Evidence That Reduced Renal Medullary Nitric Oxide Synthase Activity of Dahl S Rats Enables Small Elevations of Arginine Vasopressin to Produce Sustained Hypertension

Baozhi Yuan, Allen W. Cowley, Jr

Abstract—On the basis of observations supporting the functional importance of nitric oxide (NO) in the regulation of renal medullary function, and a reduced nitric oxide synthase (NOS) enzyme activity in the outer medulla of the Dahl salt-sensitive (SS/Mcw) rats, we hypothesized that these inbred rats would have reduced capacity to synthesize renal medullary NO. This reduced capacity would sensitize them to the hypertensive effects of small elevations of circulating arginine vasopressin (AVP). SS/Mcw and Brown Norway (BN/Mcw) rats with implanted arterial and venous catheters were fed a 0.4% salt diet and infused intravenously for 14 days with a subpressor dose of AVP (2 ng/kg per min). Mean arterial pressure (MAP) was measured 2 hours daily in unanesthetized rats maintained in their home cages. MAP in SS/Mcw rats increased during day 1 of AVP infusion from a control level of 127±0.9 mm Hg to an average of 147±1.6 mm Hg after 14 days. MAP did not return to control values during the 3 days after the end of AVP infusion. BN/Mcw rats showed no changes of MAP during 14 days of AVP infusion (90.4±0.6 mm Hg and 92.3±0.4 mm Hg). Northern blot analysis of renal tissue from vehicle (saline) -infused rats demonstrated that NOS I and NOS III mRNA expression was significantly less in SS/Mcw rats in the renal outer medulla compared with BN/Mcw rats. We conclude that small, normally subpressor elevations of plasma AVP can produce chronic hypertension in SS/Mcw rats and that this phenomenon is related to the reduced medullary NOS enzyme activity, which in turn reduces the AVP-stimulated NO synthesis. *(Hypertension. 2001;37[part 2]:524-528.)*

Key Words: Dahl salt-sensitive rats ■ Brown Norway rats ■ NOS isoforms ■ blood pressure ■ vasopressin

It is well known that chronic elevation of plasma arginine vasopressin (AVP) does not produce sustained hypertension in rats, dogs, and in human,1–4 despite that AVP is one of the most potent circulating vasoconstrictors and a fluid retaining hormone. However, previous studies from our laboratory have found that chronic intravenous infusions of the AVP V1 receptor agonist [Phe2, Ile8]VP resulted in sustained hypertension.5 It was found that these hypertensive actions of the V1 agonist were mediated through actions on the medulla of the kidney because chronic infusion of a selective V1R antagonist into renal medullary interstitium prevented the rise of arterial pressure. Additionally, hypertension was also achieved when the V1 agonist was infused directly into the renal medullary interstitium6 at a dose previously shown to reduce blood flow to the renal medulla.7–9

The hypertension achieved with V1R stimulation suggested that the failure of AVP to elevate the blood pressure was related to the stimulation of V2 receptors (V2R), which activated an opposing renal medullary vasodilator response. One such mechanism was identified to be the release of NO. Because AVP increased blood flow within the renal medulla by V2R-mediated production of NO,7,10 Since the V2R mRNA could not be detected within the microvasculature of renal cortex or medulla,10 it was suggested that the elevation of medullary NO concentrations in response to AVP or V2 agonist stimulation11–13 was the result of V2 receptors stimulation most likely in the medullary collecting ducts. The concept that NO responses in the medulla served to buffer AVP-V1 receptor vasoconstrictor actions was validated by recent studies in which N(G)-nitro-L-arginine methyl ester (L-NAME) was infused chronically into the medullary interstitial space of Sprague-Dawley rats to reduce NOS activity and the NO response to AVP. Even a moderate reduction of NOS activity in the renal medulla enabled small elevations of circulating AVP to produce sustained hypertension,14 demonstrating the importance of the medullary NO counter-regulatory system. Recently, studies have demonstrated that SS/Mcw rats exhibit reduced vasodilator responses to compounds that stimulate NO release (eg, acetylcholine)15 and that renal medullary nitric oxide synthase (NOS) activity is significantly reduced in SS/Mcw rats compared with the Brown Norway (BN/Mcw) rats.16
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AVP Produced Hypertension in SS/Mcw Rats

Figure 1. Northern blot analysis showing NOS III mRNA expression in tissue of the renal cortex, outer medulla, and inner medulla in SS/Mcw and BN/Mcw rats. A total of 20 μg of total RNA from cortex and outer medulla and 10 μg of total RNA from inner medulla were tested in this study. *Significant difference when compared with BN/Mcw rats (P<0.05).

