Effects of Exogenous Heme on Renal Function
Role of Heme Oxygenase and Cyclooxygenase

Francisca Rodriguez, Rowena Kemp, Michael Balazy, Alberto Nasjletti

Abstract—We examined the effects of heme administration (15 mg/kg IV) on indexes of renal carbon monoxide production and contrasted the renal functional response to heme in anesthetized rats pretreated and not pretreated with stannous mesoporphyrin (40 μmol/kg IV) to inhibit heme oxygenase or sodium meclofenamate (5 mg/kg IV plus infusion at 10 μg/kg per minute) to inhibit cyclooxygenase. In rats without drug pretreatment, heme administration decreased renal vascular resistance and increased renal blood flow, urine volume, and sodium excretion associated with augmented urinary excretion of 6-keto-PGF₁α and enhanced concentration of carbon monoxide in the renal cortical microdialysate. Pretreatment with stannous mesoporphyrin did not prevent heme from producing renal vasodilation and increasing renal blood flow but abolished the diuretic and natriuretic responses. Conversely, pretreatment with sodium meclofenamate blunted the renal vasodilatory effect of heme but affected neither the diuretic nor the natriuretic effect. We conclude that heme-induced renal vasodilation is a cyclooxygenase-dependent response involving increased synthesis of PG₁₂, whereas heme-induced diuresis and natriuresis are heme oxygenase–dependent responses involving inhibition of tubular reabsorption of sodium and water through undefined mechanisms. (Hypertension. 2003;42[part 2]:680-684.)

Key Words: kidney • renal circulation • prostaglandins • sodium • heme oxygenase • heme • carbon monoxide

Heme (ferroprotoporphyrin IX) undergoes metabolism by heme oxygenase (HO) isoforms, yielding biliverdin, ferrous iron and carbon monoxide (CO). Heme is the prosthetic group of proteins involved in many different regulatory functions, for example, oxygen transport, mitochondrial respiration, processing of reactive oxygen species, and manufacture of biologically active substances. Furthermore, HO-derived products such as biliverdin and iron are believed to subserve antioxidant and prooxidant mechanisms, respectively, whereas HO-derived CO has been implicated in vasoregulatory functions.

Renal vascular and tubular structures express HO. We and others investigators have shown that treatment of rats with an HO inhibitor decreases renal blood flow acutely, implying that the renal heme-HO system supports the renal circulation via formation of a vasodilatory HO product, presumably CO. If so, an intervention that enhances the formation of HO products may be expected to promote renal vasodilation and to increase renal blood flow.

The acute administration of exogenous heme may be such an intervention, since treatment with heme elicits many acute effects, such as elevation of plasma bilirubin levels. Moreover, exposure to exogenous heme was shown to elicit HO-dependent dilation of rat gracilis muscle arterioles treated ex vivo with a nitric oxide (NO) synthesis inhibitor and of pial arterial vessels of newborn pigs in vivo. Therefore, the goals of the present study were (1) to examine the effect of exogenous heme on indexes of renal CO production in vivo and (2) to contrast the renal functional response to heme administration in rats pretreated and not pretreated with stannous mesoporphyrin (SnMP), an inhibitor of HO. In addition, because heme was reported to stimulate endothelial cell prostaglandin production through a mechanism independent of HO, we also contrasted the effects of heme on renal function in rats pretreated and not pretreated with sodium meclofenamate, a cyclooxygenase inhibitor.

Methods

Drugs and Solutions
SnMP was obtained from Frontier Scientific and all other drugs from Sigma Chemical Co. SnMP was dissolved in 50 mmol/L Na₂CO₃, sonicated, and filtered immediately before use. Hemin (ferroprotoporphyrin IX chloride) was dissolved in 0.1 mol/L NaOH, and the pH was adjusted to 7.8 with 0.1 mol/L NaOH, and the pH was adjusted to 7.8 with 0.1 mol/L HCl before use. All other drugs were dissolved in 0.15 mol/L NaCl.

