Renal endothelin in chronic angiotensin II hypertension

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Sasser, Jennifer M., Jennifer S. Pollock, and David M. Pollock. Renal endothelin in chronic angiotensin II hypertension. Am J Physiol Regulatory Integrative Comp Physiol 283: R243–R248, 2002; 10.1152/ajpregu.00086.2002.—To determine the influence of chronic ANG II infusion on urinary, plasma, and renal tissue levels of immunoreactive endothelin (ET), ANG II (65 ng/min) or saline vehicle was delivered via osmotic minipump in male Sprague-Dawley rats given either a high-salt diet (10% NaCl) or normal-salt diet (0.8% NaCl). High-salt diet alone caused a slight but not statistically significant increase (7 ± 1%) in mean arterial pressure (MAP). MAP was significantly increased in ANG II-infused rats (41 ± 10%), and the increase in MAP was significantly greater in ANG II rats given a high-salt diet (59 ± 1%) compared with the increase observed in rats given a high-salt diet alone or ANG II infusion and normal-salt diet. After a 2-wk treatment, urinary excretion of immunoreactive ET was significantly increased by ~50% in ANG II-infused animals and by over 250% in rats on high-salt diet, with or without ANG II infusion. ANG II infusion combined with high-salt diet significantly increased immunoreactive ET content in the cortex and outer medulla, but this effect was not observed in other groups. In contrast, high-salt diet, with or without ANG II infusion, significantly decreased immunoreactive ET content within the inner medulla. These data indicate that chronic elevations in ANG II levels and sodium intake differentially affect ET levels within the kidney and provide further support for the hypothesis that the hypertensive effects of ANG II may be due to interaction with the renal ET system.

dietary sodium chloride; blood pressure; kidney

THE RENIN-ANGIOTENSIN SYSTEM plays an important role in the regulation of arterial pressure and renal function. It has traditionally been thought that ANG II exerts its vasoconstrictor and sodium-retaining actions via action on the angiotensin type 1 receptor (AT1) and, therefore, directly participates in the pathogenesis of cardiovascular and renal diseases (10). However, recent studies indicate that ANG II may exert some of its effects via interaction with the endothelin (ET) system. One possible mechanism for this interaction is that ANG II may regulate ET-1 synthesis in the kidney. Several lines of evidence support this hypothesis. First, it has been reported that ANG II stimulates release of ET-1 by cultured vascular smooth muscle cells, endothelial cells, and mesangial cells (5, 12, 19). ANG II has also been shown to stimulate the expression of preproendothelin-1 mRNA in rat vascular smooth muscle cells and in rat and bovine endothelial cells (5, 9, 19). Second, in vivo studies showed that rats with chronic ANG II hypertension have elevated ET-1 levels in renal tissue but not in myocardial tissue and enhanced preproendothelin mRNA expression in the renal cortex and medulla (2, 4). Finally, the hypertension associated with chronic ANG II infusion can be attenuated by a mixed ET type A (ETA) and ET type B (ETB) receptor antagonist and by selective ET A receptor antagonists (3, 4, 8, 18). ET A receptor antagonists have also been shown to prevent some of the changes in endothelial function observed in chronic ANG II hypertension (6).

Within the kidney, ET-1 has opposing hemodynamic effects to produce vasoconstriction within the renal cortex while vasodilating within the renal medulla (7). There is also good evidence that ET-1 directly inhibits sodium reabsorption in the distal nephron (11, 15). Our laboratory has recently provided evidence that ET-1 plays an important role in the response to high salt (HS) and that ET-1 excretion is elevated in rats on a HS diet (17). Given that ANG II-induced hypertension is salt dependent, we conducted experiments designed to determine whether the changes in arterial pressure and renal function observed during chronic ANG II hypertension are associated with increases in urinary, plasma, or renal tissue levels of immunoreactive ET.

METHODS

Animal experiments. Experiments were performed by using male Sprague-Dawley rats (200–250 g; Harlan Laboratories, Indianapolis, IN) in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved and monitored by the Medical College of Georgia Committee for Animal Use in Research and Education. Animals were housed under conditions of constant temperature and humidity and exposed to a 12:12-h light-dark cycle. All rats were given free access to regular rat chow (0.8% NaCl) during a 1-wk baseline period.

