MECHANISMS OF SALT-SENSITIVE HYPERTENSION: ROLE OF RENAL MEDULLARY INDUCIBLE NITRIC OXIDE SYNTHASE

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ABSTRACT

The goal of this study was to determine the role of renal medullary inducible nitric oxide synthase (iNOS) in the arterial pressure, renal hemodynamic and renal excretory changes that occur in Dahl/Rapp salt-resistant (R) and salt-sensitive (S) rats during high Na intake. Forty R and S rats, equipped with indwelling arterial, venous and renal medullary catheters were subjected to high (8%) Na intake, and selective iNOS inhibition was achieved with continuous IV or renal medullary interstitial infusion of aminoguanidine (AG, 3.075 mg/kg/h). After 5 days of AG, mean arterial pressure increased to 132 ± 2 % control in the S-high Na, AG-intramedullary rats compared to 121 ± 4 % control (P<0.05) in the S-high Na alone rats and 121 ± 2 % control (P<0.05) in the S-high Na, AG-IV rats. AG did not change arterial pressure in R rats. AG also caused little change in renal hemodynamics, urinary Na or H2O excretion or acetylcholine-induced aortic vasorelaxation in R or S rats. The data suggest that during high Na intake, nitric oxide produced by renal medullary iNOS helps to prevent excessive increases in arterial pressure in the Dahl S rat but not the R rat.
About one-half of the population of human hypertensive patients is "salt-sensitive", which refers to the dependence of blood pressure on sodium intake (38), but the cause of the salt-sensitivity is not clearly understood. We recently showed that nitric oxide (NO) produced by iNOS helps to prevent salt-sensitive hypertension in Dahl R rats, and decreases the salt-sensitivity in the Dahl S rats (32). In this study the highly salt-resistant Dahl R rat became hypertensive when a high sodium intake was combined with systemic iNOS inhibition with aminoguanidine (AG). Also, the arterial pressure of the Dahl S rat on high sodium intake and systemic AG was significantly higher than the S rat on high sodium intake alone. Yet, the major source of NO produced by iNOS which decreased salt-sensitivity in the Dahl R and S rats in this study is not known.

Renal NO production has been shown to be important in the regulation of arterial pressure and renal hemodynamics (14), and renal medullary tissue has a much greater capacity to synthesize NO than the renal cortex (3,34,36,39,40). Also, the NO concentration in the renal medulla is higher than in the cortex (40), especially during high salt intake. Therefore, the renal medulla is a likely source of NO in Dahl R and S rats during increases in sodium intake.

Recent studies have shown that increases in Na intake cause a variable response of NOS proteins in the kidney. Some investigators have found that an increased Na intake caused an increase in renal medullary inducible NOS (iNOS) protein in Sprague-Dawley rats (20), and others found a decrease in renal NOS proteins in Sprague-Dawley (27) and the outbred Brookhaven strain of Dahl S rats (26). In preliminary studies we found that renal medullary iNOS proteins increased after a one week exposure to a high Na diet in Dahl R/Rapp rats but did not change after 2 or 3 weeks on high Na intake, and there were
no significant changes in Dahl S/Rapp rats (35). Although several investigators have measured iNOS protein mass and in vitro activity in renal tissue, only a few have studied the function of iNOS protein in the kidney (5,22), and there is almost no information available about the function of iNOS in the renal medulla. We hypothesize that the NO produced by iNOS in the renal medulla in the Dahl R and S rats helps to prevent salt-sensitivity, and thus iNOS inhibition in the renal medulla will increase the salt-sensitivity of both R and S rats. Studies were conducted in Dahl R and S rats, Rapp strain, using chronically implanted arterial, venous and renal medullary interstitial catheters, during a 5-day control period and a 5-period of iNOS inhibition with continuous intramedullary or IV infusion of AG at 3.075mg/kg/hr. Rats were subjected to either low or high Na intake, and cardiovascular and renal functional measurements were made throughout the experiment.

METHODS

Animal Preparation, Experimental Measurements, and Instrumentation.

