Heme oxygenase induction attenuates afferent arteriolar autoregulatory responses

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Rats were treated with hemin and SnCl₂ to induce HO-1, and Aff-Art autoregulatory responses were evaluated using the rat blood-perfused juxtamedullary nephron preparation. Renal HO-1 expression was significantly increased in hemin- and SnCl₂-treated rats, while HO-2 was not altered. Aff-Art autoregulatory constrictor responses to increases in RPP from 100 to 150 mmHg were attenuated in hemin- and SnCl₂-treated rats compared with control rats (+1.1 ± 3.3, n = 9 and +4.4 ± 5.3, n = 9 vs. −14.2 ± 1.5%, n = 10, respectively) (P < 0.05). Acute HO inhibition with chromium mesoporphyrin (CrMP; 15 µmol/l) restored Aff-Art autoregulatory responses in hemin- and SnCl₂-treated rats. Superfusing Aff-Arts from control rats with 100 mol/l biliverdin did not alter autoregulatory responses; however, superfusion with 1 mmol/l CO significantly attenuated autoregulatory responses to increases in RPP from 100 to 150 mmHg (+3.3 ± 5.4 vs. −16.6 ± 3.8%, n = 6) (P < 0.05). Acute soluble guanylate cyclase inhibition with 10 µmol/l ODQ restored Aff-Art autoregulatory responses in hemin-treated rats. Immunohistochemistry shows HO-2 to be expressed mainly in epithelial cells with weak staining in proximal tubules, interlobular arteries, and Aff-Arts. In hemin- and SnCl₂-treated rats, HO-1 was induced in tubular epithelial cells but not interlobular arteries and Aff-Arts. We conclude that induction of renal HO-1 attenuates Aff-Art constrictor responses to increases in RPP via increasing CO production from tubular epithelial cells, suggesting that an augmented HO system in pathophysiological conditions modulates renal autoregulation.

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The kidney has the ability to autoregulate blood flow over a wide range of arterial blood pressure (AP). In response to increases in AP, preglomerular vascular resistance increases to maintain glomerular filtration rate and renal blood flow. Afferent arterioles (Aff-Art) are the major resistance vessels in the kidney responsible for renal autoregulation (11). At least two mechanisms contribute to renal autoregulation: 1) myogenic tone and 2) tubuloglomerular feedback (32). The synthesis of multiple paracrine factors, including nitric oxide (NO), cyclooxygenase-derived eicosanoids, 20-hydroxyeicosatetraenoic acid (20-HETE), and superoxide, is catalyzed via heme-dependent enzymes (i.e., nitric oxide synthase, cyclooxygenase, cytochrome P-450, and NADPH oxidase) (46). These paracrine factors modulate the myogenic and/or tubuloglomerular feedback components of the Aff-Art autoregulatory responses (27, 32).

The catabolism of heme is catalyzed by heme oxygenase (HO), causing heme to be converted to carbon monoxide (CO), biliverdin, and free iron (44). Two isoforms of HO are expressed in the kidney (HO-1 and HO-2) (15, 19). The kidney has a relatively low basal level of HO activity (8, 15) that is mainly derived from constitutive HO-2 (1, 28, 29). Renal HO-2 is localized to epithelial cells of the proximal tubule, thick ascending limb and distal tubule, and connecting tubule, and principal cells of the collecting ducts (20). Renal HO-1 is induced under certain pathological conditions and in response to several agents (19, 31, 40, 41). Acute treatment with hemin stimulates HO activity and increases renal cortical dialysate CO concentration and causes diuresis and natriuresis (37). Increases in renal perfusion pressure (RPP) induce renal CO production, and HO inhibition prevents the pressure-dependent increase in CO (26). This suggests that HO-derived products could modulate Aff-Art autoregulatory responses to increases in perfusion pressure.

Exogenous CO administration dilates renal Aff-Art from normal rats (6, 45). Furthermore, endogenously produced CO exerts a vasodilatory influence on the renal circulation, and inhibition of HO decreases renal blood flow and renal function (2, 4, 34, 38, 48). Bilirubin is also produced from heme metabolism by HO and biliverdin reductase and is an endogenous antioxidant (42). Bilirubin scavenges reactive oxygen species (14, 17, 33) and inhibits angiotensin II-mediated activation of NADPH oxidase (16, 35), also potentially dilating the renal microvasculature. Uregulation of renal HO-1 increases CO and biliverdin/bilirubin production, reduces NADPH oxidase-mediated oxidative stress (16), inhibits cortical 20-HETE synthesis (5), and inhibits thromboxane synthase (39). Inhibition of HO enhances pressure-induced constriction of isolated pressurized renal interlobular arteries (38). These results implicate HO-derived metabolites as important modulators of renal microcirculatory function.