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Telemetry transmitters (Data Sciences, St. Paul, MN) were implanted according to manufacturer’s specifications into male Sprague-Dawley rats while under pentobarbital sodium anesthesia (65 mg/kg ip; Abbott Laboratories, North Chicago, IL). In brief, a midline incision was used to expose the abdominal aorta that was briefly occluded to allow insertion of the transmitter catheter. The catheter was secured in place with tissue glue. The transmitter body was sutured to the abdominal wall along the incision line as the incision was closed. The skin was closed with staples that were removed 7–10 days after the incision healed. Rats were allowed to recover from surgery and were returned to individual housing for at least 1 wk before initiation of data acquisition. The individual rat cages were placed on top of the telemetry receivers, and arterial pressure waveforms were continuously recorded throughout the study. After baseline measurements, rats were divided into three groups: animals on HS diet (10% NaCl), ANG II-infused animals on normal salt diet (ANG/NS), and ANG II-infused animals on a HS diet (ANG/HS). While the rats were under ether anesthesia, an osmotic minipump was implanted for chronic subcutaneous infusion of saline vehicle or ANG II at a rate of 65 ng/min for 2 wk (Alzet model 2002, Alza Scientific, Palo Alto, CA).

In separate experiments, rats were divided into four groups: animals on NS diet, animals on HS diet, ANG/NS, and ANG/HS. ANG II (65 ng/min) or saline vehicle was delivered for 2 wk via subcutaneous osmotic minipump. On the last day of each week, rats were placed in metabolic cages to facilitate 24-h urine collection and monitoring of food and water intake. After the 2-wk treatment period, animals were anesthetized with inactin (5-sec-butyl-5-ethyl-2-thiobarbituric acid, 100 mg/kg ip), and a terminal blood sample was taken from the abdominal aorta.

Measurement of tissue ET-1. At the time of blood withdrawal, kidneys were removed from rats; sliced into cortical, outer medullary, and inner medullary sections; quick frozen in liquid nitrogen; and stored at −80° C. ET-1 was extracted from renal tissue samples by using a slightly modified protocol from that described by Yorikane et al. (20). Cortical and outer medullary sections were weighed and pulverized while still frozen and then homogenized in 10 vol of 1 M acetic acid containing 10 μg/ml of pepstatin A. Inner medullary samples were weighed and homogenized in 100 vol of 1 M acetic acid containing 10 μg/ml of pepstatin A. Samples were then heated to 100°C for 10 min, chilled, and centrifuged at 23,000 g at 4°C for 30 min. The soluble extract was removed, aliquoted, and frozen at −80°C. The pellet fraction was resuspended in 3 ml of 1 M acetic acid containing 10 μg/ml of pepstatin A. To determine the total protein content of the tissue samples, protein concentrations in the soluble and pellet fractions were determined by standard Bradford assay (BioRad Laboratories, Hercules, CA) with bovine serum albumin as standard. Immunoactive ET concentrations in the soluble fraction were determined by chemiluminescent immunnoassay (R & D Systems, Minneapolis, MN). Values were reported as picograms of ET per milligram of total protein.

Assays and chemicals. Urinary concentrations of electrolytes were determined by ion-selective electrodes (Synchron EL-ISE, Beckman Instruments, Brea, CA). Urinary immunoactive ET concentrations were measured by radioimmunoassay (Amersham Pharmacia Biotech, Arlington Heights, IL), and plasma and tissue immunoactive ET concentrations were measured by chemiluminescent immunnoassay (R & D Systems). The antibody used for measuring urine concentrations has 100% cross-reactivity with ET-1 and ET-2 but <0.001% cross-reactivity with ET-3. The assay used for plasma and tissue ET has 100% cross-reactivity with ET-1, 45% with ET-2, and 14% with ET-3. All normal and special NaCl content rat chow was obtained from Harlan Teklad (Madison, WI). ANG II and inactin were obtained from Sigma Chemical (St. Louis, MO).

Statistical analysis. ANOVA for repeated measures combined with post hoc contrasts was used for statistical evaluation of mean values each week (SuperANOVA and StatView, Abacus Concepts, Berkeley, CA). Values are reported as means ± SE with P < 0.05 considered significant; n = 4 in all telemetry groups and n = 6 in all other groups.

RESULTS

Figure 1 illustrates mean arterial pressure (MAP) in HS, ANG/NS, and ANG/HS rats over the baseline period and the 2-wk treatment period. MAP was significantly increased in ANG II-infused rats. The increase in MAP was significantly greater in ANG II rats given a HS diet compared with the increase in rats given a HS diet alone or ANG II infusion and NS diet. HS diet
alone caused a slight but not statistically significant increase in MAP.