Experiments were conducted in 40 conscious 7- to 8-week-old male Dahl R or S rats, Rapp strain (Harlan Sprague Dawley, Indianapolis, IN). The project had the approval of the local Institutional Animal Committee and followed the newest guiding principles for research (1). Rats were received when they were 5 to 6 weeks old, and surgery was performed when the rats reached a weight of 200g. Using aseptic surgery with isoflurane anesthesia (1%), the right kidney of these rats was removed first in all groups.

Systemic studies: Chronic arterial and venous catheters were implanted through the femoral artery and vein, respectively. Both catheters were exteriorized at the dorsal nape of the neck. The animals were then connected to a swivel apparatus that allowed the rats
free movement without tangling of the catheters. A catheter from the swivel was
connected to a Cobe transducer (Lakewood, CO) for measurement of arterial pressure
and in turn was connected to a digital amplifier. Arterial pressure and heart rate were
determined from pulsatile arterial pressure signals sent to a digital computer at 500Hz for
4 s of each minute throughout the entire 24-h period.

*Intramedullary studies:* In addition to the femoral arterial and vein catheters, an
intramedullary catheter (Polyethylene No.10 material) was inserted directly into the
medullary interstitial space of the left kidney through a 26 gauge needle hole. The tip of
catheter was placed at or near the intersection of outer and inner medulla (at a depth of 3-
4 mm) as previously done (17,19,21). The catheter also passed through mersilene mesh
on the surface of kidney, and the mersilene mesh was sutured to the renal capsule with 6-
0 Prolene suture. Finally, the exit hole was sealed and the mersilene mesh secured with
cyanoacrylate glue. The intramedullary catheter exited the body at the nape of the neck
together with the arterial and venous catheter, and it was used for continuous
intramedullary infusion of chemicals and antibiotics. The IV and intramedullary infusion
rate was 10.4µl/min. At the end of experiment, the kidneys were examined, and rats with
improperly placed catheters or significant renal damage were excluded from the study.

All rats were placed in a temperature-controlled room with a 12:12-h light-dark cycle.
Starting 0.5 hour after the catheters were implanted and continuing throughout the
experiment, the rats received prophylactic antibiotics (Levaquin, 8.75mg/kg) in a
continuous IV or intramedullary infusion using a Harvard apparatus syringe pump (South
Natic, MA). All rats were allowed to recover for 7 days before the control period began
when the rats had weight of ≈220g.
Water intake, urinary volume output and food intake were measured daily. Urine Na concentration was determined by flame photometry, and sodium and water balances were calculated for each experiment day. Urinary nitrate plus nitrite excretion (UNOx) was determined every 3 to 4 days using the Griess reaction and nitrate reductase from Escherichia coli to reduce nitrate to nitrite as we have done before (15,32,33).

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), were determined every 3-4 days in conscious rats as we have done before (32,33) by measuring the radioactivity and aminohippurate (30) concentration of a 4-hour fasted plasma sample after a 12-hour period of intravenous infusion of $^{125}$I-iothalamate and aminohippurate Na (4,6).

**Experimental protocols.** The following 6 groups of Dahl rats were studied including the intramedullary groups: R-high Na alone (n=7); R-high Na, AG (n=7); S-high Na alone (n=5); and S-high Na, AG (n=7) and the intravenous groups: R-high Na, AG (n=7); and S-high Na, AG (n=7). All these groups received a low Na (0.3%) diet for the first 5 days of the recovery period and a high Na (8%) diet for the last 2 days of recovery and the entire experimental period. Immediately after the recovery period, data were collected during 5-day control period followed by a 5-day period of either vehicle (0.9% isotonic sodium chloride solution) infusion or iNOS inhibition with intramedullary or intravenous infusion of AG at 3.075mg/kg/hr.

To confirm our AG results in R rats a highly specific iNOS inhibitor N-[3-(aminomethyl) benzyl] acetamidine HCl (1400W) was infused IV in two R rats and into the renal medulla in two R rats at 0.004 mg/h (29). The rats had measurements taken over a 5 day control period and a 4 day experimental period and received an 8% Na
intake. 1400W has a 285- and ~7000-fold greater selectivity for iNOS than nNOS or eNOS, respectively (13,25). ACh dose vasorelaxation curves on aortic segments were run on the rats using the protocol described below.

