l-NNAME

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(50 mg/kg ip) (15). NOS blockade in this model has been established by Johnson and Freeman (15). To minimize postprandial sodium excretion variability, animals were deprived of food for 12 h before experiments. 

Experimental procedures. Rats were anesthetized with a single injection of thiobutabarbital sodium (120 mg/kg ip) and a tracheal tube was inserted to maintain an open airway. Catheters (PE-50 tubing filled with heparinized saline) were implanted into a carotid artery and a jugular vein to allow for continuous monitoring of mean arterial pressure (MAP)/heart rate (HR) and for intravenous administration of drugs, respectively. The arterial catheter was connected to a pressure transducer (model TSD104A, Biopac Systems, Santa Barbara, CA) and the venous catheter was connected to a Sage microinjection pump (model M361, Orion Research, Boston, MA) set at 1 ml/h saline infusion rate. A bladder cannula was inserted to allow urine collection for determination of urinary volume and urinary concentrations of sodium/potassium (Flame Photometry, Instrumentation Laboratories) (15). A flank incision was made to expose the left kidney and renal artery. RBF was measured with a blood flow probe (Transonic, Ithaca, NY) placed around the renal artery and connected to a Transonic-T206 synchronized flow meter coupled to a recording system (model MP100, Biopac System).

Following surgical procedures, rats were allowed to stabilize for 45 min. After this initial stabilization period, a 30-min control period was performed and urine and plasma samples were collected. L-NAME-treated and -untreated animals were then administered CrMP (45 μmol/kg ip) or vehicle (1 ml 50 mmol/l ip Na2CO3 solution) and a 30-min treatment period was performed. MAP, HR, and RBF were measured continuously. After the experimental protocols were completed, renal vascular resistance (RVR) was calculated and expressed as mmHg·ml ·1·min−1.

Glomerular filtration rate. In a small subset of studies (n = 6), anesthetized animals were fitted with an additional catheter into the right femoral vein to infuse inulin. Plasma and urinary sodium and potassium concentrations were determined, and inulin concentrations were measured colorimetrically to determine glomerular filtration rate (GFR) (29). Fractional sodium excretion (FENa) was calculated according to standard formulas.

PRA. PRA was measured via a commercially available assay kit (Gamma Coat PRA Assay Kit). Briefly, in subsets of animals (n = 9–11), CrMP was administered to untreated and L-NAME-pretreated rats and PRA was measured to determine whether altered CO levels had any effect on renin release. Quantitative PRA was determined by the radioimmunoassay generation of ANG I.

Determination of ability of CrMP to inhibit CO excretion. Matched awake Sprague-Dawley rats that did not receive any surgical treat-
on MAP, HR, RVR, or RBF (Fig. 3) but significantly decreased UV, UNaV, and UKV (Fig. 4). In rats used for GFR measurements, the solution containing albumin, inulin, and physiological saline caused higher baseline values for urinary volumes, and sodium and potassium excretion rates compared with the data shown in Fig. 4. However, similar to results from that group, CrMP infusion decreased UV, UNaV, and UKV. Furthermore, acute administration of CrMP did not alter GFR (Table 1). In the animals pretreated chronically with L-NAME, CrMP administration did not elicit significant effects on MAP, RVR, or RBF (Fig. 5). Although the baseline MAP was increased in the L-NAME-pretreated animals (Figs. 3 and 5), there were no differences in GFR (Table 1). While CrMP did not affect GFR in L-NAME-treated rats, CrMP significantly decreased urine flow and sodium and potassium excretion rates (Δ−69.2 ± 3.1%, −78.4 ± 1.8%, and −58.3 ± 6.9%; Fig. 6). Vehicle treatment did not cause any changes in either group.

PRA. In untreated animals, CrMP promoted a modest, but significant increase in PRA (Δ18.1 ± 0.9%; Fig. 7). In L-NAME-treated rats, PRA values were similar to the controls but the CrMP-induced increases in PRA were prevented (Fig. 7).

DISCUSSION

The present findings show that intraperitoneal administration of an inhibitor of HO, CrMP, decreases expired CO levels and acutely promotes sodium and water reabsorption in a manner that is independent of a functional NO system. CrMP did not affect systemic or renal hemodynamic parameters even in L-NAME-treated animals. However, CrMP increased PRA, an effect that was abolished by L-NAME pretreatment. In addition, higher doses of CrMP (80 μmol/kg ip) administered to similarly treated animals also led to decreased sodium and water excretion in the absence of systemic or renal hemodynamic changes (unpublished results, Jackson et al.).