Experimental Procedure and Design
Studies were conducted on male Sprague-Dawley rats (Charles River; 300 to 325 g body weight) anesthetized with thiobutabarbital (50 mg/kg IP) and ketamine (30 mg/kg IM), with the use of protocols approved by the Institutional Animal Care and Use Committee.
Polyethylene canulas were placed in the trachea (PE-205) to aid ventilation, the bladder (PE-60) for urine collection, the left femoral vein (PE-50) for administration of fluid and drugs, and the left femoral artery (PE-90) for blood sampling and measurement of blood pressure. The left kidney was exposed through a midline incision, and some rats were instrumented with a 2-mm flow probe placed around the renal artery for measurement of flow with a transit-time flowmeter (model T206, Transonic System Inc). Other rats were instrumented with a microdialysis probe (CMA Microdialysis; 0.5-mm-tip diameter and 20-kDa transmembrane diffusion cutoff), inserted into the renal cortex to a depth of 1.5 mm and perfused continuously (3 μL/min) with 0.15 mol/L NaCl to collect microdialysate samples for analysis. Once the animals were instrumented, an infusion (2.7 mL/h IV) of 0.15 mol/L NaCl containing 10 mg/mL bovine serum albumin was initiated and maintained throughout the study. In some experiments, [3 H] inulin was included in the infusion (1 μCi/mL) for measurement of glomerular filtration rate, as reflected by the clearance of inulin. Data collection was initiated after a 60-minute equilibration interval.

Experiments were designed to examine the effect of heme (15 mg/kg IV) on renal function in rats pretreated and not pretreated with SnMP (40 μmol/kg IV) to inhibit HO isoforms or sodium meclofenamate (5 mg/kg IV bolus injection plus 10 μg/kg per minute IV infusion) to inhibit prostaglandin synthesis. Thirty minutes after the onset of vehicle or drug pretreatment, basal line data on mean arterial pressure and indexes of renal hemodynamic and excretory function were collected over two 15-minute periods before the administration of heme to rats pretreated with salinevehicle only (n=11), SnMP (n=8), or sodium meclofenamate (n=11); experimental data were collected over two additional 15-minute periods commencing 30 minutes after heme administration. An identical protocol was used to collect data on renal function before and after the administration of heme vehicle only in rats without drug pretreatment (n=8). Samples of urine and renal microdialysate obtained before and after heme administration were analyzed for CO and 6-keto-PGF1α, the nonenzymatic derivative of PGI2.

Analytical Procedure

The concentration of [3 H] inulin in plasma and urine was determined by liquid scintillation counting. Plasma and urinary sodium and potassium were measured by flame photometry; 6-keto-PGF1α was determined by enzyme immunoassay, with the use of a kit available commercially (Cayman Chemical). CO was measured by gas chromatography–mass spectroscopy, as previously described, in specimens of urine (about 100 μL) and renal microdialysate (about 100 μL) collected into amber vials (2 mL) capped with rubberized Teflon liners perforated with one G-23 and one G-30 needle, which, respectively, allowed the specimens to flow into the vials under isobaric conditions. Immediately after completion of sample collection, the needles were removed, the perforations were sealed, and the samples were analyzed.

Data Analysis

Results are expressed as mean±SEM. Data on renal hemodynamics and excretory functions are the average of two consecutive 15-minute observation periods. Data on renal blood flow, glomerular filtration rate, urine volume, and urinary excretion of sodium, potassium, 6-keto-PGF1α, and CO are factored by kidney weight. Results were analyzed by 1- or 2-way ANOVA followed by the Newman–Keuls post hoc test or the Fisher test. The null hypothesis was rejected at a value of P<0.05.

Results

As shown in Figure 1, the intravenous administration of heme to rats without drug pretreatment caused significant (P<0.05) lowering of mean arterial pressure, elevation of renal blood flow, and reduction of renal vascular resistance. Heme administration also increased (P<0.05) urine volume and urinary sodium excretion in animals without drug pretreatment while having no effect on glomerular filtration rate, urinary potassium excretion, and plasma concentrations of sodium and potassium (Table). In comparison, the administration of heme–vehicle only did not affect significantly any indexes of renal hemodynamic or excretory functions (Figure 1 and Table).

