Renal Intramedullary Infusion of L-Arginine Prevents Reduction of Medullary Blood Flow and Hypertension in Dahl Salt-Sensitive Rats

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Abstract—A role for reduced renal nitric oxide production has been proposed as a mechanism responsible for hypertension in Dahl “salt-sensitive” rats. The present study had 2 goals: first, to determine the relationship between changes in mean arterial pressure and renal cortical and medullary blood flows in unanesthetized Dahl/Rapp salt-sensitive (S) and Dahl/Rapp salt-resistant (R) rats as daily salt intake was increased from 0.4% to 4.0%; second, to determine if delivery of L- or D-arginine into the renal medulla of Dahl S rats would change the responses to high salt. Optical fibers were implanted into the renal cortex and inner medulla for daily recording of cortical and medullary blood flows using laser-Doppler flowmetry. Indwelling aortic catheters were used to record arterial pressure. Increasing salt intake to 4.0% in Dahl S rats increased mean arterial pressure from 128±2.0 to 155±5.0 mm Hg by day 5 of high salt diet; medullary blood flow was reduced 13% by day 2, 24% by day 3 (P<0.05), and 31% by day 5 (P<0.05), whereas cortical blood flow was unchanged. In Dahl R rats, mean arterial pressure averaged 117±5 mm Hg during the 0.4% salt control period and remained unchanged (as did cortical and medullary blood flows) during 5 days of 4.0% salt intake. Dahl S rats that received medullary L-arginine (300 μg·kg⁻¹·min⁻¹) exhibited no changes of mean arterial pressure or regional renal blood flow during the 5 days of 4.0% salt intake. Medullary infusion of D-arginine (300 μg·kg⁻¹·min⁻¹) did not prevent the development of hypertension in Dahl S rats that received 4.0% salt. The results are consistent with the view that Dahl S rats have a reduced capacity to generate nitric oxide within the renal medulla under conditions of high salt, which is a feature of Dahl S rats, Dahl salt-sensitive.8,9 NO inhibition specifically within the renal medulla was shown to decrease renal medullary blood flow and to reduce sodium and water excretion in the absence of changes in cortical blood flow. Chronic infusion of N⁶-nitro-L-arginine methyl ester into the renal medulla of Sprague-Dawley rats reduced medullary blood flow (MBF), resulted in sodium retention, and produced hypertension.11 Even intravenous administration of N⁶-nitro-L-arginine methyl ester at low doses produced a selective reduction of medullary blood flow and resulted in hypertension, whereas cortical blood flow (CBF) remained constant.12 These studies have shown that NO in the renal medulla plays an important role in the regulation of MBF and in the long-term control of blood pressure.

A number of studies have suggested that Dahl S rats have a defect in NO production. Administration of L-arginine (L-Arg) either intraperitoneally, intravenously, or even orally can prevent high salt-induced hypertension in Dahl S rats.13,14 Recently, we...
have demonstrated that continuous delivery of L-Arg (300 μg · kg⁻¹ · min⁻¹) into the renal medullary interstitial space of Dahl S rats prevented salt-induced hypertension, suggesting a deficit in medullary NO production.

The purpose of the present study was to first determine the effects of high salt intake on both renal medullary and cortical blood flow in unanesthetized Dahl S and Dahl R rats. The second purpose was to determine the effects of chronic renal medullary interstitial infusions of L-Arg on renal medullary blood flow and the relationship of such changes to the development of hypertension in Dahl S rats. To carry out these studies techniques were used which enabled the delivery of compounds chronically into the renal medulla using implanted medullary interstitial catheters. Daily changes in renal cortical and medullary blood flow in unanesthetized rats were determined using implanted optical fibers and laser-Doppler flowmetry techniques and arterial pressure was determined with implanted aortic catheters.

Methods

Animals

Experiments were performed using Dahl/Rapp S rats (Dahl S; 335 to 400 g) and Dahl/Rapp R rats (Dahl R; 440 to 470 g) purchased from Harlan Sprague Dawley Laboratories (Madison, Wis). All animals were housed individually in the Animal Resource Center at the Medical College of Wisconsin and maintained on a low (0.4% NaCl) diet with water provided ad libitum. All protocols were approved by the Institutional Animal Care Committee.