One week after surgery, daily 2-hour measurements of MAP were begun and continued for 3 to 4 days for the control period and 14 days for the experiment period using an on-line data collection and analysis system described previously. Intravenous infusion of AVP diluted in isotonic saline was then begun at a rate of 2 ng/kg per minute and infused continuously (0.25 mL/h) for 14 days. The infusion solution was changed back to saline after 14 days of AVP and blood pressure was measured for another 3 days. An AVP control group of SS/Mcw rats received a 14-day intravenous chronic infusion of saline (0.25 mL/h). The AVP dose used in these studies was found in previous studies to increase plasma AVP levels to 10 to 12 pg/mL, but did not cause sustained hypertension when infused intravenously for 14 days to normal Sprague-Dawley rats.

Statistical Analysis

Data are expressed as means±standard error. Within-group changes in MAP were evaluated with a 1-way ANOVA for repeated measures and Duncan’s post hoc test. Northern Blots were analyzed using ImagQuant software from Molecular Dynamics. The t test was used to examine the differences in mRNA expression between strains. The level of significance was P<0.05.

Results

NOS 3 Isoform mRNA Expression in SS/Mcw and BN/Mcw Rats

Figure 1 shows NOS III mRNA was present in the outer and inner medulla of both SS/Mcw and BN/Mcw rats receiving a low salt diet. The outer medulla of SS/Mcw rats contained significantly less NOS III mRNA when compared with BN/Mcw rats. However, there was no significant difference for NOS III expression between SS/Mcw and BN/Mcw rats in the inner medulla.

Similar results were obtained for NOS I mRNA (Figure 2) with the outer medulla of SS/Mcw showing significantly less mRNA expression than BN/Mcw rats, whereas no significant differences were found in the inner medulla.

Northern blot analysis was also performed for NOS II mRNA in SS/Mcw and BN/Mcw rats, but this isoform could not be detected in any renal regions from either strain. The mRNA levels for the three NOS isoforms were also very low and undetectable in renal cortex from both strains, as indicated in Figures 1 and 2. These observations do not suggest an absence of these isoforms but do indicate a significantly reduced total tissue expression of the mRNA because the Northern blot membranes were exposed for similar times.

Methods

Twelve-week-old male inbred Dahl S (SS/JrHsdMcw) and Brown Norway (BN/SSsMcw) rats were selected from colonies maintained at the Medical College of Wisconsin. These rats were inbred for at least 6 generations. Dahl S rats were maintained throughout life on a low 0.25% sodium diet (0.25% NaCl) and MAP was measured daily for 72 hours for iNOS at 32°C, 4°C, 4°C, respectively. The signals were analyzed by densitometry scanner and ImagQuant software (Molecular Dynamics).

Renal Regional NOS mRNA Expression Studies in Dahl S and BN/Mcw Rats

Male adult SS/Mcw and BN/Mcw rats maintained throughout life on a low 0.4% salt intake were killed with an overdose of sodium pentobarbital; the kidneys were quickly removed, dissected, and rinsed free of blood with ice cold normal saline. The renal cortex, outer medulla, and inner medulla were separated, and the tissues were frozen quickly on dry ice and transferred to −80°C freezer until use. Total RNA was extracted using TRIzol Reagent (Life Technologies) according to the manufacturer’s protocol. A total of 20 μg of total RNA from cortex, outer medulla, and 10 μg of total RNA from inner medulla were separated on 5% formaldehyde-MOPS denaturing agarose gel by electrophoresis. The RNA was then transferred onto a positively charged nylon membrane (Amersham Pharmacia) by overnight capillary transfer action that was then UV cross-linked (Stratagene). The specific probes for neuronal NOS (NOS I), inducible NOS (NOS II), and endothelial NOS (NOS III) used in subsequent Northern blot analysis were generated by reverse transcriptase-polymerase chain reaction (RT-PCR) using specific primers described previously.18 PCR products were purified (PCR purification kit, QIAGEN, Germany), and the probes were labeled with 32P (RepPrime II DNA Labeling Kit, Amersham). The membrane was prehybridized (Rapid-Hyb buffer, Amersham Pharmacia) for 1 hour, and then 25 ng of labeled probe was added to the hybridization solution (Rapid-Hyb buffer, Amersham Pharmacia) and hybridized for 2.5 hours. The membrane was washed with 2× SSC (3.0 mol/L sodium chloride and 0.3 mol/L sodium citrate) and 0.1% SDS (sodium dodecyl sulfate solution) for 20 minutes, then washed twice for 30 minutes with 1× SSC and 0.1% SDS, and 0.1× SSC and 0.1% SDS, respectively. The membrane was then exposed to the x-ray film with intensifying screen, 48 hours for eNOS, nNOS, and 72 hours for iNOS at −80°C, respectively. The signals were analyzed by densitometry scanner and ImagQuant software (Molecular Dynamics).