Specificity of iNOS inhibition. At the end of experiment, to test the selectivity of iNOS inhibition by AG, aortic segments were taken for measuring their relaxation in response to acetylcholine stimulation. On the day of the experiment, the rats were anesthetized by inhalation of isoflurane. The thoracic aorta was rapidly excised, placed in oxygenated Krebs solution, and cleaned of connective tissue. The aorta was cut transversely into 3 mm wide rings. Aortic rings were cut open into strips. Extreme care was taken throughout the procedure to avoid injury to the endothelium.

One end of the aortic strip was attached to a glass hook using a thread loop and the other end was connected to a force transducer (Grass FT03). Aortic strips were stretched to Lmax (1.5 the unloaded initial length, L). The strips were allowed to equilibrate for 1 h in a water-jacketed, temperature-controlled tissue bath filled with 50 ml Krebs solution continuously bubbled with 95% O2 and 5% CO2 at 37oC. The changes in isometric contraction were recorded on a polygraph (Grass Model 7D). A control contraction was elicited by applying phenylephrine (Phe, 10-5 mol/L) to the tissue bath solution. Once the Phe contraction reached a plateau, the tissue was rinsed with Krebs solution 3 times, 10 min each. The whole procedure of contraction and washing was repeated two times. A contraction to submaximal concentration of Phe (3x10-7 mol/L) was then elicited. Increasing concentrations of acetylcholine (ACh) were added and the extent of vascular relaxation was measured.
**Statistical analysis.** Comparison of data from animals on low and high salt diets were first performed using 2-way analysis of variance for repeated measures followed by a 1-way analysis of variance for repeated measures for each group and a Fisher LSD test for post hoc analysis at each experimental time point. Differences were considered to be statistically significant if $P<0.05$. All data are expressed as mean ± SE.

**RESULTS**

*Arterial pressure responses to iNOS inhibition.* The top panel of Figure 1 shows that during high Na intake, intramedullary iNOS inhibition significantly increased mean arterial pressure in Dahl S rats when compared to the high Na group or the AG-IV group. By day 10 arterial pressure reached a value of $132 ± 2$ % control in the AG-intramedullary (IMED) group, and at this time pressure was $121 ± 4$ % control ($P<0.05$) in S-high Na alone rats. Meanwhile, the same dose of AG in the IV group did not increase the arterial pressure when compared to the S high Na group, and the pressure in the IV group was $121 ± 2$ % control. The bottom panel of Figure 1 shows that there were no significant changes in mean arterial pressure when comparing the R-high Na IMED group, the R-high Na, AG IMED group and the R-high Na, AG-IV group, and mean arterial pressure values on day 10 were $102 ± 1$, $101 ± 1$, $102 ± 2$ % control, respectively.

Table 1 shows the absolute control values for mean arterial pressure, GFR, and ERPF. Except for the ERPF in the S rats, there were no significant changes in arterial pressure or GFR the High Na IMED group, the High Na, AG-IMED group, and the High Na, AG-IV group in either R or S rats. There were also no significant changes among these three groups in R rats in their ERPF values.
In the rats that received 1400W to inhibit iNOS, the control arterial pressure in the IV group was \(93 \pm 4\) mmHg, and the value after 4 days of 1400W infusion was \(93 \pm 0\) mmHg. In the IMED group control pressure was \(95 \pm 2\) mmHg, and after 4 days of 1400W infusion was \(97 \pm 6\) mmHg. The aortic ACh vasorelaxation curves were not changed by 1400W in either the IV or IMED groups when compared to a high Na alone group.

**GFR and ERPF responses to iNOS inhibition.** The top and bottom panels of figure 2 and 3 show that GFR and ERPF in all 6 groups did not significantly change during iNOS inhibition. By day 9, neither GFR nor ERPF of the S or the R rats in the Na, AG intramedullary group was significantly different from either the corresponding S or R High Na IMED group, or their high Na, AG -IV group.

**Urinary sodium output responses to iNOS inhibition.** Figure 4 shows that AG did not significantly affect the urinary sodium excretion in Dahl S rats. Likewise, except for the day 8, the urinary Na excretion in the R-High Na, AG IMED group was not significantly different from the R-High Na IMED or the R-High Na, AG-IV groups.

