Augmentation of Heme Oxygenase Promotes Acute Angiotensin II Induced Hypertension

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Abstract

The enzyme heme oxygenase (HO) degrades heme to form equimolar amounts of iron, biliverdin and carbon monoxide (CO). CO is a key regulatory factor of vascular tone. Thus, the current study was performed to evaluate the hypothesis...
that acute infusion of angiotensin II (AngII) promotes hypertension via an induction in heme oxygenase-1 (HO-1), which increases carbon monoxide (CO). Male Sprague Dawley rats (200 – 300g) were acutely treated with AngII (5ng/kg/min, IV), delta-aminolevulinic acid (DALA) (80 μmol/kg, IV) and zinc deuteroporphyrin 2, 4-bis glycol (ZnDPBG) (20mg/kg, IV) or vehicle. Hemodynamic and renal functions were evaluated. Blood pressure was significantly elevated in rats treated with AngII and AngII + DALA. HO-1 concentrations in hearts and kidneys were also elevated. Acute angiotensin II administration produced a significant increase in endogenous CO when compared to control. Urine flow (UF), glomerular filtration rate (GFR), sodium excretion ($U_{Na}V$), and potassium excretion ($U_{K}V$) were significantly decreased in AngII infused rats when compared to control. However, there were no significant changes in renal blood flow (RBF) among the groups. Animals treated with the HO-1 inhibitor, ZnDPBG (20mg/kg, IV), had reduced blood pressure and increased UF, GFR, $U_{Na}V$, $U_{K}V$ when compared to AngII and AngII + DALA treated rats. Also, HO-1 protein levels in heart and kidney and carboxyhemoglobin levels were reduced in ZnDPBG treated animals. These results demonstrate that AngII infusion promotes hypertension via induction in HO-1, which increases carbon monoxide levels and inhibition of HO-1 with ZnDPBG decreases the blood pressure.

**Keywords:** Carbon monoxide, heme oxygenase-1, renal function

### 1.1. Introduction

Endogenous carbon monoxide (CO) is formed by the enzymatic degradation of heme by the enzyme heme oxygenase (HO) [18]. The two major active isoforms of HO are (i) the inducible heme oxygenase-1 and (ii) the constitutive heme oxygenase-2 [25]. Several researchers have shown that oxidative stress, ischemia, hypoxia and hypertension are known factors that lead to an increase in HO-1 levels [3, 13, 32]. Also, it has been demonstrated that HO is expressed in numerous tissues [25], including vascular endothelial, smooth muscle cells and renal tissue [8, 12]. CO has been demonstrated to relax vascular smooth muscle [2, 11], but can also attenuate the vasodilator effects of the nitric oxide system (NOS) [36, 39] and promote endothelium-dependent vasoconstriction [20, 33]. Furthermore, there is evidence that CO is a key regulatory factor of normal vascular tone.

Angiotensin II (Ang II) promotes hypertension by directly increasing vascular tone or perhaps through the activation of an additional pathway, such as heme oxygenase induction, which increases CO and promotes a decrease in nitric oxide (NO) bioavailability [5, 7]. Impaired endothelial function reduces NO availability and is one of the key factors promoting microvascular disease in resistance vessels, which contributes to hypertension [10]. Previous literature has demonstrated that pathological conditions [9], such as AngII-induced
hypertension and DOCA-salt hypertension [19] increase vascular heme oxygenase-1 expression [9, 15]. An induction of HO-1 has been demonstrated to produce significant increases in CO, which can exacerbate oxidative stress; where normal to slightly increased CO levels produce dilation and attenuation of hypertension [1, 34].

Current literature would support a relationship between oxidative stress and an induction in endogenous CO production; where HO activity is increased via superoxide levels [6, 10]. It has been reported that Ang II induces HO-1 directly, leading to an increase in endogenous CO levels [3, 24]. Thus, the deleterious effect of significant increases in endogenously formed CO levels could be mediated through an induction of the oxidative stress pathway and/or reduction in NO. Also, it has been reported that a reduction in NO bioavailability increases superoxide formation and is believed to be associated with end-organ damage [10, 17]. Thus, the regulation of vascular tone by CO appears to be concentration dependent, where substantial elevations of CO levels inhibit NOS and contribute to endothelial dysfunction in several models of hypertension [29, 35]. Alternatively smaller elevations in the concentration of CO promote a vascular smooth muscle mediated decrease in tone and blood pressure [23, 26]. Thus, the regulation of endogenously formed CO will help to improve endothelial function, promoting a reduction in blood pressure and an improvement in cardiovascular and renal function.