Food intake during the 2-wk treatment period was similar among the NS, HS, and ANG/NS groups but was significantly decreased during the second week in the ANG/HS group (Fig. 2). Water consumption, urine volume, and sodium excretion were significantly elevated in rats given a HS diet with and without ANG II infusion compared with baseline measurements (week 0) while given normal chow (Fig. 2). In rats given a NS diet, ANG II treatment had no significant effect on food intake or sodium excretion but did cause an increase in water intake and urine volume in week 2 compared with week 0. When ANG II treatment was combined with a HS diet, increases in water intake and urine volume were significantly greater than those observed in rats given a HS diet alone. In contrast, sodium excretion in the ANG/HS group did not increase to the same level as that in the HS group, presumably due to lower sodium intake in this group. During the 2-wk treatment period, rats in the NS, HS, and ANG/NS groups showed significant weight gain each week. Rats in the ANG/HS group had no change in body weight during the study (Fig. 2). Figure 3 shows that urinary protein excretion in ANG/HS rats was increased from 15/1006 to 47/1006 mg/day (P < 0.0001).

Immunoreactive ET excretion after 2 wk of ANG II infusion was significantly greater than baseline levels (1,092/1006 ± 80 and 742/1006 ± 31 fmol/day, respectively, P = 0.02; Fig. 4). HS diet, alone or in combination with ANG II treatment, caused a dramatic increase in immunoreactive ET excretion (2,707/1006 ± 167 and 2,513/1006 ± 233 fmol/day, respectively) compared with immunoreactive ET excretion in NS control animals (942/1006 ± 43 fmol/day, P < 0.0001). Plasma immunoreactive ET levels were similar in all groups at the end of the 2-wk treatment period. As shown in Fig. 5, 2-wk treatment with ANG II alone did not significantly affect renal tissue levels of immunoreactive ET. The HS diet alone and in combination with ANG II infusion caused a significant decrease in immunoreactive ET content in the renal inner medulla compared with control levels. The combination of a HS diet and ANG II infusion resulted in increased renal cortical and outer medullary immunoreactive ET content compared with control values.

DISCUSSION

Previous studies indicate that ANG II may exert some of its effects via interaction with the ET system. The current study extends these findings to explore the influence of ANG II and a HS diet on immunoreactive

![Fig. 2. Food and water intake, urine volume, body weight, and sodium excretion in conscious rats given a normal (NS)- or high-salt (HS) diet and/or ANG II (ANG) infusion. After a 1-wk baseline period, rats were given either ANG II or saline vehicle infusion and a NS or HS diet. Values are means ± SE for a 24-h urine collection period. *P < 0.0001 vs. baseline measurements, #P < 0.05 vs. baseline measurements, and +P < 0.05 vs. HS.](http://ajpregu.physiology.org/lookup/suppl/doi:10.1152/ajpregu.00327.2001/-/DCSupplemental/AJP-Regulatory%20Integrative%20Comp%20Physiol%20%20Vol%20283%20%20July%202002%20%20www.ajpregu.org)

![Fig. 3. Effects of ANG II infusion and/or HS diet on protein excretion. Values are means ± SE. *P < 0.0005 vs. baseline measurements.](http://ajpregu.physiology.org/lookup/suppl/doi:10.1152/ajpregu.00327.2001/-/DCSupplemental/AJP-Regulatory%20Integrative%20Comp%20Physiol%20%20Vol%20283%20%20July%202002%20%20www.ajpregu.org)
ET levels in the kidney. Chronic ANG II infusion combined with a HS diet increased the renal cortical and outer medullary immunoreactive ET content. However, a HS diet, with or without ANG II infusion, reduced inner medullary immunoreactive ET content. A HS diet also caused a large increase in urinary excretion of immunoreactive ET. These data indicate that chronic elevations in ANG II levels and sodium intake produce a differential effect on immunoreactive ET levels within the kidney.

One possible mechanism for the interaction between ANG II and the ET system is that ANG II regulates renal synthesis of ET-1. Increased ET-1 release and preproendothelin expression in response to ANG II have been shown in vitro (5, 9, 12, 19). In vivo studies also showed that rats with chronic ANG II hypertension have enhanced preproendothelin mRNA expression and elevated ET-1 levels in renal tissue (2, 4). Our data are consistent with the hypothesis that ANG II-induced hypertension causes an increase in ET-1 synthesis at least within the renal cortex and outer medulla. Tissue immunoreactive ET levels in these regions of the kidney were increased after a 2-wk treatment with ANG II and HS. The observation that immunoreactive ET levels within the inner medulla were decreased in rats given a HS diet was somewhat surprising. It is not clear whether this reflects a decrease in ET-1 synthesis or enhanced release from tissue stores of ET-1 in response to salt loading as a means of reducing sodium reabsorption. Because plasma immunoreactive ET levels were unchanged and urinary excretion of immunoreactive ET was much greater in HS-treated rats, we propose that the observed increase in urinary immunoreactive ET excretion reflects increased renal synthesis and release of ET-1 in response to a HS diet.