**Urinary volume output responses to iNOS inhibition.** Figure 5 shows that AG did not significantly affect the urinary volume output in the Dahl S groups. Likewise, except for day 8, the urinary volume output in the R-High Na, AG IMED group was not significantly different from the R-High Na IMED or the R-High Na, AG-IV groups.

**Heart rate and UNOx excretion responses to iNOS inhibition.** Table 2 shows that heart rate was not significantly affected by iNOS inhibition in either the Dahl R or S rats. Urinary nitrate + nitrite excretion, an index of whole body NO production, tended to decrease in all AG groups but the changes did not reach significance.
**Changes in thoracic aorta vasorelaxation during iNOS inhibition.** Figure 6 shows that the acetylcholine-vasorelaxation curve was significantly depressed in the S high Na group when compared to the R high Na group, suggesting endothelial dysfunction. Inhibition of iNOS had no significant effect in any of the R or S groups.

**DISCUSSION**

The major new finding in this study is that iNOS inhibition in the renal medulla increases the salt-sensitivity of the Dahl S rat but not the Dahl R rat. This indicates that NO produced by iNOS in the renal medulla normally prevents some of the increase in arterial pressure that occurs during high sodium intake in S rats. Indeed figure 1 shows that mean arterial pressure in the high sodium S rats with intramedullary AG-treatment increased more than the high sodium alone group.

In a previous study from our laboratory, mean arterial pressure increased in Dahl S rats with IV-AG treatment suggesting that NO produced by iNOS prevented some of the increase in arterial pressure that occurs in these rats during increased sodium intake (32). However, this previous study did not indicate which area of the body produced the iNOS–dependent NO. The present study suggests that during high sodium intake the renal medulla of Dahl S rats is a source of iNOS that actively produces NO thus preventing excessive increases in arterial pressure.

The previous study in our laboratory also showed that AG treatment IV made the salt-resistant R rat salt-sensitive (32). However, renal medullary infusion of AG in R rats in the present study did not affect salt-sensitivity. There are several possibilities why systemic but not intramedullary AG did not increase salt-sensitivity. First, combining the previous results with those from the present study suggests that NO produced by iNOS is
important in preventing salt-sensitive hypertension in the R rat, but the source of this NO derived from iNOS is unlikely to be in the renal medulla. This is confirmed by our preliminary studies that showed that renal medullary iNOS protein was upregulated after a one week exposure to a high Na diet but not after a two or three week high Na diet. At the end of the present study the rats had been exposed to high Na for 12 days, and iNOS protein in the medulla may not be upregulated at his time. Second, the increased pressure during systemic AG in R rats could have been caused by a direct effect on the renal cortex or by a systemic or central effect which in turn had an effect on the kidney.

To confirm our AG intramedullary studies in R rats, a highly selective iNOS inhibitor, 1400W, (13,25,29) was infused IV and into the renal medulla of R rats using the same protocol as in the present experiment, and no increase in arterial pressure occurred. Also no change occurred in the ACh vasorelaxation curve in aortic segments. This is further evidence that our AG inhibited iNOS effectively in R rats without affecting other NOS isoforms.

In S rats both systemic and intramedullary AG increased arterial pressure suggesting that part of the increase in arterial pressure during systemic AG is due to blockade of renal medullary iNOS. The arterial pressure of the S rat seems to be highly dependent on NO produced by renal medullary iNOS protein. The increase in salt-sensitivity in the S rats treated with AG occurred without any change in GFR, renal plasma flow, urine volume or urinary volume output. However, a higher arterial pressure was required to excrete the high sodium diet, indicating that a change in pressure natriuresis occurred.