Figure 1 illustrates the effect of heme administration in rats without drug pretreatment on the urinary excretion rate of CO and 6-keto-PGF1α, as well as on the concentration of these substances in the renal cortical microdialysate, which is presumed to reflect their concentration in the renal parenchyma, at least at the point of probe placement. Heme treatment did not affect the urinary excretion of CO or the concentration of CO in urine (988±87 and 809±94 pmol/mL before and after heme, respectively) but increased (P<0.05) the concentration of CO in the renal microdialysate from 632±68 to 808±106 pmol/mL. Conversely, heme treatment increased (P<0.05) the urinary excretion of 6-keto-PGF1α from 107±12 to 236±70 pg/min per gram but had no effect on the concentration of 6-keto-PGF1α in the renal microdialysate. In rats pretreated with SnMP (n=4), the basal concentration of CO in the renal microdialysate (310±62 pmol/mL) was reduced (P<0.05) relative to the basal concentration in rats without pretreatment (632±68 pmol/mL) and did not increase in response to the administration of heme (364±71 pmol/mL). The urinary excretion of CO also was decreased (P<0.05) in rats pretreated with SnMP, relative to the excretion in rats without pretreatment (1.34±0.27 versus 2.89±0.56 pmol/min per gram). In rats pretreated with sodium meclofenamate (n=4), the basal urinary excretion of 6-keto-PGF1α (31±6 pg/min per gram) was decreased (P<0.05) relative to corresponding values in rats without drug pretreatment (107±12 pg/min per gram) and did not increase significantly after heme administration (97±54 pg/min per gram).
Comparison of the renal functional response to heme administration in rats pretreated and not pretreated with SnMP offers information on the contribution of HO-derived products to the effects of heme on renal hemodynamic and excretory functions (Figure 1 and Table). Relative to corresponding data in rats pretreated with saline only, basal values of renal vascular resistance and glomerular filtration rate were, respectively, increased \((P<0.05)\) and decreased \((P<0.05)\) in rats pretreated with SnMP.

Resembling the results obtained in rats without drug pretreatment, heme significantly \((P<0.05)\) decreased mean arterial pressure, increased renal blood flow, and reduced renal vascular resistance in rats pretreated with SnMP. Yet, at variance with the results in rats without drug pretreatment, the administration of heme did not increase urine volume and sodium excretion in rats pretreated with SnMP.

Comparison of the renal effects of heme administration in rats with and without sodium meclofenamate pretreatment yields information on the contribution of cyclooxygenase-derived products to heme-induced changes in renal function (Figure 1 and Table). Relative to corresponding data in rats pretreated with saline only, basal values of glomerular filtration rate and urine volume were decreased \((P<0.05)\) in rats pretreated with the inhibitor of cyclooxygenase. Unlike results in rats without drug pretreatment, which respond to acute heme administration with elevation of renal blood flow and reduction of renal vascular resistance, heme did not affect renal hemodynamics in rats pretreated with sodium meclofenamate. These animals, however, like animals without drug pretreatment, responded to the administration of heme with significant \((P<0.05)\) elevation of urine volume and sodium excretion.

**Discussion**

Previous studies have demonstrated expression of HO-1 and HO-2 in normal kidneys, with the level of expression increasing from cortex to medulla.\(^5,7\) HO-2 is constitutively expressed in renal arterial vessels and in tubules including the proximal tubules, medullary thick ascending limb, distal convoluted tubules and collecting tubules.\(^3,5\) Renal vascular and tubular structures also express HO-1, particularly in response to injurious conditions.\(^3,5\) The present study documents for the first time occurrence of CO in rat urine and renal microdialysate. That the administration of heme increases the concentration of CO in the renal microdialysate suggests augmentation of CO levels in the renal parenchyma, presumably caused by enhanced CO production because of increased availability of heme to HO. Surprisingly, the administration of heme increased neither the urinary concentration nor the excretion rate of CO. Hence, the level of CO in the renal parenchyma may not be reflected by the urinary concentration or excretion rate of CO. This interpretation is tentative, since our estimates of CO levels in the renal parenchyma are limited to one region of the kidney only.

