Mechanisms of salt-sensitive hypertension: role of inducible nitric oxide synthase

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Tan, Dunnyong Y., Shumei Meng, Garrick W. Cason, and R. Davis Manning Jr. Mechanisms of salt-sensitive hypertension: role of inducible nitric oxide synthase. Am J Physiol Regulatory Integrative Comp Physiol 279: R2297–R2303, 2000.—The goal of this study was to determine the role of inducible nitric oxide synthase (iNOS) in the arterial pressure, renal hemodynamics, renal excretory, and hormonal changes that occur in Dahl/Rapp salt-resistant (R) and salt-sensitive (S) rats during changes in Na intake. Thirty-two R and S rats, equipped with indwelling arterial and venous catheters, were subjected to low (0.87 mmol/day) or high (20.6 mmol/day) Na intake, and selective iNOS inhibition was achieved with intravenous aminoguanidine (AG, 12.3 mg·kg⁻¹·h⁻¹). After 5 days of AG, mean arterial pressure increased to 121 ± 3% control in the R-high Na AG rats compared with 98 ± 1% control (P < 0.05) in the R-high Na alone rats, and S-high Na rats increased their arterial pressure to 123 ± 3% control compared with 110 ± 2% control (P < 0.05) in S-high Na alone rats. AG caused no significant changes in renal hemodynamics, urinary Na or H₂O excretion, plasma renin activity, or cerebellar Ca-dependent NOS activity. 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.

A LARGE POPULATION OF HUMAN hypertensives is “salt sensitive,” which refers to the dependence of blood pressure on Na intake, but the cause of the salt sensitivity is not known. A recent study indicated that the forearm circulation of salt-sensitive humans release less nitric oxide (NO) during NO agonist administration compared with salt-resistant essential hypertensives (10). Studies in our laboratory and others (4, 12) showed that NO production is decreased in Dahl salt-sensitive (S) rats during high Na intake compared with Dahl salt-resistant (R) rats. Increasing NO production with L-arginine administration in Dahl S rats prevented salt-sensitive hypertension (4, 12). Therefore, a deficiency in NO production may be partly responsible for salt-sensitive hypertension in humans and Dahl S rats. We recently showed that NO produced by neuro-

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A Harvard apparatus syringe pump (South Natick, MA) infused saline solutions through a 0.22-μm filter (Cathivex, Millipore, Bedford, MA). The arterial catheter was filled with 1,000 U/ml heparin and connected to a Cole pressure transducer (Lakewood, CO) and in turn to a pressure amplifier. Heart rate and arterial pressure were determined from pulsatile arterial pressure signals sent to a digital computer at 500 Hz for 4 s of each minute throughout the entire 24-h period.

Water intake and urinary volume output were measured daily. Urine and plasma Na concentrations were determined by flame photometry and plasma renin activity (PRA) by radioimmunoassay. Urinary nitrate plus nitrite excretion (UNOx) was determined using the Griess reaction and nitrate reductase from Escherichia coli as we have done before (12).

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were determined as before (21) by measuring the radioactivity and aminohippurate concentration of a 4-h fasted plasma sample after a 24-h period of intravenous infusion of [125I]iothalamate (Glofil, Isotec Diagnostics, Friendswood, TX) and aminohippurate Na.

ROS activity was determined by measuring the rate of formation of radiolabeled citrulline from l-arginine based on the technique of Brown et al. (3). NOS activity was measured at the end of the 5-day iNOS inhibition period for R-high Na AG rats (n = 3), R-high Na alone rats (n = 3), S-high Na AG rats (n = 3), and S-high Na alone rats (n = 4). Rats were anesthetized with isoflurane, and the cerebellum was surgically removed and snap-frozen in liquid nitrogen. The same day, the excised cerebellum was homogenized in 20% (wt/vol) HEPES buffer, pH 7.5, containing 100 mM EGTA added to determine Ca-independent activity. Ca-activated samples were processed without Ca/calmodulin and with 4 mM FAD, 1 mM NADPH, 1 mM CaCl2, 300 nM calmodulin, 100 μM BH4, and 80 nM [3H]arginine. The reaction was stopped by 1 ml of 20 mM HEPES stop buffer, pH 5.5, containing 2 mM EGTA. Then, 1 ml of the solution was applied to a Dowex AG 50W-X8 column (Na+ form) and radioactivity of the eluent was determined on a liquid scintillation counter. Parallel samples were processed without Ca/calmodulin and with 4 mM EGTA added to determine Ca-independent activity. Ca-dependent activity was determined by subtracting Ca-independent activity from total activity.