Surgical Preparation and Chronic MAP Measurement in Dahl S, Dahl R, and BN/Mcw Rats

Rats were surgically prepared with the femoral arterial and femoral vein catheters for the measurement of arterial pressure and intravenous infusion as described previously.17 Rats were allowed to recover for 5 to 7 days while saline was infused intravenously at a rate of 0.25 mL/h throughout the recovery and experimental control period.

Taken together, these findings provided the rationale for the present study. Specifically, studies were carried out to determine whether small elevations of circulating AVP that failed to produce hypertension in normotensive BN/Mcw rats (as shown in Sprague-Dawley rats14) would lead to chronic hypertension in SS/Mcw rats even when maintained on a low salt diet (0.4%). Northern blot analyses were performed to determine whether NOS mRNA of the three NOS isoforms were reduced in the medulla of SS/Mcw rats.

Statistical Analysis

Data are expressed as means±standard error. Within-group changes in MAP were evaluated with a 1-way ANOVA for repeated measures and Duncan’s post hoc test. Northern Blots were analyzed using ImagQuant software from Molecular Dynamics. The t test was used to examine the differences in mRNA expression between strains. The level of significance was P<0.05.

Results

NOS 3 Isoform mRNA Expression in SS/Mcw and BN/Mcw Rats

Figure 1 shows NOS III mRNA was present in the outer and inner medulla of both SS/Mcw and BN/Mcw rats receiving a low salt diet. The outer medulla of SS/Mcw rats contained significantly less NOS III mRNA when compared with BN/Mcw rats. However, there was no significant difference for NOS III expression between SS/Mcw and BN/Mcw rats in the inner medulla.

Similar results were obtained for NOS I mRNA (Figure 2) with the outer medulla of SS/Mcw showing significantly less mRNA expression than BN/Mcw rats, whereas no significant differences were found in the inner medulla.

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Effects of Long-Term Intravenous Infusion of AVP on MAP in SS/Mcw and BN/Mcw Rats

Figure 3 summarizes the effects of intravenous infusion of subpressor dose of AVP (2 ng/kg per min) on MAP in SS/Mcw and BN/Mcw rats. The SS/Mcw rats showed a significant higher MAP during the control period (averaged 125.0 ± 3.9 mm Hg (n = 7), P < 0.0001) when compared with the control levels of MAP in BN/Mcw rats (90.0 ± 2.7 mm Hg). Moreover, MAP in SS/Mcw rats was increased significantly to an average of 143.6 ± 5.7 mm Hg after 14 days of administration of AVP when compared with the control levels. The blood pressure was increased significantly even after 1 day of AVP infusion. MAP did not return back to the control level for 3 days after ending the AVP infusion, averaging 149.1 ± 7.4 mm Hg during this post-AVP infusion period. BN/Mcw exhibited no significant changes of MAP during the 14 days of AVP infusion. The control MAP averaged 90.0 ± 2.7 mm Hg in BN/Mcw rats (n = 6), and 92.3 ± 2.3 mm Hg during the intravenous AVP infusion. These results clearly indicate different sensitivity to AVP for these two strains of rat.

Discussion

Our previous studies have shown that NO in the renal medulla protects from vasopressin-induced hypertension and that there is a reduced capacity of SS/Mcw rats to produce NO in response to stimuli such as angiotensin II.13,16 The present study was designed to determine whether AVP could produce hypertension in rats that exhibited reduced endogenous medullary NOS and NO production.

Evidence of Reduced Medullary NOS Gene Expression in Dahl S Rats

Figure 2. Northern blot analysis showing NOS I mRNA expression in renal cortex, outer medulla, and inner medulla in SS/Mcw and BN/Mcw rats. n = 5 for each group. *Significant difference when compared with BN/Mcw rats (P < 0.05).