Hypertension induced by chronic infusion of Ang II is an established model of hypertension where subcutaneous infusion of Ang II produced an increase in mean arterial pressure [22]. In addition, an acute increase in blood pressure was observed during low dose infusion of Ang II [40]. Therefore, the goal of the present study was to evaluate the effect of acutely administered Ang II on hemodynamic function. The current study was performed to examine the hypothesis that acute intravenous infusion of Ang II promotes an increase in blood pressure via an induction of HO, which increases CO levels.

1.2. Materials and Methods

1.2.1. Animals:
Male Sprague-Dawley rats (200-300g; n= 86, Harlan, Indianapolis, IN) were used. The following studies were approved by the University of Louisiana at Monroe Institutional Animal Care and Use Committee. Prior to experiments, rats were housed in a controlled environment and had free access to commercial rat chow and tap water.
DALA was purchased from Frontier Scientific (Logan, UT). Inactin (thiobutabarbital sodium), and para-aminohippuric acid (PAH) were obtained from Sigma-Aldrich (St. Louis, MO). Albumin was purchased from EMD Biosciences Inc. (San Diego, CA). Inulin was purchased from Fresenius Kabi UK Ltd. (Runcorn, Cheshire). All other chemicals were purchased from Fisher Scientific (Houston, TX).
1.2.2. **AngII Dose Response.**
Male Sprague-Dawley rats (200-300g) were weighed and anesthetized with a single injection of thiobutabarbitral sodium (Inactin, 120mg/kg ip) and a tracheal tube was inserted to maintain an open airway. Catheters (PE-50 tubing filled with heparinized saline) were implanted into the carotid artery and the jugular vein to allow for continuous monitoring of mean arterial pressure (MAP) and for intravenous administration of AngII, 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 micro infusion pump (HARVARD apparatus, PHD 2000,) set at a 1.2 ml/hour saline infusion rate. In addition, a catheter was introduced into the bladder to permit the collection of urine for determination of urinary flow, electrolyte analysis (Flame Photometry; Instrumentation Laboratories, IL 943) and evaluation of renal function. Following the surgical procedures, animals were acutely treated with various doses of AngII (5ng/kg/min, 10ng/kg/min, 20ng/kg/min; n=7 each) or vehicle (control) intravenously for 90 min and mean arterial pressures (MAP) were measured.

1.2.3. **Experimental Procedures.**
Following the surgical procedures, rats were allowed to stabilize for 30min. After this initial stabilization period, a 60-minute control period was performed and urine samples were collected. Following the control period, a subset of animals were acutely treated with AngII (5ng/kg/min, IV; n=13), ZnDPBG (20mg/kg, IV; n=11), DALA (80 μmol/kg, IV; n=11) or vehicle (1 ml stock, IV), and an additional 60 minute treatment period was performed. Mean arterial pressures (MAP), systolic blood pressures (SBP), diastolic blood pressures (DBP), heart rates (HR), were measured during both the 60 minute control and treatment periods. Blood was collected in EDTA tubes, plasma was collected and centrifuged. Plasma samples were aliquoted and stored at -20°C until analyzed. The heart and kidneys were extracted and stored in -80°C until analyzed.

1.2.4. **Carboxyhemoglobin measurements:**
At the conclusion of the previously described experiments, a 1ml arterial blood sample was drawn from rats acutely treated with AngII, ZnDPBG, DALA or vehicle. Blood samples were analyzed for endogenous CO content via a blood gas analyzer (Bayer Rapidlab 865 Blood Gas Analyzer, Diamond Diagnostic, Holiston, Massachusetts).

1.2.5. **Glomerular Filtration Rate:**
Inulin, para-amohippuric acid and albumin were administered intravenously during the surgery through the jugular vein. PAH and inulin concentrations were measured colorimetrically to determine renal plasma flow and glomerular filtration rate (GFR), respectively. Flame photometry was used to determine sodium and potassium concentration in urine and plasma samples.
1.2.6. **ELISA:**

**Heme oxygenase-1 protein measurement:**
Following the previously delineated experiments, the thoracic and abdominal cavities were opened and the hearts and kidneys were extracted. Opening of the thoracic cavity is an Institutional Animal Care and Use Committee approved method of sacrificing an animal. The extracted hearts and kidneys were snap frozen with liquid nitrogen and stored at -80°C until analyzed for HO-1 content. HO-1 levels were measured by commercially available ELISA kits purchased from Stressgen. Briefly, hearts and kidneys from control, AngII, ZnDPBG and DALA treated rats were extracted, flash frozen in liquid nitrogen and suspended in 1X extraction reagent, and protease inhibitor. Once the kidney and heart tissues were homogenized, the ELISA sandwich assay was preformed and the level of HO-1 present was determined by comparison to a standard curve.