Previous studies demonstrated the presence of HO-2 primarily in the tubules of control animals with no detection of HO-1 (5). In contrast, other studies indicated the presence of HO-1 and HO-2 predominantly in the renal medulla with a smaller presence in the renal cortex (10, 37). Still others suggested the presence of HO-1, but not HO-2 in the nephron (24). While variations in staining may arise from particular combinations of antibody lots and immunohistochemical methods, the literature supports the presence of HO in the kidney tubules consistent with our hypothesis that HO inhibition elicits a direct tubular action.

CrMP is a potent photostable inhibitor of HO (32) that has been shown to decrease CO formation in the kidney (18). Our current findings show that the inhibitor of endogenous CO

![Figure 3](image1.png)

Fig. 3. In anesthetized rats, neither vehicle nor CrMP (45 μmol/kg ip) had any effect on blood pressure or renal hemodynamics (P < 0.05; n = 10 each). HR, heart rate; MAP, mean arterial pressure; RVR, calculated renal vascular resistance; RBF, renal blood flow.

![Figure 4](image2.png)

Fig. 4. In anesthetized rats, with respect to vehicle time controls, CrMP (45 μmol/kg ip) decreased urinary volume, sodium and potassium excretion. *P < 0.05; n = 10 each.
formation, CrMP, acutely decreases CO excretion in intact animals when given intraperitoneally. In addition, CrMP acutely decreases renal HO-1 levels. The present study determined that renal HO-1 protein levels decreased; however, HO-1 activity was not determined as previously reported (21). In addition, previous studies observed an increase in HO protein during administration of Sn-protoporphyrin while the current study observed a decrease in HO-1 protein with CrMP (27). This difference appears to be temporal in that it is only observed after 4 h of administration of Sn-protoporphyrin, suggesting that inhibition of HO activity after several hours leads to feedback production of HO-protein. However, the findings in the current study confirm that the dose and route of administration of CrMP successfully decrease CO production acutely.

Previous studies found that increasing HO activity by heme formation increases water and sodium excretion (25). Our current study extends these findings to show that HO inhibition by CrMP acutely decreases UV, UNaV, and UKV without altering systemic or renal hemodynamic parameters. We further found that CrMP decreases FENa. These results suggest that CrMP most likely exerts its effects by inhibition of a direct tubular action of a HO metabolite. In our studies, the acute decrease in water and electrolyte excretion was still present after inhibition of NOS by L-NAME, indicating that the effects on renal excretory functions are independent of the NO system.

Our current study failed to show any changes in systemic or renal hemodynamic parameters. In contrast, other studies showed that HO inhibition decreased medullary blood flow, without significantly affecting cortical blood flow (19, 37). Intravenous administration of SnMP was observed to increase RVR, and this effect was enhanced by L-NAME pretreatment (26). Additional studies performed on isolated renal interlobular arteries demonstrated a vasoconstriction in response to SnMP a response that was also enhanced in L-NAME-pretreated vessels (26). A study using another HO inhibitor, cobalt protoporphyrin, showed increases in RVR and decreases in GFR (3). While it is possible that higher doses of CrMP could have elicited renal vasoconstriction, the present study provides evidence that the reductions in sodium excretion are independent of RBF and GFR. While it is impossible to determine the reason for the different outcome in our present study, it may be due to the selection of pharmacological agents (CrMP vs. SnMP) and/or manner of drug administration (ip vs. iv). Even so, it remains clear that the antinatriuretic/antidiuretic effects of the HO inhibitor can occur independently of changes in RBF and GFR. Furthermore, our results are in agreement with a study showing that the HO inhibitor zinc protoporphyrin had no effect on baseline RBF (23).