Changes in renal NOS, including changes in renal iNOS, can have profound effects on renal excretory ability and thus arterial pressure(7,15,22,33). Evidence that renal
iNOS may play an important role in salt-sensitive hypertension has been provided by both biochemical and physiological studies. Messenger RNA (mRNA) for iNOS is located in renal tubular and vascular segments. The highest level of iNOS mRNA is in the medullary thick ascending limb and the inner medullary collecting duct (2,31), indicating that a decrease in medullary iNOS could increase sodium reabsorption resulting in salt-sensitive hypertension. Indeed, Mattson et al.(22) found that IV infusion of AG in Sprague-Dawley rats decreased renal medullary iNOS activity and urinary Na excretion for two days. Therefore, in the present experiment medullary iNOS inhibition in the S rat may have increased Na retention in the medullary part of the nephron thus increasing blood volume. Arterial pressure in turn would increase thus increasing Na excretion back to normal. Therefore, iNOS inhibition would result in normal Na excretion but at an elevated arterial pressure, and indeed, this occurred in the present experiment, since an increased arterial pressure in the S rat was necessary to excrete the high Na load during medullary AG infusion. These data suggest that NO produced by iNOS in the renal medulla of S rats prevents excessive increases in arterial pressure in the Dahl S rat subjected to high sodium intake.

An interesting finding in this study is that the S rats show considerable endothelial dysfunction. The release of NO by ACh during the high sodium diet significantly decreased in the aorta of S rats compared to R rats. L-NAME (18,28) but not D-NAME (18) blocks the vasorelaxation to ACh in aortic rings indicating that this response is mediated by NO. Since ACh causes the eNOS in endothelial cells to produce NO, this suggests that the release of NO by eNOS is decreased in the S rat.
Another important result that was found is that AG treatment of R rats did not significantly change the ACh vasorelaxation curve in the intramedullary and IV groups. Likewise, AG treatment of S rats also did not significantly change the ACh vasorelaxation curve in the intramedullary and IV groups. Importantly, this likely shows that iNOS inhibition did not affect aortic eNOS production of NO in the R and S rats.

Even though the present study is the first to examine the functional role of renal medullary iNOS in Dahl salt-sensitive hypertension, others have studied other aspects of iNOS in salt-sensitive hypertension. Molecular genetic linkage analysis showed that the locus for iNOS but not constitutive NOS (12) cosegregates with blood pressure in Dahl S rats. In a follow-up study Deng et al. (11) showed that abnormalities in the chromosome containing the NOS II gene in the S rat were not primarily responsible for the salt-sensitive hypertension but concluded that NOS II could have secondary effects that caused the hypertension. Ikeda et al. (16) found that renal NOS I activity decreased but NOS II and III did not change after 4 weeks of a high Na diet in Dahl S/Iwai rats, but the interpretation of these results are complicated by another study which showed that 3 weeks of high Na intake in Dahl S rats caused renal damage (9).

Other investigators studied the functional role of iNOS in arterial pressure regulation. Hypertension was prevented in S rats on high sodium intake by the administration of L-arginine (8,15), but this blood pressure lowering effect of L-arginine in the S rat was prevented by infusion of dexamethasone, a nonselective inhibitor of iNOS activity(8). Rudd et al. (29) showed that selective systemic iNOS inhibition in Dahl R and S rats on high Na intake with an IV infusion of AMT caused an increase in systolic pressure. Dr. Baylis’ laboratory (5) gave AG once daily by gavage at a rate of 10.4
mg/kg/h to Sprague-Dawley rats on a 6% Na diet for 14 days. In this study arterial pressure did not increase, cerebellar NOS activity did not change, and NOS activity from the soluble fraction of the renal cortex and medulla (ie iNOS plus nNOS) did not change. This dose of AG decreased UNOx and decreased the hypotensive effect of LPS suggesting that iNOS was inhibited (5). Dr. Mattson’s laboratory (22) used a systemic dose of 10 mg/kg/h AG in Sprague-Dawley rats on a 4 % Na diet and found a significant decrease in renal medullary iNOS activity and arterial pressure increased. Since the kidney receives about 25% of cardiac output we wanted to give at least 25% of Mattson’s systemic dose. We gave slightly more than this for safety. In addition, this amount infused into the renal medulla, which has a much lower flow than the total renal flow, should be more than sufficient to inhibit iNOS. Our previous studies with systemic iNOS inhibition with IV administration of AG (32) also caused an increase in arterial pressure in Dahl R and S rats on high sodium intake. The above studies show that iNOS may be an important component of salt-sensitive hypertension in the S rat, but the specific functional role of medullary iNOS has been clarified by the present study.

