Role of Abnormal Nitric Oxide Systems in Salt-Sensitive Hypertension

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A large percentage of human hypertensive patients are salt sensitive, referring to the dependence of hypertension on sodium intake, but the cause of the salt sensitivity is not known. Although several mechanisms may contribute to salt-sensitive hypertension, the nitric oxide (NO) system appears to play a major role. Studies in humans and Dahl salt-sensitive (S) rats indicate that NO production is decreased during hypertension. Intravenous L-arginine infusion in Dahl S rats increases NO production and prevents salt-sensitive hypertension. In the Dahl salt-resistant (R) rat, NO production by both inducible NO synthase (iNOS) and neuronal NOS (nNOS) help to prevent salt-sensitive hypertension. Experimental evidence is summarized, indicating that the Dahl S rat has a deficient production of NO by nNOS, although NO production by iNOS appears to moderately decrease salt sensitivity. Other evidence about the importance of NO in salt-sensitive hypertension is reviewed, including the role of the renal NO system.

Key Words: Mean arterial pressure, Dahl rats, inducible nitric oxide synthase, neuronal nitric oxide synthase, pressure natriuresis.
is decreased in the Dahl S rat. Therefore, although several mechanisms may contribute to salt sensitivity in the Dahl rat, the NO system seems to play a major role.

**Role of NO and NOS Isoforms in Salt-Sensitive Hypertension**

**Decreased NO Production Can Cause Salt-Sensitive Hypertension**

A recent study in humans showed that agonist-induced release of NO was lower in salt-sensitive essential hypertensives compared to salt-resistant essential hypertensives. Because NO aids the kidney in the excretion of sodium, a decrease in NO could cause sodium retention. Administration of nonpressor doses of $N\textsuperscript{0}$-nitro-L-arginine methyl ester (L-NAME) caused decreases in urinary sodium excretion in dogs. Studies in our laboratory and in others have shown that in Sprague-Dawley and Dahl R rats elevated sodium intake normally increases UNOx, an index of NO production, and enhances the renal hemodynamic response to NO synthesis inhibition. However, this increase in NO production during increased sodium intake is blunted in the Dahl S rat, and therefore, decrements in NO in these rats could lead to sodium retention and thus salt-sensitive hypertension.

**L-Arginine Administration Can Prevent Salt-Sensitive Hypertension**

Chen and Sanders and Hu and Manning have shown that salt-sensitive hypertension in the Dahl S/Rapp rats was completely prevented by parental and oral administration of L-arginine but not d-arginine. Their data also suggested that an increase in dietary sodium chloride increased NO production in salt-resistant (R) rats but not in S rats by using $N\textsuperscript{G}$-monomethyl-L-arginine (L-NMMA) as a probe to estimate the NO production. Patel et al. reported that long-term L-arginine administration normalized short-term pressure natriuresis in anesthetized S rats. These studies supported the idea that salt-sensitive hypertension might be a state of deficient NO production. However, the NO production measurements in these studies were made indirectly using L-NMMA as a probe or from anesthetized rats over a short period. To determine whether L-arginine administration in S rats will prevent any hypertensive shift in the long-term pressure natriuresis relationship by increasing NO production, a study was designed in our laboratory to determine the role of NO in the development of hypertension and the regulation of long-term pressure natriuresis relationship in salt-induced hypertension. We examined the antihypertensive effects of continuous intravenous infusion of the NO precursor L-arginine in R and S rats that received low, normal, and high sodium intakes, sequentially. Mean arterial pressure was continuously monitored 21 h a day through indwelling arterial catheters over a 16-day period. In addition, UNOx were measured to quantitate the whole body NO production in response to the changes in sodium intake.

The top panel of Fig. 1 shows the mean arterial pressure (MAP) response to low, normal, or high sodium intake in control S and R rats without any L-arginine infused. The bottom panel shows S and R rats that were infused intravenously with 4 mg/kg/min of L-arginine. In Dahl S rats in this study, UNOx was significantly lower in S high sodium rats compared to R high sodium rats, and the L-arginine infusion increased UNOx in S high sodium rats to a value not different from R high sodium rats.