### 1.3. Statistics

Data were expressed as mean ± SE. Data were analyzed by repeated measure analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test when appropriate (INSTAT 3). P < 0.05 was accepted as statistically significant.

### 1.4. Results

**1.4.1. AngII dose response.**
A substantial and sustained increase in MAP was observed in rats acutely treated with AngII (5ng/kg/min) (MAP = 151 ± 1.17 mmHg; n=10) when compared to control (MAP = 123 ± 0.2 mmHg; n=10). Additionally, the MAP in the 5ng/kg/min treatment group was maintained over a period of 90min (Figure 1). Acute treatments with AngII (10 and 20ng/kg/min; n=10each) produced an increase in MAP; however this increase was attenuated shortly after acute AngII infusion.

**1.4.2. Hemodynamic response to AngII infusion.**
Acute intravenous administration of AngII (5ng/kg/min) produced a substantial increase in MAP (151 ± 0.1 mmHg; n=13) when compared to control (MAP = 123 ± 0.2 mmHg; n=13). There was a significant increase in SBP (AngII=174 ± 0.3 mmHg) and diastolic blood pressure (AngII= 128 ± 0.3 mmHg) during acute AngII administration as compared to control (SBP= 142 ± 0.2 mmHg, DBP=104 ± 0.4 mmHg) (Figure 2). There was a significant increase in HR (353 ± 0.2 BPM) in AngII treated rats as compared to control (HR= 316 ± 1.4 BPM) (Figure 3).

**1.4.3. Hemodynamic response to AngII + DALA (80 μmol/kg) and AngII + ZnDPBG (20mg/kg) infusion.**
Acute administration of AngII + DALA (80 μmol/kg) produced a significant increase in MAP (146 ± 1.5 mmHg; n=11), SBP (162 ± 0.9 mmHg) and DBP (125 ± 0.51 mmHg) as compared to control. However, no significant differences between AngII + DALA and AngII alone treatments were observed. ZnDPBG (20mg/kg) treatment attenuated the AngII and AngII + DALA mediated increase in MAP, SBP, and DBP. Co-administration of AngII + ZnDPBG significantly lowered the MAP (133 ± 0.1 mmHg; n = 11), SBP (147 ± 0.19 mmHg) and DBP (110 ± 0.11 mmHg) as compared to AngII treated animals (Figure 2). ZnDPBG significantly reduced the HR as compared to rats treated with AngII (Figure 3). There were no significant differences between ZnDPBG and control groups.

1.4.4. Renal Function.

Urinary flow.

AngII treatment produced a significant reduction in urine flow (20 ± 1.04µl/min) as compared to control (30.2 ± 2.1µl/min). There was a significant reduction in urine flow (16.01 ± 0.3 µl/min) during treatment with AngII + DALA. ZnDPBG treatment produced an increase in urine flow (34.2 ± 3.2µl/min). There were no significant differences in urine flow observed between the rats treated with ZnDPBG and control (Figure 4).

Urinary sodium (UNaV) and potassium (UKV) levels.

AngII significantly decreased sodium (UNaV = 0.23 ± 0.02 µmol/min) and potassium (UKV= 0.14 ± 0.01 µmol/min; n=13) excretion as compared to control (UNaV= 0.5 ± 0.03 µmol/min, UKV= 0.5 ± 0.01 µmol/min; n=13). AngII + DALA treatment also caused a significant reduction in urinary sodium and potassium (UNaV= 0.1 ± 0.02 µmol/min, UKV= 0.14 ± 0.02 µmol/min; n=11) excretion. ZnDPBG treatment attenuated the AngII reduction in sodium and potassium excretion and produced an increase in sodium and potassium excretion (UNaV= 0.5 ± 0.01 µmol/min, UKV= 0.5 ± 0.02 µmol/min; n=11) as compared to AngII and AngII + DALA treated rats (Figure 5).

1.4.5. Glomerular filtration rate (GFR)

AngII (2.24 ± 0.12 ml/min) and AngII + DALA (1.85 ± 0.06 ml/min) treatments produced a significant decrease in GFR as compared to control (5.30 ± 0.42 ml/min). ZnDPBG administration significantly increased the GFR (6.29 ± 0.04ml/min) (Table 1). There were no observed differences in renal blood flow (RBF) between the treatment groups (Table 1).

