Inhibition of Renal Outer Medullary 20-HETE Production Produces Hypertension in Lewis Rats

David E. Stec, David L. Mattson, Richard J. Roman

**Abstract** Recent studies have indicated that a deficiency in the production of 20-hydroxyeicosatetraenoic acid (20-HETE) in the outer medulla of the kidney may contribute to the abnormalities in the renal handling of sodium and the development of hypertension in Dahl salt-sensitive rats. To determine whether a reduction in 20-HETE production in the outer medulla is sufficient to induce hypertension, an inhibitor of the renal metabolism of arachidonic acid by P450 enzymes, 17-octadecenoyl acid (17-ODYA), was chronically infused directly into the outer medulla of the left kidney of nephrectomized Lewis rats fed a high salt diet. Renal medullary interstitial infusion of 17-ODYA (400 pmol/min) reduced the formation of 20-HETE in the outer medulla of the infused kidney by 70% compared with values seen in the right kidney collected when the rat was nephrectomized, but it had no effect on the production of 20-HETE in the renal cortex. After 5 days, mean arterial pressure rose from 115±2 to 142±2 mm Hg (n=6) in the rats infused with 17-ODYA, while mean arterial pressure was not significantly altered in the rats infused with vehicle alone (116±1 versus 117±2 mm Hg, n=6). These results suggest that inhibition of the renal metabolism of arachidonic acid by P450 enzymes in the outer medulla of the kidney is sufficient to induce the development of hypertension in Lewis rats fed a high salt diet and support the view that P450 metabolites of arachidonic acid play an important role in the regulation of renal function and the long-term control of arterial pressure. (Hypertension. 1997;29[part 2]:315-319.)

**Key Words** • cytochrome P450 • kidney • eicosanoids • P450 inhibitors

Renal transplantation studies have indicated that some form of renal dysfunction underlies the development of hypertension in Dahl SS rats; however, the factors altering renal function and the genes involved remain to be determined. We and others have reported that SS rats require a higher renal perfusion pressure to excrete the same amount of sodium and water as normotensive rats and that this is associated with an elevation in Cl− reabsorption in the TAL. Recent observations suggest that an abnormality in the renal metabolism of AA by enzymes of the P4504A family may contribute to the development of hypertension in this model. In this regard, the formation of 20-HETE is reduced, and the levels of P4504A proteins are lower in the OM of the kidney of SS rats compared with normotensive rats. Since previous studies have indicated that 20-HETE is the primary metabolite of AA produced in the TAL, and that this substance serves as a potent endogenous inhibitor of Na,K,2Cl− cotransporter, a deficiency in the production of this substance may contribute to the elevation in loop Cl− transport and the development of hypertension. The recent finding that in vivo perfusion of the loop of Henle of SS rats with 20-HETE normalizes loop Cl− transport is consistent with this possibility. Moreover, this hypothesis is also supported by the observations that a genetic marker within CYP4A2 gene cosegregates with blood pressure in an F2 cross of SS/Jr and Lewis rats and that induction of P4504A expression in the kidney prevents the development of hypertension in SS rats.

The purpose of the present experiments is to determine whether inhibition of the metabolism of AA by P450 enzymes in the OM of rats is sufficient to induce hypertension. In these experiments, the production of P450 metabolites of AA was selectively reduced in the OM of the kidney of normotensive Lewis rats fed a high salt diet by infusing 17-ODYA directly into the renal medullary interstitium, and changes in blood pressure were determined.