This study was designed to examine the hypothesis that renal HO-1 induction attenuates Aff-Art autoregulatory responses to increases in RPP. To test this hypothesis, we used the blood-perfused juxtamedullary nephron preparation. In this preparation, the intimate tubulovascular relationships are not disturbed and autoregulatory mechanisms are intact (i.e., myogenic tone.

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and tubuloglomerular feedback) (13). Accordingly, the aims of this study are 1) to determine the effect of HO-1 induction on Aff-Art autoregulatory responses, 2) to determine the effect of acute HO inhibition on Aff-Art autoregulatory responses to increases in RPP, and 3) to determine the effects of HO-derived products (biliverdin and CO) and effects of soluble guanylate cyclase (sGC) inhibition on Aff-Art autoregulatory responses.

**METHODS**

**Chemicals and drug preparation.** Hemin (50 mg, Sigma-Aldrich, St. Louis, MO) was dissolved in 1 ml of 0.1 M NaOH, the solution was diluted using deionized water, pH was adjusted to 7.8, and the final volume was adjusted to 5 ml. Tin chloride (SnCl₂; Sigma-Aldrich) was dissolved in 0.1 M sodium citrate solution. Chromium mesoporphyrin (CrMP; Frontier Scientific, Logan, UT) was dissolved in 0.1 M sodium citrate solution. Chromium (11–14 wk old), were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and perfused with Tyrode's solution containing 1% albumin at 37°C; however, 1% albumin was not included when kidneys were superfused with CO or Tyrode’s solution containing 1% albumin at 37°C; however, 1% albumin was not included when kidneys were superfused with CO or biliverdin to avoid foaming. The tissue was transilluminated under a microscope with a water-immersion objective (×40). Video images of the microvessels were transferred to a video monitor and recorded on a DVD for later analysis. Aff-Art diameters were measured using a digital image-shearing monitor. A 10-min equilibration period was allowed before the initiation of each experimental protocol. Basal diameters were measured during superfusion with appropriate vehicle for at least 5 min. Aff-Art diameters were averaged for each minute.

**Tissue preparation.** Left kidneys were immediately collected and frozen at −80°C until used for Western blotting. Tissues were homogenized in buffer, pH 7.4, containing 0.25 M sucrose. The homogenates were centrifuged in an Eppendorf centrifuge at 10,000 g for 10 min at 4°C to remove unbroken cells, and the supernatant was stored at −80°C. Protein concentration was determined according to the method of Bradford (Bio-Rad, Hercules, CA) (10).

**Western blot analysis.** As previously described (5), cell-free homogenates (10,000-g supernatant) of kidney preparations were separated by SDS-PAGE and transferred to a hydrophobic polyvinylidene difluoride (PVDF) transfer membrane (Amersham-GE Biosciences, Piscataway, NJ). The membranes were incubated with Odyssey blocking reagent (LI-COR Biosciences, Lincoln, NE) at 4°C overnight. The membranes were incubated for 1 h with one of the following antibodies: rabbit anti-rat HO-1 and HO-2 polyclonal antibodies (1:1,000, Stressgen Biotechnologies, Victoria, BC, Canada) or mouse anti-β-actin monoclonal antibody. The membranes were washed with phosphate-buffered saline Tween 20 (PBST) and subsequently probed with fluorescent-tagged secondary antibodies at a dilution of 1:15,000. The signal was detected using an Odyssey fluorescent scanner.

**Aff-Art responses to increases in RPP.** To determine effects of HO induction on Aff-Art autoregulatory responses, Aff-Ats from control, hemin-treated, or SnCl₂-treated rats were superfused with vehicle buffer for 5 min at a perfusion pressure of 100 mmHg. RPP was increased to 125 and 150 mmHg and was maintained for 5 min at each pressure. Then, the pressure was decreased to 100 mmHg for a recovery period of 5 min. Aff-Art responses to increases in RPP were compared between the three different groups (control, hemin, and SnCl₂) and to basal diameters at 100 mmHg within the same experimental group.