One key finding of our study is that the administration of heme reduces renal vascular resistance and increases renal
blood flow in rats without drug pretreatment and rats pretreated with SnMP but not in animals pretreated with sodium mecolfenamate. These observations imply that heme-induced renal vasodilation relies on a mechanism involving cyclooxygenase rather than HO. The notion that cyclooxygenase plays a role in the renal vasodilatory effect of heme is reinforced by the finding that heme increases the urinary excretion of 6-keto-PGF\(_{1\alpha}\), the inactive derivative of PGI\(_2\). As PGI\(_2\) is known to promote renal vasodilation,\(^{12}\) the renal vasodilatory effect of heme is attributable to an enhanced production of renal PGI\(_2\). This conclusion fits well with the results of a previous study documenting that heme promotes prostaglandin production in cultured endothelial cells.\(^{13}\) This effect of heme was not prevented by HO inhibition and consequently was ascribed to heme itself rather than to a product of its metabolism by HO.\(^{13}\) In this regard, it is known that cyclooxygenase isoforms are heme proteins and that the heme prosthetic group is essential for the expression of catalytic activity.\(^{18}\)

It is surprising that pretreatment with the HO inhibitor SnMP does not interfere with heme-induced renal vasodilation, since the administration of heme increased the level of CO at renal cortical sites and previous studies have suggested a role for endogenous CO as a mediator of renal vasodilatation in vivo.\(^{6,8}\) On the other hand, it is also known that CO inhibits NO synthase,\(^{19}\) and action that fosters vasoconstriction and eventually can overcome the direct vasorelaxing action of the gas on vascular smooth muscle.\(^{6,20}\) Hence, our study does not exclude the possibility that the expected contribution of CO to the renal vasodilatory effect of heme is offset by vasoconstriction arising from a deficit in vascular NO created by CO-mediated inhibition of NO production\(^{6,20}\) or by a decrease in NO bioavailability caused by heme-induced oxidative stress.\(^1\)

Another key finding of our study is that the administration of heme increases urine volume and sodium excretion in rats without drug pretreatment and rats pretreated with sodium mecolfenamate but not in animals pretreated with SnMP. These observations imply that heme-induced diuresis and natriuresis rely on a mechanism involving HO but not cyclooxygenase. As heme did not increase the glomerular filtration rate, the diuretic and natriuretic effects may be ascribed to inhibition of sodium and water reabsorption by a product of heme metabolism by HO. However, there is a paucity of information on the regulatory influence of CO and biliverdin/bilirubin on tubular function. CO was reported to increase the activity of the apical 70 pS K\(^+\) channel in the thick ascending limb of the loop of Henle, an action that is expected to increase rather than decrease the reabsorption of Na\(^+\) and Cl\(^-\) as the result of increased availability of K\(^+\) to the Na\(^+\)/K\(^+\) 2Cl\(^-\) cotransporter.\(^{21}\) On the other hand, it is conceivable that heme-derived CO, like NO,\(^{22,23}\) competes with O\(_2\) for sites on the mitochondrial cytochrome C respiratory chain and thus inhibits oxygen consumption and sodium reabsorption. This possibility remains to be tested.

In summary, this study demonstrates that administration of heme reduces renal vascular resistance and promotes renal vasodilation, diuresis, and natriuresis associated with augmented urinary excretion of 6-keto-PGF\(_{1\alpha}\) and enhanced concentration of CO in renal cortical microdialysate. The study also shows that heme-induced renal vasodilation is a cyclooxygenase-dependent response involving increased synthesis of PGI\(_2\), whereas heme-induced diuresis and natriuresis are HO-dependent responses involving inhibition of tubular reabsorption of sodium and water through an undefined mechanism(s). Thus changes in heme availability may affect renal hemodynamic and excretory functions through mechanisms involving both cyclooxygenase- and HO-derived products.

**Perspectives**

The results of the present study add to a growing body of evidence that place heme and the products of its metabolism by HO in the center stage as protagonists of various homeostatic mechanisms in the kidney and other organs. That upregulation of HO product generation, in response to an acute increase in heme availability, brings about diuresis and natriuresis implies that the renal heme-HO system participates in the regulation of salt and water excretion. That increased availability of heme causes HO-independent renal vasodilation through a prostaglandin-dependent mechanism is in keeping with the concept that the level of cellular heme regulates the expression of catalytically active cyclooxygenase.\(^{5,13}\) This concept may be extended to include other renal enzymes that depend on heme for their catalytic activity, for example, soluble guanylate cyclase, NO synthase, and cytochrome P450 oxygenase that manufacture 20-hydroxyeicosatetraenoic acid and other eicosanoids.\(^{1,2,5}\) If so, cellular heme availability may be regarded as a critical controlling element shared by multiple renal function regulatory systems.

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**References**


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