Chronic Surgical Preparation and Experimental Procedures

To eliminate compensatory responses from the contralateral kidney, all rats were anesthetized with ketamine (30 mg/kg, IM) and xylazine (2 mg/kg, IM) and unilaterally nephrectomized. Surgeries were performed under aseptic conditions, and 7 to 10 days were allowed for recovery. Buprenorphine (0.3 mg/kg, SC) was administered during the recovery from anesthesia to provide analgesia. Rats were then again anesthetized for catheter and optical fiber implantations. The femoral artery was exposed for insertion of the indwelling aortic catheter as described previously. The left kidney was then exposed via a flank incision, and a polyethylene catheter (tip size extruded to approximately 100 μm in diameter) was implanted into the renal medulla to a depth of about 5.5 mm as described previously.

Optical fibers were then implanted into the renal cortex (2 mm depth) and the inner medulla (5.5 mm depth) using techniques developed in our laboratory. All catheters and optical fibers were tunneled subcutaneously to the back of the neck where they were exteriorized through a mid-scapular incision and passed through a spring for protection. Rats were housed individually with the catheters attached to an infusion swivel to allow for the continuous interstitial infusion of isotonic saline or of L-Arg at a rate of 0.5 mL/h. A minimum of 7 days of surgical recovery was provided before the start of hemodynamic measurements. At the end of the experimental protocol, the animals were euthanized, and the positions of the interstitial catheter, cortical fiber, and medullary fiber were determined after fixation of the kidney in a 10% formalin solution for 24 hours.

Daily Measurement of Mean Arterial Pressure, CBF, and MBF

During the week after surgery, rats were trained to rest in a plastic tubular restrainer within their home cages for 2 hours each day. Daily 2-hour measurements of mean arterial pressure (MAP), CBF, and MBF were then begun using an on-line data collection (rate of 100 Hz) and analysis system as previously described. The flow signals from renal cortex and medulla were measured and processed by a 2-channel laser-Doppler flowmeter (Transonics). Continuously recorded signals were transformed to minute averages for analysis.

Protocol 1: Effects of High Salt Intake on MAP, CBF, and MBF in Dahl S and Dahl R Rats

Rats in this group were prepared with an indwelling arterial catheter and optical fibers as described above. After a surgical recovery, MAP, CBF, and MBF signals were measured daily for 1.5 to 2 hours over 3 to 5 days with rats maintained on a low NaCl (0.4%) diet. After obtaining 3 days of stable control values, the daily NaCl content was raised to 4% for 5 days, then returned to 0.4% for 3 days to determine short-term recovery responses.

Protocol 2: Effects of Renal Medullary Interstitial Infusion of L-Arg on MAP, CBF, and MBF in Dahl S Rats With High Salt Intake

Rats in this group were prepared with a renal medullary catheter, an arterial catheter, and optical fibers as described above. After 3 days of stable control, the saline medullary interstitial infusion was switched to L-Arg (300 μg · kg⁻¹ · min⁻¹), and MAP, CBF, and MBF were recorded daily. After 3 days of interstitial infusion of L-Arg, the daily NaCl content was raised from 0.4% to 4% for 5 days and then returned to 0.4% as in protocol 1.

Protocol 3: Effects of Renal Medullary Interstitial Infusion of D-Arg on MAP in Dahl S Rats With High Salt Intake

This protocol was the same as that used for rats in protocol 2 with the important exception that D-Arg was infused rather than L-Arg and rats were prepared with only an arterial catheter for measurement of MAP. After 3 stable control days with rats maintained on a low NaCl (0.4%) diet, the medullary infusion of isotonic saline was switched to an infusion of D-Arg (300 μg · kg⁻¹ · min⁻¹) in saline, and daily recordings of MAP continued. After 3 days of D-Arg, the daily NaCl diet was raised to 4% for 5 days and then returned to 0.4%.

Statistical Analysis

Data are expressed as mean±SEM. Within-group changes were evaluated with a 1-way ANOVA for repeated measures followed by Duncan’s multiple range test. The level of significance was P<0.05.