To test the effect of acute HO inhibition on Aff-Art responses to increases in RPP, Aff-Ats from control, hemin-treated, or SnCl₂-treated rats were superfused with 15 µmol/l CrMP (a nonspecific HO activity inhibitor) for 5–10 min at 100 mmHg, which has been shown to be an effective dose and time for HO inhibition (3, 24). RPP was increased to 125 and 150 mmHg and was maintained for 5 min at each pressure, and then it was decreased to 100 mmHg for a final recovery period of 5 min. Aff-Art responses to increases in RPP during HO inhibition were compared with responses obtained during vehicle superfusion.

To determine the effects of biliverdin and CO on Aff-Art autoregulatory responses, Aff-Art responses to increases in RPP from 100 mmHg to 125 and 150 mmHg were determined during superfusion with vehicle, biliverdin (100 µmol/l), and CO (1 mmol/l). Because the plasma bilirubin level is ~100 µM in the hyperbilirubinemic Gunn rats (35), we superperfused Aff-Ats with 100 µM biliverdin to mimic these high bilirubin levels. Treatment with heme (30 µmol/kg) increases CO blood levels to 100 µmol/l (47); however, because the superfusion chamber is exposed, we superperfused Aff-Ats with 1 µmol/l CO to compensate for gas loss over the duration of the experiment.

To determine the effects of sGC inhibition on Aff-Art autoregulatory responses, Aff-Art responses to increases in RPP from 100 to 125 and 150 mmHg were determined during superfusion with vehicle and ODQ (10 µmol/l). Aff-Art responses to increases in RPP during sGC inhibition were compared with responses obtained during vehicle superfusion.

**Immunohistochemistry.** Kidneys were perfused with PBS followed by 4% paraformaldehyde. Kidneys were fixed in 4% paraformaldehyde followed by zinc-saturated formalin for immunohistochemical studies as previously described (7, 36). Briefly, kidney sections were deparaffinized in xylene and graded alcohol, washed, and then incubated for 10 min in dual endogenous enzyme block. Then, sections were washed and incubated with diluted blocking serum for 30 min. Sections were incubated overnight with monoclonal mouse HO-1 antibody (1:250) or polyclonal HO-2 antibody (1:1,000, Assay Designs, Ann Arbor, MI). A Dakocytomation kit (Dako, Carpinteria, CA) was used for immunostaining; sections were washed and incubated for 30 min with the diluted peroxidase-labeled secondary antibody. A 3,3-diaminobenzidine + chromogen substrate solution was applied for 2 min to develop the stain. Sections were counterstained with hematoxylin, mounted, and coverslipped until microscopic anal-
RESULTS

HO-1 protein expression is induced in kidneys from hemin- and SnCl₂-treated rats. HO-1 protein expression significantly increased by 10.1 ± 3.8- and 7.3 ± 0.8-fold in kidneys from hemin- and SnCl₂-treated rats compared with kidneys from normal control rats (n = 6, P < 0.05) (Fig. 1). No differences in renal HO-2 expression between hemin- or SnCl₂-treated rats and normal control rats were detected (Fig. 1).

Renal HO-1 induction attenuates Aff-Art autoregulatory responses to increases in RPP. At 100 mmHg RPP, basal Aff-Art diameters were not significantly different among all three groups (Fig. 2). Aff-Art diameter averaged 17.9 ± 0.5 (n = 10) for control, 19.7 ± 0.8 (n = 9) for hemin-treated, and 18.4 ± 0.5 μm (n = 9) for SnCl₂-treated rats, respectively. In normal rats, Aff-Art diameter significantly decreased from 17.9 ± 0.5 to 16.3 ± 0.4 and 15.4 ± 0.5 μm in response to increases in RPP from 100 to 125 and 150 mmHg (Fig. 2). In hemin-treated rats, Aff-Art diameter did not significantly change in response to increases in RPP to 125 and 150 mmHg (19.5 ± 0.7 and 19.9 ± 0.9 μm, respectively; n = 9, P > 0.05). Similarly, in SnCl₂-treated rats, Aff-Art diameter did not change in response to increases in RPP to 125 and 150 mmHg (18.2 ± 0.6 and 19.0 ± 0.8 μm, respectively; n = 9, P > 0.05) (Fig. 2). At RPP of 125 and 150 mmHg, Aff-Art diameters in hemin and SnCl₂-treated rats were significantly greater than diameters in normal control rats (Fig. 2).