Experimental protocols. The following four groups of rats were studied: Dahl R-low Na AG (n = 7), Dahl R-high Na AG (n = 6), Dahl R-high Na AG (n = 8), and Dahl S-high Na AG (n = 11). Rats were allowed to recover for 7 days before the control period began. The R and S low Na groups received a low Na intake throughout the entire recovery period and experiment. The R and S high Na groups received a low Na intake for the first 5 days of the recovery period and high Na intake for the last 2 days of recovery and the entire experimental period. Immediately after the recovery period, data were collected during a 5-day control period followed by a 5-day period of either vehicle infusion or iNOS inhibition with intravenous infusion of AG at 12.3 mg·kg⁻¹·h⁻¹. All rats were fed a low-Na food (Teklab Test Diets, Madison, WI) throughout the experiment, and a low Na intake of 0.87 mmol/day was achieved by intravenous infusion of 15 ml/day of 0.3% NaCl plus ingestion of 0.10 mmol/day of the low-Na food. Some of the rats received a high Na intake of 20.6 mmol/day of Na (15 ml/day iv of 8% NaCl plus the low Na food).

RESULTS

Arterial pressure responses to iNOS inhibition. Figure 1, top, shows that during iNOS inhibition, mean arterial pressure (MAP) of the Dahl R rats increased significantly during high Na intake and, by day 10,
MAP reached a value of $121 \pm 3\%$ control and $98 \pm 1\%$ control ($P < 0.05$) in R-high Na alone rats. MAP of R-low Na AG rats did not significantly increase compared with R rats on low Na alone. Therefore, the R rat became highly salt sensitive during iNOS inhibition. Figure 1, bottom, shows that, by day 10, MAP increased to $123 \pm 3\%$ control in the S-high Na AG rats and $110 \pm 2\%$ control in the S Na alone groups ($P < 0.05$). Data from R and S Na alone groups have been published (21) and are shown here for comparison purposes.

Table 1 shows absolute values of MAP, GFR, and ERPF on day 5 of the control period and day 10, which is after 5 days of AG infusion, and indicates that MAP was elevated in all S rats on high Na. The absolute changes in MAP also indicate that the Dahl R rat became salt sensitive during AG infusion, and MAP of the S rat on high Na and AG significantly increased. However, the increase in MAP in the S rat on AG was likely caused by the combination of the effects of AG and the independent effects of a high Na diet. The normalized values of MAP, GFR, and ERPF in Figs. 1–3 are expressed relative to the absolute values of these data on day 5 of the control period as shown in Table 1.

GFR and ERPF responses to iNOS inhibition. Figures 2 and 3, top and bottom, show that GFR and ERPF in all groups of rats did not significantly change during the iNOS inhibition. Neither the R- or S-high Na AG groups were significantly different from either the R- or S-high Na alone groups, respectively, or their corresponding low Na-AG groups. As indicated above, the absolute values of GFR and ERPF are shown in Table 1.

Urinary Na output and plasma Na concentration responses to iNOS inhibition. Figure 4 shows that urinary Na excretion in the R-high Na AG rats was not significantly different from the Na excretion of the R rats on high Na intake alone, except on the first day of AG infusion. Likewise, AG did not significantly affect the urinary Na excretion of the R rats on low Na intake. In a similar way, AG had no significant effect on Na excretion of S rats on low or high Na intake.

Plasma Na concentration changes were measured in some of the rats on day 9, and there were no significant changes in any of the groups. Na concentrations (mmol/l) at this time were Dahl R-low Na, 142.0 ± 0.4 ($n = 4$); Dahl R-high Na, 141.8 ± 0.9 ($n = 5$); Dahl R-high Na AG, 143.9 ± 1.8 ($n = 4$); Dahl S-low Na AG, 142.1 ± 0.5 ($n = 3$); Dahl S-high Na, 141.1 ± 0.6 ($n = 6$); and Dahl S-high Na AG, 143.7 ± 1.2 ($n = 5$).

Urinary volume output responses to iNOS inhibition. Figure 5 shows that AG did not significantly affect urinary volume output in either the R or S groups on either high or low Na intake compared with their respective timed control Na alone groups. Urine volume was significantly higher in the high Na R and S groups than in the low Na groups as expected.