1.4.6. HO-1 protein level.

Sandwich ELISA was performed on the harvested hearts and kidneys. There was a significant induction in HO-1 protein during AngII (Heart= 0.09 ± 0.005 ng/ml, kidney= 0.03 ± 0.001 ng/ml; n=13 each) and AngII + DALA (Heart= 0.10 ± 0.005 ng/ml, kidney= 0.03 ± 0.005 ng/ml; n=11 each) administration as compared to control (Heart= 0.06 ± 0.002 ng/ml, kidney= 0.02 ± 0.003 ng/ml; n=13). ZnDPBG produced a significant decrease in the HO-1 protein (Heart= 0.06 ± 0.002 ng/ml, kidney= 0.01 ± 0.001 ng/ml; n=11) as compared to rats treated with AngII and
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AngII + DALA. However, there were no observed differences in HO-1 between control and ZnDPBG treated animals (Figure 6).

1.4.7. Carboxyhemoglobin levels.
Acute AngII infusion (25 ± 2.5%; n = 13) produced a significant increase in carboxyhemoglobin (CoHb) levels as compared to control (4 ± 0.5%; n = 13). Co-administration of AngII and DALA (30 ± 2.4%; n = 11) produced a significant increase in CoHb levels as compared to control (4 ± 0.5%; n = 13). However, no significant differences were observed between the AngII treated and AngII + DALA treated groups. ZnDPBG treatment (3 ± 0.2%; n = 11) attenuated the AngII mediated increase in CoHb. No significant differences were observed between the ZnDPBG treated and control groups (Figure 7).

1.5. Discussion

The current study demonstrates that acute intravenous administration of AngII promotes hypertension mediated through an induction of HO-1, which produces a significant increase in endogenous CO. Current literature would support an increase in endogenous CO production during HO-1 induction. These high levels of CO promote vasoconstriction potentially leading to hypertension [21, 28]. Therefore, blockade of HO-1 in the current study inhibited the Ang II mediated hypertension.

Our results demonstrated that acute administration of low dose AngII produces a sustained increase in MAP. Therefore, the current study demonstrates that low dose AngII infusion can produce hypertension. Aizawa T e.t al. have observed a similar hypertensive effect during chronic infusion of AngII [3, 15]. Similarly to previous studies the current study demonstrates that acute low dose infusion of AngII promotes hypertension. In addition, the current study demonstrates that the AngII mediated hypertensive effect can be maintained over the entire 90 min treatment period, when the AngII dose is slightly increased. Our results indicate that acute AngII increases MAP by the induction of HO-1, which increased endogenous CO levels. It has been documented that AngII induces HO-1 in isolated, perfused rat kidneys, in the heart and increases the release of CO [15, 24]. Several studies reported that HO-1 represents a generalized oxidative response system [27, 37]. Recent reports have demonstrated an attenuation of AngII induced hypertension via an induction in renal HO-1 [34]. While the current study appears to be in direct contradiction to this recent report, the differences in the studies appear to be in the level of CO production. The current study observed a massive increase in HO-1 and endogenous CO production, where the other study appeared to produce much smaller levels of HO-1 induction. In addition, it has been documented in in vitro and in vivo studies that there is induction of the HO system in a wide array of oxidative stresses [4, 27]. Taken together HO is enhanced under oxidative stress which can lead to endothelial dysfunction and contribute to hypertension; if there is a significant increase in CO production.
The current study demonstrated a significant increase in endogenous CO levels during an induction in HO-1. In addition, the current study observed a decrease in urine flow during AngII and AngII + DALA treatments. The AngII mediated decrease in urine flow was attenuated by ZnDPBG administration. Inhibition of HO by administration of the HO inhibitor ZnDPBG was demonstrated to lower the blood pressure in obese Zucker rats, which is an animal model of metabolic syndrome [21]. In the present study, ZnDPBG acutely decreased kidney and heart HO-1 levels and endogenous CO levels. Also, we have recently demonstrated that inhibition of HO-1 decreases endogenous CO levels [16]. Thus, one could reasonably conclude that the observed decrease in HO-1 returned the circulating endogenous CO levels to control, which attenuated the CO mediated vasoconstriction. The disparities in the current literature as it relates to HO affects on hypertension appear to be due to differences in the levels of CO and HO induction.