The top panel of Fig. 2 shows the salt-loading pressure natriuresis relationship in control S and R rats without any L-arginine infused, and MAP of the S rats was salt sensitive. The bottom panel shows S and R rats that were infused intravenously with 4 mg/kg/min of L-arginine, and the increases in salt sensitivity in the S rats was prevented. These data suggest that a deficiency in production of NO in Dahl S rats contributes to their high salt sensitivity.
Decreased Renal NO Production Can Cause Salt-Sensitive Hypertension

That renal NO production is important in the regulation of arterial pressure and renal hemodynamics has been shown by several investigators. Acute infusion of the NO synthesis inhibitor L-NAME directly into the renal artery of dogs caused renal vasoconstriction, and long-term renal artery infusion of L-NAME caused hypertension. The renal production of NO depends on one or more of the isoforms of NO synthase (NOS), which are neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). These NOS isoforms are located throughout all three parenchymal zones in the kidney. There are several isoforms of iNOS including macrophage (mac), vascular smooth muscle (vsm), and hepatic isoforms, and the kidney contains vsmNOS, located in blood vessels, and macNOS, located along the tubules. Selective inhibition of iNOS or nNOS in the renal medulla of Sprague-Dawley (SD) rats for 6 days caused hypertension during high sodium intake, but not during normal sodium intake. Also, NO synthesis inhibition decreases renal blood flow more in rats on high sodium intake than on low sodium intake. Therefore, specific isoforms of NOS in the kidney can have profound effects on renal function and salt sensitivity. However, the mechanisms by which the renal NOSs accomplish these changes are not completely understood.

Role of iNOS in Salt-Sensitive Hypertension

Both biochemical and functional studies suggest that renal iNOS may play an important role in salt-sensitive hypertension. Messenger RNA (mRNA) for iNOS has been found in renal tubular and vascular segments. The highest level of iNOS mRNA has been found in the medullary thick ascending limb and the inner medullary collecting duct suggesting that a decrease in medullary iNOS expression could lead to increased sodium reabsorption and salt-sensitive hypertension. Recently, molecular genetic linkage analysis showed that the locus for iNOS, but not eNOS, cosegregates with blood pressure in S rats. However, cosegregation of arterial pressure with a candidate gene is not proof of cause and effect; therefore, experimental studies are needed to determine whether iNOS plays an important role in the salt sensitivity of S rats.

One recent study suggested that iNOS may play a role in salt-sensitive hypertension. Chen and Sanders found that the blood pressure-lowering effect of L-arginine in the S rat on high sodium chloride intake was prevented by infusion of dexamethasone, which is believed to inhibit induction of iNOS activity. However, dexamethasone is not a selective inhibitor of iNOS, and the role of iNOS cannot be determined by measuring changes in UNOx, as nitrate and nitrite in the urine originate from both renal and extrarenal sources and can come from any of the isoforms.

Mattson and Higgins recently showed that medullary iNOS protein concentration increased markedly during high sodium intake in SD rats. In another study, unilaterally nephrectomized SD rats maintained on high sodium diet received a 6-day intravenous infusion of aminoguanidine, a selective inhibitor of iNOS, and mean arterial pressure increased 11 mm Hg; NOS activity in the renal medulla decreased 49%, but cerebellum NOS (presumably nNOS) was not affected. These experiments support a role for renal medullary iNOS in the control of arterial pressure, possibly through renal tubular effects in the SD rat and a similar role of iNOS may exist in the Dahl rat.

A recent study was performed in our laboratory to determine the role of iNOS in Dahl salt-sensitive hypertension. Dahl R and S rats, equipped with indwelling arterial and venous catheters, were subjected to high (20.6 mmol/day) sodium intake, and selective iNOS inhibition was achieved with intravenous aminoguanidine at 12.3 mg/kg/h. As seen in Fig. 3, after 5 days of aminoguanidine, MAP increased to 121 ± 3% control in the R-high sodium aminoguanidine rats compared to 98 ± 1% control (P < .05) in the R-high sodium alone rats, and S-high sodium rats increased their arterial pressure to 123 ± 3% control compared to 110 ± 2% control (P < .05) in S-high sodium alone rats. Therefore, iNOS inhibition in the S rat...
moderately increased salt sensitivity. Aminoguanidine caused no significant changes in renal hemodynamics, urinary sodium or water excretion, plasma renin activity, or cerebellar calcium-dependent NOS activity.

Another NOS inhibitor that selectively inhibits iNOS is of 2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT), and it has a $K_I$ of 4.2 nmol/L, which is similar to that of aminoguanidine, and is up to 40 times more selective for iNOS than for nNOS or eNOS. A recent study with an AMT infusion of 300 nmol/h in Dahl R rats showed that after 5 days of infusion of AMT and high sodium intake, systolic pressure increased 20%, and the MAP in R rats on high sodium and aminoguanidine in the present study increased 21%. The AMT infusion probably did not inhibit eNOS, as the dilatory responses of mesenteric arteries to methacholine were unchanged. Therefore, our study and the AMT study appear to have selectively blocked iNOS. The data suggest that nitric oxide produced by iNOS normally helps to prevent salt-sensitive hypertension in the Dahl R rat and decreases salt sensitivity in the Dahl S rat.