**Selectivity of aminoguanidine.** In the present study acetylcholine vasorelaxation was unaffected in the AG intramedullary group and the AG-IV group in both R and S rats when compared to the high sodium alone group. Therefore, this indicates that eNOS was not affected by the AG infusion. This agrees with other previous studies that showed that at low concentrations, which we used in this study, AG does not block eNOS, since it has a Ki that is 32-52 fold less for iNOS than for eNOS (24). In the present experiment, AG did not significantly affect the renal plasma flow which also suggests that eNOS activity was unaffected by AG. Other groups have also shown that AG can selectively inhibit
iNOS without affecting Ca-dependent NOS activity. AG infusion into SD rats for 40
min., at a rate 20 times the rate we used in the present experiment, caused no change in
arterial pressure or renal cortical blood flow (22). A 6-day IV infusion of AG at 10
mg/kg/h decreased renal medullary Ca-independent NOS activity without affecting Ca-
dependent activity (22).

Other studies have shown that AG at low doses does not affect nNOS. In a
previous study in our laboratory cerebellar Ca-dependent NOS activity, which is mainly
nNOS, was unaffected by intravenous AG infusion (33). However, there is a possibility
that the reason that this cerebellar NOS activity was unaffected was that AG did not cross
the blood-brain barrier. Yet, systemically administered AG caused significant
cerebroprotection in rats after brain injury due to either percussion or ischemia (10,37),
indicating that AG crosses the blood-brain barrier. In addition, a 6 day IV infusion of AG
in rats did not affect Ca-dependent NOS activity in the renal medulla (22).

Renal medullary infusion technique. Several studies have shown that the renal
medullary interstitial infusion technique is a powerful tool for assessing the role of the
renal medulla. However, proper controls must be used to eliminate potential effects from
systemic overflow of the infused substances. Initial validation of this technique showed
that renal medullary infusion of $^{14}$C-clentiazem, an analogue of diltiazem, resulted in
92% of the radioactive counts being localized in the renal medulla of the infused kidney
(17). Functional validation studies showed that renal medullary infusion of L-NAME
causde a selective decrease in medullary flow without affecting cortical blood flow or
GFR (23). To determine the effects of maximum systemic overflow of AG in the present
study, 100% of the renal medullary dose of AG was infused in the IV group. When
compared to the high Na group, the high Na, AG-IV group had no significant differences in arterial pressure, renal hemodynamics, renal excretory function or acetylcholine-induced vasorelaxation of the aorta. This suggests that the cardiovascular-renal changes that occur following renal medullary infusion of AG are due to effects in the renal medulla and not caused by overflow and recirculation of AG.

In summary, iNOS inhibition by AG infusion into the renal medullary interstitial space caused an increase in salt-sensitivity of Dahl S rat but not Dahl R rats. During high sodium intake in S rats, AG increased arterial pressure but caused little change in heart rate, renal hemodynamics and renal volume or sodium excretion. AG did not affect acetylcholine-induced vasorelaxation of the aorta and did not significantly change urinary nitrate+nitrite excretion. These data suggest that during high sodium intake, NO produced by iNOS in the renal medulla helps to prevent excessive increases in arterial pressure in Dahl S rats but not in Dahl R rats. NO produced by iNOS is important in preventing salt-sensitive hypertension in the R rat, but this iNOS is not likely to be located in the renal medulla.

ACKNOWLEDGEMENTS

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FIGURE LEGENDS

**Figure 1.** Mean arterial pressure responses in Dahl salt-sensitive (S) and salt-resistant (R) rats. Inducible nitric oxide synthase (iNOS) inhibition was achieved with aminoguanidine (AG). *P<0.05* when comparing High Na intramedullary (IMED) with High Na, AG-IMED. † P<0.05 High Na, AG-IMED compared with High Na, AG-IV values in S or R rats at the same experimental time. There were no significant differences between the High Na IMED and High Na, AG-IV groups.

**Figure 2.** Glomerular filtration rate (GFR) responses to iNOS inhibition in Dahl S and R rats. There were no significant changes in any of the experimental data points.

**Figure 3.** Effective renal plasma flow (ERPF) responses to iNOS inhibition in Dahl S and R rats. There were no significant changes in any of the experimental data points.