Results

Protocol 1: Effects of High Salt Intake on MBF, CBF, and MAP in Dahl S and Dahl R Rats

Figure 1 summarizes the average values and responses of MBF, CBF, and MAP in unanesthetized Dahl S rats (n=7) and Dahl R rats (n=6) as the dietary NaCl content was changed from 0.4% to 4% and then returned to 0.4%. MAP of Dahl S rats averaged 128±2 mm Hg during the low salt control period and was significantly increased to 146±3 mm Hg after the second day of receiving high salt. MAP then rose progressively to an average of 155±5 mm Hg after 5 days of the high salt intake. MAP remained at this level during the 3 days of measurement after rats were returned to a low salt diet. MBF, which averaged 1.7±0.3 V (n=6 rats) during the control period, was by the second day of high salt intake clearly reduced in 4 of the 6 Dahl S rats. By the third day of high salt, MBF was significantly reduced in all rats, averaging 1.3±0.3 V (P<0.05), a 24% reduction. The fifth day of high salt, MBF had continued to decline to 1.2±0.2 V (P<0.05), a 31% decrease. MBF remained reduced during the 2 days after return to a low salt intake. In contrast, CBF averaged 2.6±0.1 V during the control period (n=5 rats) and did not change significantly either during or after the period of high salt intake.
MAP of Dahl R rats averaged 116 ± 2 mm Hg during the low salt (0.4% NaCl) control period and did not exhibit significant elevations of MAP with high salt (4% NaCl) intake. MAP averaged 119 ± 2 mm Hg after 5 days of the high salt intake. The CBF signal averaged 2.6 ± 0.2 V and MBF 1.4 ± 0.1 V during control period (n = 6 rats) and was not statistically changed from the control level during the period of high salt.

Protocol 2: Effects of Renal Medullary Interstitial Infusion of L-Arg on MBF, CBF, and MAP in Dahl S Rats With High Salt Intake

Figure 2 summarizes the effects of renal medullary interstitial infusion of L-Arg (300 μg · kg⁻¹ · min⁻¹) on MBF, CBF, and MAP responses in Dahl S rats subjected to high salt (4%) intake. The dose of L-Arg that was chosen for these experiments was the threshold dose found to increase MBF, sodium excretion, and urine flow in our previous study in anesthetized Sprague-Dawley rats. It is also the medullary dose that we previously demonstrated would prevent high salt-induced hypertension in unanesthetized Dahl S rats. During the low salt (0.4% NaCl) control period (3 days) MAP averaged 129 ± 2 mm Hg (n = 8). When the medullary infusion of saline was replaced by L-Arg, MAP remained unchanged and averaged 127 ± 3 mm Hg during the next 3 days. Importantly, when the Dahl S rats receiving interstitial L-Arg were switched to the high salt intake (4% NaCl), no significant elevations of MAP were observed over the 5-day period of high salt intake. MAP averaged 130 ± 2 mm Hg after 5 days of the high salt intake.

The CBF and MBF were stable and averaged 2.6 ± 0.3 V (n = 5) and 1.4 ± 0.04 V (n = 6), respectively, during the 3-day control period with medullary infusion of saline and remained unchanged with infusion of L-Arg into the medullary interstitium. In contrast to the reduction of MBF in Dahl S rats that did not receive medullary L-Arg (Figure 1), Dahl S rats that were infused with L-Arg exhibited no reduction of MBF during the period of high salt intake (Figure 2). Likewise, no changes of CBF were observed during the high salt period. Thus, renal medullary interstitial infusion of L-Arg prevented high salt-induced hypertension and also the reductions of renal MBF.

Protocol 3: Effects of Renal Medullary Interstitial Infusion of D-Arg on MAP in Dahl S Rats With High Salt Intake

Figure 3 summarizes the effects of renal medullary interstitial infusion of D-Arg (300 μg · kg⁻¹ · min⁻¹) on MAP responses in Dahl S rats subjected to high salt intake (4% NaCl). During the low salt (0.4% NaCl) control days when Dahl S received a continuous medullary infusion of D-Arg, the MAP remained constant, averaging 124 ± 4 mm Hg (n = 5). During the period of 4% high salt intake, MAP rose progressively each day, reaching a level averaging 158 ± 7 mm Hg by the fifth day of high salt diet. The increase of MAP with high salt was not significantly different from that obtained in the absence of D-Arg (comparison shown in Figure 3). These data indicate that medullary D-Arg administration was completely ineffective in preventing salt-induced hypertension in Dahl S rats.