UNOx excretion responses to iNOS inhibition. Figure 6 shows that UNOx, an index of whole body NO production (12), significantly decreased in the R-high Na AG rats ($P < 0.05$), suggesting that iNOS is partly responsible for the increased NO production during high Na intake. This enhanced NO production in R rats is evident, because on day 5 of the control period, UNOx was 4,790 ± 801 nmol/day in R-low Na alone rats and 10,059 ± 1,416 nmol/day in R-high Na alone rats ($P < 0.05$). UNOx of the S rats did not significantly increase when Na intake was increased, nor did it significantly decrease during AG infusion.
Calcium-dependent NOS activity, heart rate, and PRA responses to iNOS inhibition. Table 2 shows that there were no significant effects of AG on cerebellar Ca-dependent NOS activity during high Na intake or on heart rate or PRA in the R or S rats during high or low Na intake compared with their respective time-controlled Na alone groups. Cerebellar Ca-independent NOS activity during high Na intake was at undetectable levels in R and S rats. As expected, PRA of R and S rats on low Na intake was higher than during high Na intake. PRA during low Na intake averaged 3.2 ± 0.3 ng·ml⁻¹·h⁻¹ in R-low Na alone rats and 2.0 ± 0.3 ng·ml⁻¹·h⁻¹ in S-low Na alone rats (P < 0.05).

DISCUSSION

The major new finding in this study is that iNOS inhibition makes the normally salt-resistant Dahl R rat salt sensitive without any effect on renal plasma flow, GFR, or PRA. This fact was confirmed by the increase in MAP in the R-high Na group during intravenous infusion of the selective iNOS inhibitor AG and the lack of increase in MAP in both the R rats on high Na alone and R rats on low Na plus AG. MAP in the S rats on high Na intake significantly increased during iNOS inhibition. However, part of the increase in MAP in these S rats was due to the effects of high Na intake, because the arterial pressure in the high Na alone group significantly increased. Inhibition of iNOS also did not significantly change urinary Na excretion or urinary volume in the R rats, but a higher MAP was required to excrete this Na, indicating that a change in renal pressure natriuresis occurred. Our laboratory also found a depressed pressure-natriuresis relationship in the Dahl S rat in a previous study (12).

Recent studies from our laboratory have shown that NO production, as estimated by UNOx, was decreased in outbred Dahl S rats on high Na intake (12) compared with R rats, and the UNOx data in Fig. 6 confirm that UNOx is decreased in the inbred Dahl S/Rapp strain used in the present study. However, it has not been clear what role iNOS played in this increased NO production in the R rat on high Na intake. Infusion of the selective iNOS inhibitor AG into R-high Na rats caused a significant decrease in UNOx, and salt sensitivity increased. During AG infusion, the UNOx of the R-high Na rat decreased to levels close to that of the S-high Na rat, suggesting that NO produced by iNOS may be important in preventing salt-sensitive hypertension in the R rat.

Several investigators have studied the role of iNOS in salt-sensitive hypertension. Deng and Rapp (9) in...
Molecular genetic linkage analysis showed that the locus for iNOS but not constitutive NOS cosegregates with blood pressure in S rats. Deng (8) subsequently showed that defects in the chromosome containing the NOS II gene in the S rat could not cause the salt-sensitive hypertension directly, but concluded that NOS II could have secondary effects leading to hypertension. Ikeda et al. (13) showed that renal NOS I activity decreased but NOS II and III were unchanged after 4 wk of high Na intake in Dahl S/Iwai rats; these results are complicated by another study that showed that 3 wk of high Na intake in Dahl S rats caused renal damage (6). On the other hand, Mattson et al. (16, 17) showed that in SD rats on high Na intake, medullary iNOS protein concentration increased markedly, and a 6-day intravenous infusion of AG, a selective inhibitor of iNOS, increased MAP to hypertensive levels and decreased renal medullary Ca-independent but not Ca-dependent NOS activity. Indeed, Mattson et al. (17) found that intravenous infusion of AG in SD rats caused a decrease in renal medullary iNOS activity and a decrease in urinary Na excretion for 2 days. Therefore, in the present experi-

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Na intake caused an increase in systolic pressure. In contrast, during AG infusion in the present experiment, MAP increased in R and S rats on high Na but not in S rats on low Na intake.

The increase in salt sensitivity in R rats during iNOS inhibition could have been due to several factors. First, increased blood angiotensin II concentration can cause an increase in salt sensitivity, but it is unlikely that this occurred, because PRA was not affected by the AG infusion. Second, renal iNOS activity may have decreased during AG infusion.