**Figure 4.** Urinary Na output responses to iNOS inhibition in Dahl S and R rats. *P<0.05* when comparing High Na-IMED with High Na, AG-IMED. † P<0.05 High Na, AG-IMED compared with High Na, AG-IV values in S or R rats at the same experimental time. There were no significant differences between the High Na, AG-IMED and the High Na, AG-IV groups in S and R rats.

**Figure 5.** Urinary volume output responses to iNOS inhibition in Dahl S and S rat. *P<0.05* when comparing High Na-IMED with High Na, AG-IMED. † P<0.05 High Na,
AG-IMED compared with High Na, AG-IV values in S or R rats at the same experimental time. There were no significant differences between the High Na, AG-IMED and the High Na, AG-IV groups in S and R rats.

**Figure 6.** Thoracic aorta segment relaxation responses to acetylcholine (ACH) in control and iNOS-inhibited Dahl S and R rats. †P<0.05 when comparing High Na-IMED Dahl S and R rats at the same ACh concentration. There were no significant effects of AG on vasorelaxation in S or R rats.
Table 1. Control Values

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<th>High Na, AG (IMED)</th>
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<tr>
<td>MAP-R rats, mmHg</td>
<td>101 ± 2</td>
<td>102 ± 1</td>
<td>98 ± 1</td>
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<tr>
<td>MAP-S rats, mmHg</td>
<td>129 ± 2</td>
<td>121 ± 5</td>
<td>119 ± 3</td>
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<td>GFR-R rats, ml/min</td>
<td>2.0 ± 0.2</td>
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<td>GFR-S rats, ml/min</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.1</td>
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<td>ERPF-R rats, ml/min</td>
<td>6.2 ± 0.5</td>
<td>6.9 ± 0.4</td>
<td>7.8 ± 0.5</td>
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<td>ERPF-S rats, ml/min</td>
<td>4.5 ± 0.5</td>
<td>4.4 ± 0.4</td>
<td>6.1 ± 0.3 †</td>
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All data are mean ± SE. MAP, mean arterial pressure (mmHg); GFR, glomerular filtration rate (ml/min); ERPF, effective renal plasma flow (ml/min); IMED, renal intramedullary infusion; AG, aminoguanidine hemisulfate salt; IV, intravenous infusion; R, Dahl resistant; S, Dahl sensitive. † p< 0.05 High Na, AG (IV) values compared with High Na, AG (IMED) values.

There were no significant changes between the High Na (IMED) and the High Na, AG (IMED) groups in either R or S rats.

Table 2. Responses of Heart Rate (HR) and Urinary Nitrate+Nitrite (UNOx) Excretion

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<td>HR, beats/min</td>
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<tr>
<td>R rats-day 5 control</td>
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<td>423 ± 5</td>
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<td>R rats-day 10</td>
<td>395 ± 5</td>
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<td>S rats- day 5 control</td>
<td>421 ± 12</td>
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<td>S rats-day 10</td>
<td>435 ± 11</td>
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<td>UNOx, nmol/d</td>
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<td>R rats- day 5 control</td>
<td>10696 ± 2166</td>
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</tbody>
</table>

All data are mean ± SE. IMED, intramedullary infusion; AG, aminoguanidine hemisulfate salt; IV, intravenous infusion; R, Dahl resistant; S, Dahl sensitive. There were no significant changes in the High Na (IMED), High Na, AG (IMED), and High Na, AG (IV) groups on day 5 of control or on day 10. Day 5 of control is the last day of the control period; Day 10, is the last day of the experimental period.
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5

The figure shows the urinary volume output (ml/day) over 10 days for Dahl Sensitive and Dahl Resistant rats in two conditions: Control and iNOS Inhib. The graphs depict the effect of high sodium (Hi Na) alone, high sodium with AG (Hi Na + AG), and high sodium with AG administered IMED and IV on urinary output.

- **Dahl Sensitive**
  - Hi Na + AG IV
  - Hi Na + AG IMED
  - Hi Na alone IMED

- **Dahl Resistant**
  - Hi Na + AG IV
  - Hi Na + AG IMED

The graphs indicate a trend where the urinary output increases over time, with notable differences between the control and iNOS inhib conditions, especially in Dahl Sensitive rats.
Figure 6