Discussion

Results of this study show that high salt intake preferentially reduced MBF in Dahl S but not Dahl R rats. Because CBF remained unchanged and because the reductions of MBF clearly paralleled the increases of MAP, the data indicate that the reduction of renal MBF participates in the development of
the hypertension in Dahl S rats. Furthermore, chronic administration of L-Arg selectively into the renal medulla prevented these reductions of MBF and prevented salt-induced hypertension in Dahl S rats. This was not observed with equimolar medullary infusions of d-Arg in Dahl S rats.

MBF Responses to High Salt Intake in Dahl S Rats

Roman and Kaldunski\textsuperscript{17} found that in Dahl S rats maintained on low salt intakes, renal blood flow, papillary blood flow, and renal interstitial hydrostatic pressure did not differ between young adult Dahl S and Dahl R rats. When Dahl S rats were fed a high (8\%) salt diet for 3 weeks, however, papillary blood flow was significantly reduced, whereas Dahl R rats remained unchanged. It was concluded from these and other studies that reductions of renal blood flow in Dahl S rats probably represented target organ damage associated with the severe glomerulosclerosis seen in the established phase of hypertension in these rats.\textsuperscript{17,18} Although these structural changes undoubtedly do contribute eventually to the reduced renal perfusion in the hypertensive Dahl S rats, the present results suggest that the early reductions of MBF may play an important role in the early development of hypertension in these rats. As recently shown, there is considerable evidence that preferential reductions of blood flow to the renal medulla of rats can initiate and sustain chronic hypertension.\textsuperscript{19} Neither the Dahl R rats nor Sprague-Dawley rats develop hypertension with high salt intake, and we have recently found that the Sprague-Dawley rats also do not exhibit a reduction of MBF with high salt intake.\textsuperscript{20} The MBF reduction appears to be a unique characteristic of the Dahl S rats.

It is not entirely clear how the organism senses a high salt intake or how this could lead to a reduction of MBF in Dahl S rats. Earlier studies in our laboratory have shown that high salt intake results in significant increases of cerebral ventricular sodium concentration in Dahl S rats but not in Dahl R or Sprague-Dawley rats.\textsuperscript{21} Such increases in CSF$_{\text{Na}}$ in Dahl S rats could account for observed increases of efferent sympathetic nerve activity in Dahl S rats.\textsuperscript{22} Yet, it was found that renal denervation of Dahl S rats did not slow the onset or the degree of hypertension in Dahl S rats.\textsuperscript{23} Alternatively, the greater increase of CSF$_{\text{Na}}$ concentrations in Dahl S rats could lead to the reported greater elevations of plasma arginine vasopressin (AVP) in Dahl S rats compared with Dahl R rats.\textsuperscript{22} We have shown that small physiological elevations of plasma AVP can significantly reduce MBF and blunt the acute pressure-natriuresis relationship,\textsuperscript{24} so it is possible that AVP could account for reduced medullary perfusion in Dahl S rats with high salt intake. These effects would be expected to be amplified if the production of NO were impaired in the renal medulla, an event that is consistent with the effects of L-Arg administration discussed below. Other studies from our laboratory have shown that if the medullary NO system is somewhat impaired, vasoconstrictors such as angiotensin II\textsuperscript{25,26} and norepinephrine\textsuperscript{27} are more vasoactive. These or yet-unknown mechanisms could explain the salt-induced reductions of MBF and will need to be more fully explored before a full understanding of these responses is achieved.

Renal Medullary L-Arg Administration Prevents Salt-Induced Reduction of MBF

The most remarkable observation of the present study was the complete abolishment of the salt-induced reduction of MBF and the prevention of hypertension in Dahl S rats with medullary interstitial infusion of L-Arg. In a previous study,\textsuperscript{15} we have shown that the antihypertensive actions of medullary L-Arg infusion were indeed due to actions of this amino acid within the renal medulla because chronic intravenous infusion of the same amount of L-Arg (300 $\mu$g $\cdot$ kg$^{-1}$ $\cdot$ min$^{-1}$) was ineffective in preventing the hypertension. That is, escape of L-Arg and systemic actions or recirculation to the renal cortex could not explain its antihypertensive effects in Dahl S rats. It has been evident from previous studies that intraperitoneal injections, intravenous administration, or even oral administration of high concentrations of L-Arg could prevent salt-induced hypertension in Dahl S rats.\textsuperscript{13,14} d-Arg was not effective in either of those studies or in the present study. L-Arg has been shown to normalize pressure-natriuresis and to improve transmission of perfusion pressure into the renal interstitium in anesthetized Dahl S rats.\textsuperscript{28} Renal blood flow of Dahl S rats on a high salt diet (8\% NaCl) treated with oral L-Arg was found to be higher than in untreated Dahl S rats.\textsuperscript{29} Studies have also suggested that Dahl S rats may have a reduced capacity to produce NO as it was observed that urinary NO$_2$/NO$_3$ excretion was lower in Dahl S rats than in Dahl R rats.\textsuperscript{14} The present results are consistent with these observations.