In the current study a substrate for heme formation DALA, which drives CO formation and induces HO-1 [31] was infused along with AngII and a significant increase in MAP was observed. However, the increase in MAP observed during the co-administration of Ang II and DALA was not significantly different than Ang II infusion alone. In addition, Ang II and Ang II + DALA decreased the urinary sodium and urinary potassium excretion. This decrease is potentially due to sodium and potassium retention. Thus, the hypertensive effects of AngII were two fold one via direct vasoconstriction and two via a decrease in sodium/urine excretion. AngII appeared to promote maximum vasoconstriction, decreased sodium and potassium excretion and HO-1 induction in that no further increase in MAP were observed during DALA administration.

Several studies have demonstrated that elevations in AngII produce alterations in renal function which leads to sodium retention and therefore plays a key role in development and maintenance of hypertension [14, 38]. The current study also demonstrates that there is a reduction in GFR. However, there was no change in RBF. The absence in a change in RBF demonstrates the kidneys ability to autoregulate renal blood flow, during substantial alterations in renal perfusion pressures. These results are in agreement with a study demonstrating that the HO inhibitor zinc protoporphyrin (ZnPP) had no effect on baseline RBF [30]. Also, chronic administration of Ang II was observed to lower GFR and this reduction in filtered load could contribute to fractional sodium reabsorption and a reduced sodium excretion [38]. Thus, AngII is known to cause hypertension by direct vasoconstriction and by a reduction in sodium and water excretion. In addition, the current study demonstrates a potential role for HO in AngII mediated hypertensive effects.

1.6. Conclusion

In summary, the current study demonstrates that acute administration of AngII promoted an increase in blood pressure via an induction of HO-1, and a
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significant increase in endogenous CO. Administration of ZnDPBG decreases the HO-1 levels in the kidney and heart and was shown to lower the blood pressure. These findings suggest that acute infusions of AngII increase HO-1 levels promoting an elevation in endogenous CO concentrations leading to CO mediated vasoconstriction and ultimately hypertension. Thus, HO-1 could prove to be a novel target for the treatment of hypertension.

REFERENCES

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Figure Legends:

Figure 1. Dose response curves for the acute administration of AngII. Acute infusion of AngII (5ng/kg/min) promotes a sustained increase in MAP (150 ± 1.17 mmHg) when compared to other treated groups (AngII (10ng/kg/min) = 143 ± 1.35 mmHg, AngII (20ng/kg/min) = 122 ± 1.3 mmHg) (*P< 0.05; n=10 each).

Figure 2. Hemodynamic response for the acute administration of AngII (5ng/kg/min).
Fig 2 (A). Infusion of AngII (5ng/kg/min) and AngII + DALA (80 μmol/kg) increases the MAP (AngII=151 ± 0.1 mmHg; n=13; AngII + DALA= 146 ± 1.5 mmHg; n=11) when compared to control (MAP= 123 ± 0.2 mmHg; n=13). Co-infusion of AngII + ZnDPBG (20mg/kg) significantly decreases the MAP (133 ± 0.1 mmHg; n = 11) (*P< 0.05).
Fig 2 (B). Acute infusion of AngII and AngII + DALA produced a significant increase in systolic blood pressure (AngII= 174 ± 0.3 mmHg; AngII + DALA= 162 ± 0.9 mmHg) when compared to control (SBP = 142 ± 0.2 mmHg). Acute administration of AngII + ZnDPBG (20mg/kg) attenuated the AngII and Ang II + DALA mediated increase in SBP (147 ± 0.19 mmHg) (*P< 0.05).
Fig 2 (C). Diastolic blood pressure (DBP) was increased by administration of AngII (DBP = 128 ± 0.3 mmHg) and Ang II + DALA (DBP= 125 ± 0.51 mmHg) when compared to control (DBP = 104 ± 0.4 mmHg) mmHg. AngII + ZnDPBG attenuated the AngII mediated increase in DBP (110 ± 0.11 mmHg) (*P< 0.05).

Figure 3. Acute administration of AngII produced a significant increase in heart rate (353 ± 0.2 BPM) when compared to control (HR= 316 ± 1.4 BPM). Treatment with ZnDPBG produced a significant decrease in heart rate (318 ± 0.6 BPM) when compared to AngII treated rats. However, there were no significant differences between the heart rates of control and AngII + ZnDPBG treated rats.