Changes in renal NOS, including changes in renal iNOS, can have profound effects on renal excretory ability (4, 12, 17, 21). Both biochemical and functional studies suggest that renal iNOS may play an important role in salt-sensitive hypertension. 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 (1, 20), suggesting that a decrease in medullary iNOS could lead to increased Na reabsorption and salt-sensitive hypertension. Indeed, Mattson et al. (17) found that intravenous infusion of AG in SD rats caused a decrease in renal medullary iNOS activity and a decrease in urinary Na excretion for 2 days. Therefore, in the present experi-

\[ \text{Fig. 5. Urinary volume output responses to iNOS inhibition in Dahl R and S rats. AG caused no significant changes in any group. Data from R and S Na alone groups have been published (21) and are shown here for comparison purposes.} \]

\[ \text{Fig. 6. Urinary nitrate + nitrite excretion responses to iNOS inhibition in Dahl R and S rats.} \]

\[ \text{*P < 0.05 when comparing R-low Na AG rats to R-low Na alone rats. Data from R and S Na alone groups have been published (21) and are shown here for comparison purposes.} \]
ment, medullary iNOS inhibition in the R rat may have increased Na retention in the distal part of the nephron, thus increasing blood volume. Arterial pressure would thus increase, which would elevate Na excretion back to normal. Therefore, iNOS inhibition would result in normal Na excretion but at the expense of an elevated arterial pressure, and indeed, this occurred in the present experiment, because an increased arterial pressure was necessary in the R rat to excrete the high Na load during AG infusion. This shifted pressure-natriuresis relationship was found in S rats previously (12).

In Dahl R rats on high Na and AG, UNOx significantly decreased during the iNOS inhibition period. We were surprised that UNOx did not significantly decrease in Dahl S rats on high Na and AG. The reason for the lack of a significant decrease in UNOx in the S-high Na rats is not clear but could have been due to a low basal NO production. There seems to be a basal UNOx in R and S rats of 5,000–7,000 nmol/day, part of which is due to food intake, and only the UNOx production due to NO release is suppressible by NOS inhibitors. Figure 6 suggests that only the upregulated NO production was blocked by AG. Supporting this concept was the fact that high Na caused an upregulation in NO production in R rats not S rats and AG only decreased UNOx in the high Na R rats.

The chronic infusion of hypertonic NaCl in the high Na groups or AG administration in the present experiment could have caused an increase in plasma Na concentration and an increase in plasma arginine vasopressin (AVP) concentration. However, plasma Na concentration was not significantly affected by either high Na intake or AG infusion. Water intake was significantly elevated in the high Na groups, which could have helped to prevent an increase in plasma Na concentration. Plasma AVP was not measured in the present experiment, but previous studies in Dahl rats exposed to high dietary Na intake demonstrated no change in plasma Na concentration (2) in S rats, an increase in plasma AVP in R and S rats (2, 15), and no arterial pressure effect of acute V1 (15) or chronic V1 or V2 blockade (11) in R and S rats. Therefore, although plasma AVP may increase in Dahl rats during high Na intake, the effects on arterial pressure may not be important.

Selectivity of AG. At low concentrations, which we used in this study, AG does not block eNOS, because it has an inhibition constant ($K_i$) that is 32- to 52-fold less for iNOS than for endothelial NOS (eNOS) (18). In the present experiment, AG did not significantly affect the renal plasma flow, and arterial pressure was unchanged in the Dahl R and S groups on low Na intake. These data suggest that eNOS activity was unaffected by AG. Also, cerebellar Ca-dependent NOS activity, which is mainly nNOS, was unaffected by AG infusion. 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 concussion or ischemia (7, 22), indicating that AG crosses the blood-brain barrier.

Other groups have also shown that AG can selectively inhibit iNOS. AG did not constrict aortic rings in vitro, although the NO synthesis inhibitors $N^G$-nitro-L-arginine methyl ester and $N^G$-monomethyl-L-arginine did (14, 23). Another study (17) showed that AG intravenous infusion into SD rats for 40 min at a rate five times the rate we used in the present experiment caused no change in arterial pressure or renal cortical blood flow, and a 6-day intravenous infusion of AG at 10 mg·kg$^{-1}$·h$^{-1}$, a rate similar to that used in the present experiment, decreased renal medullary Ca-independent NOS activity without affecting Ca-dependent activity.

Another NOS inhibitor that selectively inhibits iNOS is AMT, and it has a $K_i$ of 4.2 nM, which is similar to that of AG and is up to 40 times more selective for iNOS than for brain NOS or eNOS (19). A recent study (19) with an AMT infusion of 300 nmol/h in Dahl R rats showed that after 5 days of infusion of AMT and high Na intake, systolic pressure increased 20%; the MAP in R rats on high Na and AG in the...
The present study increased 21%. The AMT infusion probably did not inhibit eNOS, because the dilatory responses of mesenteric arteries to methacholine were unchanged. Therefore, our study and the AMT study appear to have selectively blocked iNOS.

In summary, iNOS inhibition in the Dahl R rat caused a salt-sensitive hypertension and a decrease in UNOx but no significant changes in GFR, ERPF, urinary Na excretion, urinary volume, PRA, or cerebellar Ca-dependent NOS activity. This suggests that iNOS normally plays an important role in the R rat in increasing NO production thus preventing salt-sensitive hypertension.

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REFERENCES