**Methods**

**General Procedures**

Experiments were performed on 12-week-old inbred Lewis Wistar rats purchased from Charles River Laboratories (Wilmington, Mass). The rats were housed in an animal care facility at the Medical College of Wisconsin, which is approved by the American Association for the Accreditation of Laboratory Animal Care, and they had free access to food and water throughout the study. All protocols involving animals received prior approval by the Animal Care Committee of the Medical College of Wisconsin. The rats were placed on a high salt diet (8% NaCl) for 1 week, and the rats were anesthetized with injections of ketamine (30 mg/kg IM) and acepromazine (3 mg/kg IM), and the right kidney was removed. The kidney was hemisected, separated into cortex and OM, rapidly frozen in liquid nitrogen, and stored at −80°C until assayed for P4504A activity. After a 3-day recovery period, the rats were anesthetized with ketamine and acepromazine, and a catheter was implanted in the OM of the kidney for chronic renal interstitial infusions. A catheter was also placed in the femoral artery for the measurement of arterial pressure. The renal interstitial catheter consisted of a 15-μm piece of polyvinyl tubing connected to 3.5- to 4.0-mm length of PE-10 tubing pulled to an outer tip diameter of approximately 100 μm. The renal medullary interstitial catheter was inserted 4 mm deep into the kidney so that the tip was located at the junction of the OM and inner medulla. A piece of adipose tissue was placed around the catheter on the surface of the kidney, and the catheter and the tissue were fixed to the surface of the kidney with cyanoacrylate adhesive. The catheters were exteriorized at the back of the neck and brought out through a stainless-steel spring and swivel device. After sur-
A 3-day recovery period, the rats were switched to a high salt liquid diet containing 8% NaCl by weight. Sodium intake was monitored using a calibrated feeding tube. Water intake was measured daily. Arterial pressure was recorded for 3 consecutive days while the rats underwent infusion with 0.9% NaCl solution (0.5 mL/h) into the renal medullary interstitium. Arterial pressure was directly measured using a pressure transducer and a computerized recording system for 2 hours per day. The signals were sampled at 30 Hz, and heart rate and systolic, diastolic, and mean arterial pressure were determined at 1-minute intervals and reduced to a mean value for the entire recording session. After the control period, the renal medullary interstitial infusion (0.5 mL/h) was switched to either vehicle (1% BSA in 0.9% NaCl solution) or 17-ODYA (400 pmol/min) and continued for 5 additional days. Arterial pressure was measured on days 1, 3, and 5 while vehicle or 17-ODYA was infused. At the end of the experiment, the kidneys were collected, sectioned into cortex and OM, rapidly frozen in liquid nitrogen, and stored at -80°C until assayed for P450 enzyme activity.

Preparation of Renal Microsomes

Microsomes were prepared from the cortex and OM of the contralateral control kidney and vehicle- or 17-ODYA-infused kidneys. The tissues were homogenized in 3 mL of a 10-mmol/L potassium phosphate buffer (pH 7.7) containing 250 mmol/L sucrose, 1 mmol/L EDTA, and 0.1 mmol/L PMSP. The homogenate was centrifuged at 3000 g for 15 minutes, followed by 100,000 g for 1 hour. The microsomal pellet was resuspended in a 100 mmol/L potassium phosphate buffer (pH 7.2) that contained 30% glycerol, 1 mmol/L DTT, 1 mmol/L EDTA, and 0.1 mmol/L PMSP and was stored at -80°C until P4504A enzyme activity was measured.

Renal Metabolism of AA

Renal P4504A enzyme activity was measured by incubating renal cortical or OM microsomes (0.5 mg protein) with [3H]AA (0.1 μCi, 10 mmol/L) in 1 mL of a potassium phosphate buffer (pH 7.4) containing 5 mmol/L MgCl2, 1 mmol/L EDTA, 1 mmol/L NADPH, and an NADPH-regenerating system (10 mmol/L isocitric acid and isocitrate dehydrogenase, 0.4 U/mL, Sigma). The samples were incubated at 37°C for 30 minutes following the addition of NADPH. The reactions were terminated by acidification to pH 4.0 with 0.1 mol/L formic acid, extracted twice with ethyl acetate, and dried under N2 gas. The residue was resuspended in 500 μL of 100% ethanol. The metabolites were separated using a Hitachi 655A high-performance liquid chromatography gradient system equipped with a C18 reverse-phase column (2 × 250 mm, 5 μm; Supelco) and a 2 cm guard column (Supelco). A linear elution gradient ranging from acetone-water-acetic acid (50:50:2 vol/vol/vol) to acetone-acetic acid (100:0, vol/vol) was used. The rate of change was 1.25% per minute at a flow rate of 0.5 mL/min. Metabolites were monitored using a Capillary Zone Electrophoresis (CZE) system with a DualSRS 7000 detector.(CZE) system with a DualSRS 7000 detector.

Hypertension Vol 29, No 1, Part 2 January 1997

Selected Abbreviations and Acronyms

AA = arachidonic acid
HETE = hydroxyeicosatetraenoic acid
17-ODYA = 17-octadecenoic acid
OM = outer medulla, outer medullary
SS = salt-sensitive
TAL = thick ascending limb of Henle’s loop