Acute HO inhibition does not alter Aff-Art autoregulatory responses in normal rats. In control kidneys, HO inhibition by superfusing 15 μM CrMP did not alter Aff-Art responses to increases in RPP from 100 to 125 and 150 mmHg (13.6 ± 2.9 and 16.9 ± 3.0% during CrMP vs. 8.7 ± 2.4 and 13.6 ± 1.6% during vehicle superfusion, n = 8, P > 0.05) (Fig. 3). These results indicate that basal HO activity does not modulate Aff-Art autoregulatory responses in normal kidneys.

Acute HO inhibition restores Aff-Art autoregulatory responses in SnCl₂- and hemin-treated rats. In hemin- and SnCl₂-treated rats, Aff-Art autoregulatory responses to increases in RPP from 100 to 125 and 150 mmHg were examined before and during superfusion with 15 μM CrMP. In hemin-treated rats, during superfusing with CrMP, Aff-Art diameters significantly decreased in response to increases in RPP to 125 and 150 mmHg compared with basal diameter at 100 mmHg (from 17.9 ± 0.9 to 16.0 ± 1.0 and 15.4 ± 1.1 μm, n = 8, P < 0.05). Aff-Art constrictor responses to increases in RPP to 125

![Figure 1](image1.png)  Western blot and densitometric analysis showing the effect of treatment with hemin (4.5 μmol/100 g body wt; A) or SnCl₂ (10 μmol/100 g body wt; B) on renal heme oxygenase (HO-1 and HO-2) protein expression (n = 6). Results are normalized by β-actin and expressed as fold-control and presented as means ± SE for each group. *P < 0.05 vs. control.

![Figure 2](image2.png)  Afferent arteriolar (Aff-Art) diameter responses to increases in renal perfusion pressure (RPP) from 100 to 125 and 150 mmHg in control (n = 10), hemin-treated (n = 9), and SnCl₂-treated rats (n = 9). Results are expressed as means ± SE for each group. *P < 0.05 vs. basal diameter. #P < 0.05 vs. control.
and 150 mmHg during superfusion with CrMP were significantly greater than responses during vehicle superfusion (10.9 ± 4.0 and 13.7 ± 3.0 vs. 1.7 ± 2.2 and +0.8 ± 3.7%, n = 8, P < 0.05) (Fig. 4).

Similarly, in SnCl₂-treated rats, superfusing with CrMP partially restored Aff-Art autoregulatory constrictor responses to increases in RPP. Increases in RPP from 100 to 125 mmHg did not significantly alter Aff-Art diameter (18.5 ± 0.3 vs. 17.6 ± 0.5 μm, n = 6, P > 0.05); however, Aff-Art diameters significantly decreased to 16.8 ± 0.7 μm (P < 0.05) in response to increase in RPP to 150 mmHg. Aff-Art constrictor responses to increase in RPP to 150 mmHg during superfusion with CrMP were significantly greater than responses during vehicle superfusion (−9.1 ± 3.1 vs. +9.1 ± 5.2%, n = 6, P < 0.05) (Fig. 5).

CO attenuates Aff-Art autoregulatory responses to increases in RPP. During vehicle superfusion, Aff-Art constricted by 12.0 ± 4.0 and 16.6 ± 3.8% in response to increases in RPP from 100 to 125 and 150 mmHg, respectively. Aff-Art autoregulatory responses were not altered during superfusion with biliverdin (8.9 ± 2.0 and 13.9 ± 3.0%, n = 6, P > 0.05). Superfusion with CO significantly attenuated Aff-Art constrictor responses to increases in RPP from 100 to 125 and 150 mmHg (+0.4 ± 3.8 and +3.3 ± 5.4%, n = 6, P < 0.05 compared with responses during vehicle superfusion) (Fig. 6).

Acute sGC inhibition with ODQ augments Aff-Art autoregulatory responses in control rats and restores autoregulatory responses in hemin-treated rats. In control rats, during vehicle superfusion, Aff-Art constricted by 9.3 ± 2.0 and 16.8 ± 2.1% in response to increases in RPP from 100 to 125 and 150 mmHg, respectively (n = 7, P < 0.05). Acute inhibition of sGC with ODQ significantly augmented Aff-Art autoregulatory responses to increases in RPP from 100 to 125 and 150 mmHg (−13.3 ± 2.1 and −25.1 ± 4.6%, n = 7, P < 0.05 compared with responses during vehicle superfusion) (Fig. 7).