There is considerable evidence that renal medullary NO production is of special importance in sodium and water homeostasis and in long-term blood pressure regulation of rats.\textsuperscript{11,12} There are substantially greater levels of NOS activity\textsuperscript{30} and NOS protein expression\textsuperscript{31} in the renal medulla, and medullary interstitial NO concentrations are twice that found in the cortex.\textsuperscript{32} If there is a deficiency of NO production in the renal medulla of Dahl S rats, it is interesting that it does not appear to substantially impair MBF under conditions of a low salt intake (0.4\% NaCl). This is suggested by the observation that Dahl S rats on a low salt intake did not show an increase of MBF with medullary L-Arg administration during the control period. It is possible that medullary L-Arg concentrations may be substrate limited for NO production in Dahl S rats only in conditions of high salt intake and that sufficient substrate is available during low salt intake to maintain a normal state of MBF.

Potential defects in the pathway for NO production will now need to be examined. There could be a deficit in the activity of i or more of the NOS isoforms within the vessels, tubules, or interstitial cells of the renal medulla due to defects in transcription, translation, or posttranslational processing. Mattson and Higgins\textsuperscript{33} found that normal Sprague-Dawley rats fed a high salt intake responded with an increase of NOS activity and protein expression within the renal medulla, so it is possible that Dahl S rats are unable to respond to a comparable extent. It was recently reported that NOS activity in the renal medulla of hypertensive Dahl S rats was decreased compared with normotensive Dahl S rats.\textsuperscript{18} A deficit of medullary NO production in Dahl S rats could be related to a limitation of medullary L-Arg substrate for intracellular conversion to NO. This L-Arg substrate limitation could be related to a defect in L-Arg production, metabolism, uptake, or related transduction pathways. There is evidence that NO production can be substrate limited in the renal medulla of Sprague-Dawley rats. The evidence for this is based on our observations that medullary L-Arg adminis-
tration increases interstitial medullary NO concentrations in anesthetized Sprague-Dawley rats and is consistent with observations that L-Arg concentrations are the lowest in the medulla in which NOS activity is the highest. If Dahl S rats exhibit a quantitatively greater deficit of medullary L-Arg or a reduced capacity for cellular uptake, such defects could account for the therapeutic actions of medullary L-Arg administration. Other possible explanations for the therapeutic effects of L-Arg may also be considered. For example, it has recently been reported that L-Arg and d-Arg can inhibit vasopressin-stimulated increases of intracellular Ca2+ in cultured rat mesangial cells. This effect did not appear to be mediated by metabolism of L-Arg to either NO or l-ornithine, and it was concluded that the response was due to a charge effect related to the cationic structure of L-Arg (guanidine in particular). Such effects do not appear to be able to account for the effects of L-Arg in the present study because d-Arg, which is not metabolized to NO, had no therapeutic effects. Finally, although the emphasis of the present study was on the role of NO in the regulation of MBF, it should be recognized that NOS mRNA and protein are present in the tubules of the renal medulla including medullary collecting ducts. There is evidence that tubular NO production may act to directly increase the excretion of sodium. So, it is also possible that medullary L-Arg administration to Dahl S rats may enhance sodium excretion both indirectly via effects on MBF and by influencing tubular NO production directly.

In summary, the present study demonstrated that Dahl S rats respond to a high salt diet with a rapid decline of blood flow to the renal medulla and a parallel increase of arterial pressure. This response and the ensuing hypertension was prevented by providing greater amounts of L-Arg to the renal medulla. The results are consistent with the view that Dahl S rats have a reduced capacity to generate NO within the renal medulla under conditions of a high salt intake, which the administration of L-Arg can normalize.

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