It should be noted that, although statistically significant, the magnitude of the changes in intrarenal ET is not large, and so we cannot conclude with complete certainty that these changes account for the salt sensitivity associated with chronic ANG II infusion. However, there is no clear information available concerning the biological activity of tissue concentrations of ET. ET is a very potent peptide, and one could easily speculate that small changes would produce significant functional responses. Previous studies showed that elevations in blood pressure associated with ANG II infusion have been reduced with ET-receptor antagonists (3, 8, 18). ET is also known to play a role in the response of the kidney to HS (7), indicating an important role of ET in this response. Therefore, the current observations of an increase in ET-1 content in the renal cortex, outer medulla, and inner medulla could be important in the regulation of ET levels in the kidney.

Fig. 4. Effects of ANG II infusion and/or HS diet on urinary endothelin (ET)-1 excretion (A) and plasma ET-1 concentration (B). Values are means ± SE. *P < 0.02 vs. baseline measurements.

Fig. 5. Effects of ANG II infusion and/or HS diet on renal cortical (A), outer medullary (B), and inner medullary (C) tissue ET-1 content. Values are means ± SE. *P < 0.03 vs. NS group.
cortex and outer medulla of rats on HS given ANG II provide further support for the involvement of ET in this setting. The finding that urinary immunoreactive ET excretion was increased by a HS diet is consistent with previous studies from our laboratory (17). In the present study, immunoreactive ET excretion was increased in both normotensive rats given a HS diet alone and in hypertensive rats given a HS diet and ANG II infusion, indicating that urinary excretion of immunoreactive ET is elevated in response to increased salt intake, regardless of the MAP level. These data are consistent with the hypothesis that the ET system plays an important role within the medulla in response to sodium loading. Indeed, there is increasing evidence that ET-1 acts on the inner medullary collecting duct to inhibit the reabsorption of sodium via ET\textsubscript{B} receptors (11, 16).

The elevations in immunoreactive ET content in the cortex and outer medulla appear to be unique to hypertension associated with elevated salt intake, as they were not seen in animals that were hypertensive due to ANG II infusion or in normotensive animals given a HS diet alone. These increases in immunoreactive ET levels may lead to hemodynamic changes that contribute to the observed proteinuria. The actions of ET\textsubscript{1} in the cortex may contribute to the decline in renal function and exaggerated hypertension observed during ANG II and HS treatment. The increased cortical immunoreactive ET levels seen during combined HS and ANG II treatment may cause vasoconstriction of the cortical vasculature via ET\textsubscript{A} receptors and reduction in cortical blood flow (1, 14). ET\textsubscript{A}-receptor blockade reduces the rise in MAP and decline in glomerular filtration rate associated with ANG II-induced hypertension (2–4, 18), indicating an important role for ET\textsubscript{1} in mediating the renal hemodynamic response via ET\textsubscript{A} receptors during ANG II-induced hypertension.

In the outer medulla, it is also possible that the increased immunoreactive ET content during ANG II and HS treatment may serve to enhance medullary blood flow and sodium excretion. Studies showed that infusion of exogenous ET-1 increases medullary blood flow by causing dilation of the medullary vasculature via ET\textsubscript{B} receptors (7, 16). ET-1 also has inhibitory effects on tubular reabsorption in the outer medulla (14–16).

The elevation in MAP in ANG II-treated rats and the further elevation in rats given a HS diet with ANG II confirm that chronic infusion of ANG II is a model of salt-sensitive hypertension. Urinary protein excretion is significantly increased in rats given a combination of a HS diet and ANG II infusion, indicative of renal injury in this model. These results are consistent with previously published reports of the effects of ANG II and HS treatment (13).

In conclusion, our results are consistent with the hypothesis that regulation of the renal ET system plays an important role in mediating the effects of ANG II. The combination of a HS diet and ANG II infusion resulted in an increase in MAP, a decline in renal function, and increases in renal cortical and outer medullary immunoreactive ET content. HS diet caused a decrease in renal inner medullary immunoreactive ET content and an increase in urinary immunoreactive ET excretion. These data indicate that chronic elevations in ANG II levels and sodium intake produce a differential effect on immunoreactive ET levels within the kidney.

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