**Role of nNOS in Salt-Sensitive Hypertension**

Several laboratories using techniques including immunohistochemistry, reverse transcription polymerase chain reaction in microdissected renal vessels and tubules, and in situ hybridization have demonstrated the presence of nNOS protein and mRNA in the inner and outer medullary collecting ducts, glomerulus, macula densa, vasa recta, arcuate artery, and renal nerves. Functionally, nNOS blunts the tubuloglomerular feedback response of the afferent arteriole and mediates the macula densa control of renin secretion. However, the effect of sodium intake on nNOS synthesis and expression in different renal parenchymal zones is controversial. Messenger RNA for nNOS has been shown to increase in the renal cortex during low sodium intake. Yet, other investigators showed that nNOS protein increased markedly in the inner medulla during increased sodium intake. In support of the latter finding, renal medullary infusion of 7-nitroindazole (7NI), a selective inhibitor of nNOS, decreased medullary nNOS activity by 37% and increased arterial pressure over a 6-day period in SD rats on high sodium intake but not on normal sodium intake. This suggests that medullary nNOS may enhance renal sodium excretion and thus help to prevent salt-loading hypertension.

The role of nNOS regulation of the sodium sensitivity of arterial pressure and the mechanisms that contribute to these changes in salt-sensitive hypertension in Dahl rats have not been well understood and was the focus of a recent study in our laboratory. Dahl R and S rats/Rapp strain of 7 to 8 weeks of age with indwelling arterial and venous catheters were subjected to high (20.6 mmol/day) sodium intake beginning 2 days before the start of the control period. Measurements were made during a 5-day control period followed by a 5-day period of nNOS inhibition with intravenous 7NI (1.67 mg/kg/h) or vehicle infusion. As seen in Fig. 4, after 5 days of 7NI, MAP increased to 120% of control in the R-high sodium, 7NI rats compared to 98% of control ($P < .05$) in the R-high sodium alone rats. The data demonstrate that the highly salt-resistant Dahl R rat became salt sensitive during nNOS inhibition with 7NI. However, the arterial pressure of the S rat was not affected by 7NI. This suggests that NO produced by nNOS in the Dahl R rat normally helps to prevent salt-sensitive hypertension and that low functional levels of nNOS in the S rat may contribute to its salt sensitivity.

**The Regulation of NOS Isoforms During Increased Sodium Intake**

The mechanisms by which NO production is stimulated with increased sodium intake are not clear, and few studies have been performed on the changes in NOS isoforms in the kidney during changes in dietary sodium intake. Increasing sodium intake in SD rats caused large increases in

![FIG. 3. Mean arterial pressure responses in Dahl salt-resistant and salt-sensitive rats. Inducible nitric oxide synthase (iNOS) inhibition was achieved with aminoguanidine. *P < .05 when comparing salt-resistant high sodium, aminoguanidine rats with salt-resistant high sodium rats at the same experimental time. The same statistics apply to salt-sensitive rats. The arterial pressure on day 10 in the salt-sensitive high sodium alone rats was 40 mm Hg higher than the control pressure of the salt-sensitive low sodium alone group demonstrating the salt sensitivity of the salt-sensitive rats. Data is redrawn from a previous publication (Ref. 40).](https://academic.oup.com/ajh/article-abstract/14/S3/68S/205757)
eNOS, iNOS, and nNOS protein, particularly in the renal inner medulla. During increased sodium intake in SD rats, eNOS and iNOS mRNA were unchanged in the cortex, and nNOS mRNA decreased in the cortex but was unchanged in the inner medulla. The eNOS and iNOS mRNA levels in the inner medulla were not reported in this study. During high sodium intake for 4 weeks, renal nNOS activity decreased, and eNOS and iNOS activities did not change in the Dahl Iwai salt-sensitive rat compared to the R rat. However, this study analyzed NOS activity in the whole kidney; therefore, changes in the medulla may have been overlooked using this approach. Another factor complicating interpretation of the above study is that a recent study has shown that 3 weeks of high sodium intake in S rats caused moderate renal damage including vascular and glomerular damage, both of which could affect NO production. The role of the renal NOS isoforms have not been elucidated.

### Conclusion

Although several mechanisms may contribute to salt-sensitive hypertension, the NO system appears to play a major role. Studies in humans and experimental animals indicate that NO production is decreased during hypertension. That this decrease in NO is important in salt-sensitive hypertension was confirmed by studies showing that L-arginine administration increased NO production and prevented hypertension in Dahl S rats during high sodium intake. In the Dahl R rat NO production by both iNOS and nNOS help to prevent salt-sensitive hypertension. In the Dahl S rat, there may be a deficient production of NO by nNOS, and NO production by iNOS moderately decreases salt sensitivity.

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