Figure 3. Measurement of MAP in SS/Mcw and BN/Mcw infused with 2 ng/kg per minute of AVP intravenously for 14 days. All animals were infused with normal saline and had blood pressure measured for 3 stable control days; then the infusion solution was changed to AVP on day 1 and continuously infused for 14 days. Blood pressure was measured for another 3 days after infusion solution was changed back to normal saline. *Significant difference when compared with respective controls (P < 0.05). Plus signs indicate significant difference between SS/Mcw and BN/Mcw rats (P < 0.0001).

Figure 4. Measurement of MAP in SS/Mcw rats with intravenous infusion of normal saline. All animals were fed 0.4% low salt diet during the entire experimental period. Blood pressure was measured daily for 2 hours. n = 6. *Significant difference when compared with the average of the first 3 MAP measurement (P < 0.05).

of saline. However, the increases of MAP reached a level of statistical significant only on days 11 and 12 of isotonic saline infusion, whereas significant changes in MAP with AVP occurred in the first day.
rats may be responsible for the reduction of NO production induced by different stimuli such as AVP. Previous studies in our laboratory have shown that AVP stimulates NO production in normal Sprague-Dawley rats and that NO counteracts the vasoconstrictor effects of AVP. This study further defined the significance of this AVP-stimulated NO production in the long-term control of arterial pressure. The results also demonstrated a deficit in the NOS RNA levels in the SS/Mcw rats. Together, we conclude that decreased outer medullary NOS expression primarily contributes to a reduction of NO production in response to AVP, which abolishes the counteracting effect of NO on medullary vasoconstriction resulting in AVP-induced hypertension.

Although we and others have reported previously that NOS II mRNA and proteins are present in rat renal medulla, the inducible NOS isoform was not detectable by Northern blot analysis in the present study in either SS/Mcw or BN/Mcw kidneys. The failure to demonstrate the NOS II mRNA in the renal outer and inner medulla in the present study could simply be due to the limitation of tissue mRNA amount (20 μg for outer medulla and 10 μg for inner medulla) in the Northern blot study. The present data indicate only that SS/Mcw and BN/Mcw rats express much less NOS II mRNA than NOS III and NOS I under normal physiological conditions.

In light of other recent data from our laboratory, the present study provides the basis for a more integrated understanding of the overall impairment of the renal NO system in Dahl S rats. Because the concentration of NO does not appear to differ in the medulla of SS/Mcw and BN/Mcw rats under basal conditions, the deficits in NOS enzyme expression of the medulla seem to attenuate the AVP-stimulated increases of tissue NO concentration. We have also recently found in preliminary studies an inability of angiotensin II to increase NO production with AVP stimulation and are unable to overcome the medullary vasoconstrictor effects of AVP, SS/Mcw rats are susceptible to the hypertensive actions of small elevations of circulating AVP.

The saline infusion study summarized in Figure 4 served as a vehicle control experiment, because we added AVP in saline for the intravenous infusion. With a very low infusion rate (0.25 mL/h), the rats received 54 mg NaCl/24 hours, which amounts to approximately 0.1% salt diet. Because the diet of these rats was 0.4%, the total daily intake was equivalent to a 0.5% salt diet. It seems that this small amount of intravenous NaCl may contribute to the slight elevation of MAP in the AVP-infused SS/Mcw rats on the last 2 days of the infusion period, but this contribution of the saline (vehicle) to the rapid and sustained increase of MAP in the AVP-infused SS/Mcw rats seems to be minimal in this study.

It is not clear what causes the NO deficiency in SS/Mcw rats. Chen et al identified a single nucleotide mutation in the NOS II gene in SS/Mcw rats. However, because most investigators find the level of NOS II in the outer and inner medulla is quite low, the functional significance of this variation remains to be determined. Deng and Rapp reported that NOS III does not segregate with the trait of blood pressure in a genetic linkage study. An alternative hypothesis to explain reduced NO production with AVP stimulation could be a reduced expression of AVP V2 or V2-like receptors, because they modulate vasodilatory effects in the renal medulla by increased production of NO. However, this would not explain why SS/Mcw rats are also hyporesponsive to angiotensin II and norepinephrine. These observations would suggest a generally reduced capacity to produce NO for reasons that remain to be determined.

In conclusion, we have shown that SS/Mcw rats, like the medullary L-NAME infused rats, exhibit an abnormal NO system in the renal medulla. This defect in the NO system makes them more vulnerable to vasoconstrictor effect of AVP, such that long-term elevation of plasma AVP causes sustained hypertension.

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