In hemin-treated rats, during vehicle superfusion, Aff-Art diameters did not change in response to increases in RPP from 100 to 125 and 150 mmHg (−2.9 ± 1.5 and −7.0 ± 3.2%, n = 5, P > 0.05). Acute inhibition of sGC with ODQ restored and augmented Aff-Art autoregulatory responses to increases in RPP from 100 to 125 and 150 mmHg (−14.5 ± 5.5 and −25.4 ± 3.0%, n = 5, P < 0.05 compared with vehicle) (Fig. 8).

Renal HO-2 and HO-1 immunohistochemistry and HO-1 immunofluorescence. To evaluate HO-2 and HO-1 expression in Aff-Art, immunohistochemistry was performed using kidneys from control rats. HO-2 was expressed in control kidneys and was mainly expressed in tubular epithelial cells. Weak staining was detected in proximal tubules, glomeruli, interlobular...
arteries, and Aff-Arts (Fig. 9A). HO-1 was not detected in kidneys from control rats (Fig. 9B). In hemin-treated rats, HO-1 was expressed in tubular epithelial cells with prominent staining of proximal tubules (Fig. 9C). HO-1 was not detected in interlobular arteries or Aff-Arts (Fig. 9, C and D). In SnCl2-treated rats, HO-1 was detected in medullary tubular epithelial cells, and few proximal tubular cells showed staining. HO-1 was not detected in interlobular arteries or Aff-Arts (Fig. 9E).

DISCUSSION

Although basal HO activity in normal kidneys exerts a minor role in regulating renal hemodynamics (6, 34, 38), HO-1 plays a major role when induced under certain pathophysiological conditions (2, 8, 31, 34). To examine the effects of augmented HO on Aff-Art autoregulatory responses, two different inducers of HO were used; hemin, which is the substrate for HO (44), and SnCl2, which is a potent inducer of renal HO-1 (23, 30). Hemin induces HO via increasing substrate availability and also by transcriptional activation of the \textit{hmox-1} gene (46). In contrast, SnCl2 induces HO only via transcriptional activation of the \textit{hmox-1} gene, leading to an increase in HO-1 protein expression, and a decrease in heme levels (15). Because hemin treatment increases the activity of the heme-dependent cyclooxygenase (COX) (18, 37) while HO-1 induction with SnCl2, CoCl2, or CoPP decreases the activity of COX (5, 8, 18), hemin and SnCl2 were selected to induce HO-1 and to assess effects associated with HO-1 induction regardless of the changes in other heme-dependent enzymes. In rats treated with hemin or SnCl2, Aff-Art autoregulatory constrictor responses to increases in RPP were attenuated compared with responses in normal kidneys. These results indicate that increasing renal HO activity and/or renal HO-1 induction modulates Aff-Art autoregulatory responses.

Several mechanisms could be responsible for attenuation of Aff-Art autoregulatory responses during HO-1 induction. Induction of HO results in increased production of CO, causing a renal vasodilatory influence (2, 4, 34, 38, 48) and dilation of Aff-Art (6, 45). In addition to induction of HO-1, increases in RPP may stimulate HO-mediated CO production (26), which would lead to Aff-Art dilation and attenuation of autoregulatory constrictor responses. HO-1 induction in tubular epithelial cells may increase CO production in tubular fluid, which may interfere with NaCl delivery or NaCl-sensing mechanisms at macula densa cells, leading to attenuation of tubuloglomerular feedback. To determine the effects of CO on Aff-Art autoregulatory responses, we acutely superfused Aff-Arts from control kidneys with a high dose of CO to mimic prolonged exposure to high CO levels in kidneys from hemin-treated or SnCl2-treated rats. Aff-Art constrictor responses are attenuated during

Fig. 6. Aff-Art diameter responses to increases in RPP from 100 to 125 and 150 mmHg during superfusion with vehicle, biliverdin, or CO in kidneys from control rats (n = 6). Results are expressed as means ± SE for each treatment. *P < 0.05 vs. basal diameter. #P < 0.05 vs. vehicle.

Fig. 7. Aff-Art diameter responses to increases in RPP from 100 to 125 and 150 mmHg during superfusion with vehicle or 10 μmol/l ODQ in kidneys from control rats (n = 7). Results are expressed as means ± SE for each treatment. *P < 0.05 vs. basal diameter. #P < 0.05 vs. vehicle.