Immunoblots

Microsomal protein prepared from the OM (100 μg) of contralateral kidneys and from kidneys infused with vehicle or 17-ODYA was separated by electrophoresis on a 7.5% SDS gel (15X15 cm) for 14 hours at 80 V. Proteins were transferred electrophoretically to a nitrocellulose membrane (Trans-Blot, Bio-Rad) at 40 V in a buffer consisting of 25 mmol/L Tris-HCl, 192 mmol/L glycine, and 20% methanol for 4 hours at 4°C. The membrane was blocked for 2 hours by immersion in a buffer (TBST-20) containing 10 mmol/L Tris-HCl, 150 mmol/L NaCl, 0.8% Tween 20, and 5% nonfat dry milk and incubated with a 1:2000 dilution of a polyclonal antibody raised against a 20-mer synthetic peptide in the rat CYP4A1 sequence that recognizes the CYP4A1, 1, and 3 isoforms. The membrane was washed in TBST-20 buffer and incubated for 1 hour with a 1:1000 dilution of an alkaline phosphatase-coupled goat anti-rabbit secondary antibody (Zymed). The samples were incubated at 37°C for 30 minutes followed by 100,000 g for 1 hour. The microsomal pellet was resuspended in a 100 mmol/L potassium phosphate buffer (pH 7.2) that contained 30% glycerol, 1 mmol/L DTT, 1 mmol/L EDTA, and 0.1 mmol/L PMSP and was stored at -80°C until P450 enzyme activity was measured.

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Results

Effects of 17-ODYA on Blood Pressure

Control mean arterial pressures of the rats infused with vehicle and 17-ODYA averaged 116±1 versus 115±3 mm Hg, respectively (Fig 1). On the first day of 17-ODYA infusion into the renal medullary interstitium, mean arterial blood pressure rose from 115 ±3 to 132±3 mm Hg, whereas no change in mean arterial pressure was observed in rats infused with vehicle alone (Fig 1). After 5 days of infusion with 17-ODYA, mean arterial pressure rose to 142±2 mm Hg versus a value of 117±2 mm Hg observed in the vehicle-infused group (Fig 1). Control sodium intake was similar in both vehicle- and 17-ODYA-treated rats.

FIG 1 Effect of renal medullary interstitial infusion of 17-ODYA (400 pmol/min) on blood pressure in Lewis rats fed a high salt diet (8% NaCl). Mean arterial pressure was measured in conscious unrestrained rats. Control values represent the average of 3 days of blood pressure recording while saline was infused through the interstitial catheter. After the control period, rats were either infused with vehicle (1% BSA) or 17-ODYA (400 pmol/min) for 5 days. Values are presented as mean±SE. Numbers in parentheses indicate number of animals in each group.*Significant difference (P < 0.05) from corresponding value in vehicle-infused group; †significant difference (P < 0.05) from the control value.
Effects of 17-ODYA on the Renal Metabolism of AA

To assess the degree of inhibition of P4504A activity produced by infusion of 17-ODYA into the renal medullary interstitium, the metabolism of AA by microsomes prepared from the cortex and OM of the contralateral control (referred to as untreated at the time of nephrectomy), 17-ODYA- and vehicle-infused kidneys was determined. In control incubations, OM microsomes produced primarily 20-HETE and lesser quantities of 11- and 15-HETE when incubated with AA. In some incubations, small quantities of 11,12- and 14,15-dihydroxyeicosatetraenoic acids (DiHETes) could also be detected. The production of 20-HETE in the OM fell in rats infused with 17-ODYA by 70 ± 8% relative to that measured in the contralateral kidney. It also reduced the formation of DiHETes by about the same extent. In contrast, outer medullary 20-HETE production was not significantly different in the control and vehicle-infused kidneys (Fig 2). The inhibitory effect of 17-ODYA on the metabolism of AA of P4504A5 appeared to be restricted to the OM, since the production of 20-HETE (Fig 3) was not significantly different between the renal cortex of control kidneys and kidneys infused with 17-ODYA. Similarly, the production of epoxygenase metabolites of AA in the renal cortex averaged 46 ± 8 pmol min⁻¹ mg protein⁻¹ in control kidneys and 51 ± 4 pmol min⁻¹ mg protein⁻¹ in the OM of kidneys undergoing chronic infusion with 17-ODYA.

Effects of 17-ODYA on P4504A2 Protein Levels

Immunoblot experiments were performed in microsomes prepared from the OM of control kidneys and kidney. The inhibitory effect of 17-ODYA on the metabolism of AA of P4504A5 appeared to be restricted to the OM, since the production of 20-HETE (Fig 3) was not significantly different between the renal cortex of control kidneys and kidneys infused with 17-ODYA. Similarly, the production of epoxygenase metabolites of AA in the renal cortex averaged 46 ± 8 pmol min⁻¹ mg protein⁻¹ in control kidneys and 51 ± 4 pmol min⁻¹ mg protein⁻¹ in the OM of kidneys undergoing chronic infusion with 17-ODYA.