Numerous studies suggest that CO contributes an important dual role regulating normal vascular tone (13, 14, 16). Specifically, CO has been shown to promote relaxation of vascular smooth muscle (7), but it can also cause endothelium-dependent vasoconstriction through inhibition of NOS (13). Thus, the renal vascular responses to increased CO are complex. One group (31) showed that CO elicits a biphasic response in isolated renal resistance arteries and juxtamedullary nephron afferent arteries: 1) promotes vasodilation at very low con-

Table 1. Acute effects of CrMP (45 μmol/kg) on GFR, UV, UNaV, FENa, and UKV

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>UV, μl/min</th>
<th>GFR, ml/min</th>
<th>UNaV, μmol/min</th>
<th>FENa, %</th>
<th>UKV, μmol/min</th>
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<tbody>
<tr>
<td>No pretreatment</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>6</td>
<td>8.6 ± 0.12</td>
<td>1.15 ± 0.13</td>
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<td>6</td>
<td>8.6 ± 0.01</td>
<td>1.13 ± 0.15</td>
<td>0.81 ± 0.14</td>
<td>0.40 ± 0.21</td>
<td>0.19 ± 0.01</td>
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<tr>
<td>Control</td>
<td>6</td>
<td>8.6 ± 0.01</td>
<td>1.12 ± 0.18</td>
<td>0.79 ± 0.16</td>
<td>0.56 ± 0.24</td>
<td>0.18 ± 0.04</td>
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<td>CrMP</td>
<td>6</td>
<td>4.6 ± 0.02*</td>
<td>1.14 ± 0.25</td>
<td>0.10 ± 0.01*</td>
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<td>0.05 ± 0.11*</td>
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<tr>
<td>Control</td>
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<td>8.0 ± 0.12</td>
<td>1.21 ± 0.12</td>
<td>0.68 ± 0.03</td>
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<tr>
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<td>1.24 ± 0.15</td>
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<td>0.42 ± 0.12</td>
<td>0.19 ± 0.02</td>
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<tr>
<td>CrMP</td>
<td>6</td>
<td>3.4 ± 0.24*</td>
<td>1.22 ± 0.11</td>
<td>0.12 ± 0.02*</td>
<td>0.03 ± 0.14*</td>
<td>0.07 ± 0.12*</td>
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Values are means ± SE. In anesthetized rats, infusion of albumin, inulin, and physiological saline for the purpose of measuring glomerular filtration rate (GFR)-increased sodium and potassium excretions compared with previous animals. However, no significant differences in GFR were observed in any of the experimental groups (*P < 0.05, n = 6 each). CrMP, chromium mesoporphyrin; UV, urinary volume; UNaV, sodium excretion; FENa, fractional excretion of sodium; UKV, urinary potassium.

Fig. 5. In anesthetized rats, CrMP (45 μmol/kg ip) did not affect HR, MAP, or renal hemodynamics in chronic L-NAME rats (50 mg·kg⁻¹·day⁻¹ for 12 h). P < 0.05; n = 10 each.

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centrations (100 nmol/l) but 2) elicits vasoconstriction and attenuates endothelium-dependent vasodilation by inhibiting NOS at higher levels (1–10 μmol/l). Similarly, another group found (26) that CO (1 μmol/l) causes vasoconstriction in isolated renal interlobular arteries, which is converted to vasodilation in vessels pretreated with a NOS inhibitor. However, a recent study (4) reported that CO (10 μmol/l) promotes dilation of juxtaglomerular nephron afferent arterioles. Our results suggest that modest inhibition of endogenous CO production does not change total RBF and GFR. While these results do not exclude the possibility of local changes of vascular resistance within the kidney in response to HO inhibition, they argue against it being the primary mechanism responsible for the changes in sodium and potassium excretion and urine flow.

The current findings also demonstrate a small increase in PRA during acute administration of CrMP. Given the importance of the renin-angiotensin system in regulating renal function (22), it might be speculated that the PRA response may account for the observed decreases in excretory functions. However, in L-NAME-treated animals, CrMP had no effect on PRA, but it still promoted sodium/water reabsorption. The fact that CrMP still promotes water/sodium reabsorption in the L-NAME-treated animals, in which PRA is unaffected, suggests that the excretory effects of the HO inhibitor are also independent of changes in renin activity.

In previous studies, the interaction between the NO and CO systems has been documented (16). However, the present study using relatively low doses does not support an interaction between these two systems in regulating changes in whole kidney vascular resistance or GFR. Even so, the inhibitor of CO production still acutely promotes sodium and water reabsorption and decreases FE_Na, suggesting that endogenous CO likely has direct effects on the nephron to alter water and electrolyte reabsorption.

In summary, the present results indicate that intraperitoneal administration of CrMP decreases endogenous CO production, decreases renal HO-1 levels, and promotes water and sodium reabsorption, even in the absence of changes in RBF, GFR, or NO production. The findings suggest that a metabolite of HO, potentially CO, may promote water and sodium excretion by a direct tubular action.

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ENDOGENOUS CO BLOCKADE AND RENAL FUNCTION

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