Fig. 8. Aff-Art diameter responses to increases in RPP from 100 to 125 and 150 mmHg during superfusion with vehicle or 10 μmol/l ODQ in kidneys from hemin-treated rats (n = 5). Results are expressed as means ± SE for each treatment. *P < 0.05 vs. basal diameter. #P < 0.05 vs. vehicle.
superfusion with CO, indicating that attenuation of Aff-Art autoregulatory mechanisms in hemin- and SnCl2-treated rats is mediated via increased renal CO levels. Because sGC is a major target for CO and because sGC activation attenuates Aff-Art autoregulatory responses (9), we examined the effects of acute sGC inhibition with ODQ on Aff-Art autoregulatory responses in kidneys from hemin-treated rats. Since NO is the major activator of sGC, we also examined effects of acute sGC inhibition in kidney from control rats. sGC inhibition enhanced Aff-Art autoregulatory responses to increases in RPP to 150 mmHg by 18.4 ± 4.7% in hemin-treated rats (n = 5), while responses were only enhanced by 8.4 ± 4.5% in control rats (n = 7). These data indicate that activation of sGC mediates attenuation of Aff-Art autoregulatory responses in hemin-treated rats.

Induction of HO-1 results in increased production of biliverdin, which gets converted to the antioxidant bilirubin (42). Increased bilirubin production may attenuate tubuloglomerular feedback. Induction of HO-1 inhibits NADPH oxidase (16), which is the major source of superoxide, via decreasing cellular heme levels (43) and via increased production of bilirubin (25). Bilirubin can scavenge superoxide (14, 17, 33) and may attenuate superoxide-mediated Aff-Art constriction, leading to attenuation of Aff-Art autoregulatory responses to increases in RPP. To determine the effects of biliverdin on Aff-Art autoregulatory responses, we acutely superfused Aff-Arts from control kidneys with a high dose of biliverdin to mimic increased biliverdin production in kidneys from hemin-treated or SnCl2-treated rats. Superfusion with biliverdin did not alter Aff-Art constrictor responses to increases in RPP, indicating that increased biliverdin production does not modulate Aff-Art autoregulatory mechanisms.

Because inhibition of 20-HETE attenuates Aff-Art autoregulatory constrictor responses (21, 22), HO-1 induction may attenuate Aff-Art autoregulatory responses via inhibition of 20-HETE production (5, 15). Although HO inhibition with CrMP attenuates pressure-natriuresis in normal rats (26), our results demonstrate that acute HO inhibition with CrMP does
not alter Aff-Art autoregulatory responses to increases in RPP in normal kidneys, indicating that basal HO activity in the normal kidney does not modulate Aff-Art autoregulation. The reason could be that the normal kidney has relatively low basal HO activity that is derived from tubular HO-2 expression (15, 20). Although HO inhibitors may have nonspecific effects not related to HO inhibition such as nitric oxide synthase (NOS) or sGC inhibition (3), it is unlikely that we have these nonspecific effects for two reasons. 1) We used one of the most selective inhibitors of HO activity which does not inhibit NOS or sGC (3). 2) The dose used did not alter Aff-Art diameters in normal kidneys while inhibition of NOS or sGC should cause Aff-Art constriction, indicating that we did not have any NOS or sGC CrMP-mediated inhibition. In heme- and SnC12-treated rats, basal Aff-Art diameters at 100 mmHg RPP did not change in response to acute HO inhibition, indicating that HO-1 does not play a direct role in modulating basal Aff-Art tone. However, induction of HO-1 may indirectly modulate the expression or activity of heme enzymes that regulate Aff-Art tone, thus contributing to regulation of Aff-Art vasoactivity. HO-1 induction has been shown to inhibit NOS, cytochrome P-450 4A (CYP450-4A), COX, and NADPH oxidase. The net effect on Aff-Art would be inhibition of vasodilators (NO, prostacyclin) and vasoconstrictors (20-HETE, thromboxanes, superoxide). In this case, it is unlikely that acute inhibition of HO would restore normal activities of these enzymes. In heme- and SnC12-treated rats, acute HO inhibition restored Aff-Art autoregulatory responses to increases in RPP. This indicates that acute HO inhibition interfered with the activation of vasodilatory mechanisms, or with the ability of HO-mediated products to inhibit vasoconstrictor mechanisms that are activated in response to increases in RPP.

The present study demonstrates that pharmacological renal HO-1 induction attenuates Aff-Art constrictor responses to increases in RPP. Under several pathophysiological conditions, HO-1 is induced in the kidney (31), and renal HO-1 induction under these circumstances might play a role in attenuating Aff-Art autoregulatory constrictor responses to prevent or counteract excessive constriction of Aff-Art.

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