Discussion

Previous studies have indicated that the pressure-natriuretic relationship is shifted towards higher pressures in SS/Jr rats and that this is associated with an elevation in Cl⁻ reabsorption in the TAL of SS/Jr rats. Recently, an abnormality in the renal metabolism of AA has been identified in the OM of SS/Jr rats. Since 20-HETE is the primary metabolite of AA produced by TAL cells, where it serves as an endogenous inhibitor of the Na⁺,K⁺,2Cl⁻ cotransporter, a deficiency in the renal production of 20-HETE may play a role in altering this transport and the development of hypertension in SS/Jr rats. This hypothesis is further supported by the recent findings that induction of renal 20-HETE production with clofibrate prevents the development of hypertension in SS/Jr rats and that a marker within the cytochrome CYP4A2 gene cosegregates with blood pressure in an F2 population derived from a cross between SS/Jr and Lewis rats.

To determine whether a deficiency in the production of 20-HETE or some other endogenous P450 metabolite of AA in the OM can promote the development of hypertension, experiments were performed in normotensive Lewis rats in which the metabolism of AA by P450 in the OM was selectively reduced by infusion of 17-ODYA directly into the renal medullary interstitium. Chronic medullary infusion of 17-ODYA for 5 days increased mean arterial pressure in Lewis rats fed a high salt diet. Mean arterial pressure rose 15 mm Hg on the first day following infusion.
of 17-ODYA and by 27 mm Hg over the 5-day course of the experiment. The time course and the magnitude of the rise in arterial pressure observed in Lewis rats on a high salt diet infused with 17-ODYA closely resemble those that we have previously reported in Dahl SS rats fed the same diet.\textsuperscript{12,15,16}

In the present study, we confirmed that infusion of 17-ODYA into the renal medullary interstitium inhibited the metabolism of AA by P450. The production of 20-HETE was 70% lower in microsomes prepared from the OM of the control kidney of Lewis rats; lanes 2, 4, and 6 were loaded with microsomes (100 μg) prepared from the OM of Lewis rats after medullary interstitial infusion of 17-ODYA (400 pmol/min) for 5 days; and lane 7 was loaded with microsomes (15 μg) prepared from the liver that expresses all three CYP4A isoforms. Lanes 8, 10, and 12 were loaded with microsomes (100 μg) prepared from the OM of the control kidney of Lewis rats; lanes 9, 11, and 13 were loaded with microsomes (100 μg) prepared from OM of kidneys infused with vehicle (1% BSA) for 5 days. Data are presented as percent of levels of CYP4A2 protein found in the contralateral kidney of the same animal.

The mechanism by which renal medullary infusion of 17-ODYA produces hypertension in Lewis rats fed a high salt diet remains to be determined. The present results are consistent with the hypothesis that 17-ODYA, by inhibiting the endogenous production of 20-HETE in the TAL, may promote sodium retention in this segment and produce hypertension secondary to volume expansion. On the other hand, the present results do not exclude participation of other P450 metabolites of AA in this response. In this regard, 17-ODYA also inhibited the production of DiHETEs in the OM, and Capdevila et al.\textsuperscript{19} and Makita et al.\textsuperscript{20} have reported that blocking epoxygenase activity with clotrimazole alters renal function and increases arterial pressure in Sprague Dawley rats fed a high salt diet.

In summary, the results of the present study indicate that infusion of 17-ODYA (400 pmol/min) directly into the renal medullary interstitium selectively inhibits the metabolism of AA by P450 enzymes in the OM and that this is associated with an increase in mean arterial blood pressure in Lewis rats fed a high salt diet. These results, taken together with the previous observation that induction of renal 20-HETE production with clofibrate prevents the development of hypertension in SS/Jr rats,\textsuperscript{9} provide evidence that chronic manipulation of this system in vivo influences arterial pressure. Overall, these results further support the hypothesis that diminished production of 20-HETE or some other P450 metabolite of AA in the OM can lead to the development of salt-sensitive hypertension and provide additional support for the view that the CYP4A2 locus is a candidate gene for salt-sensitive hypertension in the Dahl SS rat.

Acknowledgments
This work was supported by National Institutes of Health grant HL 36279 and by a predoctoral fellowship for D. Stec from the American Heart Association Wisconsin Affiliate.

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_Hypertension_. 1997;29:315-319
doi: 10.1161/01.HYP.29.1.315

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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