Figure 4. Urine flow. Fig 4(A). AngII infusion decreased urinary flow (20 ± 1.04 µl/min) when compared to control (30.2 ± 2.1 µl/min) (*P< 0.05).
Fig 4 (B). Infusion of AngII + DALA also significantly decreased urinary flow (16.01 ± 0.3 µl/min) when compared to control (30.2 ± 2.1 µl/min) (*P< 0.05).
Fig 4 (C). Co-administration of AngII + ZnDPBG (UF= 34.2 ± 3.2µl/min) produced no significant differences in urinary flow as compared to control.
Fig 4 (D). Acute administration of AngII + ZnDPBG significantly increased urinary flow when compared to AngII and AngII + DALA treated rats (*P< 0.05).
Figure 5. Renal function. Fig 5 (A) Urinary sodium excretion of AngII (0.23 ± 0.02 µmol/min; n=13) and AngII + DALA (0.1 ± 0.02 µmol/min; n=11) treated rats was significantly reduced when compared to control (0.5 ± 0.03 µmol/min; n=13). Infusion of AngII + ZnDPBG increased urinary sodium excretion (0.5 ± 0.01 µmol/min; n=11) (*P< 0.05).

Fig 5 (B). Urinary potassium excretion of AngII (0.14 ± 0.01 µmol/min) and AngII + DALA (0.14 ± 0.02 µmol/min) treated rats was significantly reduced when compared to control (0.5 ± 0.01 µmol/min). Infusion of AngII + ZnDPBG increased urinary potassium excretion (0.5 ± 0.02 µmol/min) (*P< 0.05).

Figure 6. HO-1 protein concentration was measured. There was a significant reduction in HO-1 levels in the hearts and the kidneys of animals treated with AngII + ZnDPBG (Heart= 0.06 ± 0.002 ng/ml, kidney= 0.01 ± 0.001 ng/ml) when compared to AngII and AngII + DALA treated rats (*P< 0.05). There were no significant differences between control and AngII + ZnDPBG treated rats.

Figure 7. Carboxyhemoglobin (CoHb) concentration was measured. There was a significant increase in the levels of carboxyhemoglobin (CoHb) in animals treated with AngII (25 ± 2.5 %) and AngII + DALA (30 ± 2.4%) when compared to control (4 ± 0.5%). This increase in endogenous CO was attenuated during ZnDPBG treatment (3 ± 0.2%) (*P< 0.05).

Table 1. GFR was increased in AngII and AngII + DALA treated rats. This increase in GFR was attenuated by the infusion of ZnDPBG (Values are mean ± SE, P < 0.05). However, there were no significant differences in RBF when compared to control. Fractional excretion of sodium was significantly decreased in AngII and AngII + DALA treated rats (Values are mean ± SE, P < 0.05). AngII + ZnDPBG treatment did not alter the fractional sodium excretion.
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Figure 1

Acute AngII Dose Response

- Control
- 5ng/kg/min
- 10ng/kg/min
- 20ng/kg/min

MAP (mmHg)

Time (min)

* * * * * * * *
Figure 2
Augmentation of heme oxygenase

**Figure 3**

**Heart Rate (BPM)**

- Control
- AngII *
- AngII + DALA
- AngII + ZnDPBG

![Heart Rate (BPM) graph]

**Heart Rate (BPM)**

- Control
- Ang II
- Ang II + DALA
- Ang II + ZnDPBG

![Heart Rate (BPM) bar graph]
**A**

Control vs. Ang II

- Control (n=13)
- Ang II (n=13)

Urine Flow (μl/min)

**B**

Control vs. Ang II + DALA

- Control (n=13)
- Ang II + DALA (n=11)

Urine Flow (μl/min)

**C**

Control vs. Ang II + ZnDPBG

- Control (n=13)
- Ang II + ZnDPBG (n=13)

Urine Flow (μl/min)
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Figure 4
Figure 5
Augmentation of heme oxygenase

Figure 6

Rat HO-1 PROTEIN

- Control
- AngII
- Ang II + DALA
- AngII + ZnDPBG

Concentration ng/mL

Heart
Kidney

* *
Figure 7

**Carboxyhemoglobin**

- **Control**
- **Ang II**
- **Ang II + DALA**
- **AngII + ZnDPBG**

![Bar chart showing carboxyhemoglobin levels for different groups.](chart.png)
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### Table 1. Effect of Ang II (5ng/kg/min), Ang II + DALA (80 µmol/kg), Ang II + ZnDPBG (20mg/kg) on glomerular filtration rate (GFR), renal blood flow (RBF), Fractional excretion (FENa)

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