Renal P450 Metabolites of Arachidonic Acid and the Development of Hypertension in Dahl Salt-Sensitive Rats

Richard J. Roman, Magdalena Alonso-Galicia, and Thomas W. Wilson

Renal transplantation studies indicate that some form of renal dysfunction underlies the development of hypertension in Dahl salt-sensitive (S) rats; however, the factors responsible for altering kidney function remain to be determined. Previous studies have indicated that Dahl S rats require a higher renal perfusion pressure to excrete the same amount of sodium and water as normotensive rats and that this is due largely to an elevation in Cl⁻ transport in the thick ascending limb of the loop of Henle. There are now five lines of evidence that suggest an abnormality in the renal metabolism of arachidonic acid by enzymes of the P4504A family may contribute to the increase in loop Cl⁻ transport and the development of hypertension in Dahl S rats. In this regard, the formation of 20-HETE and the levels of P4504A protein are reduced in the outer medulla of Dahl S rats. Perfusion of the loop of Henle of Dahl S rats with exogenous 20-HETE normalizes the elevated loop Cl⁻ transport. In addition, a genetic marker in the P4504A2 gene, which encodes for the enzyme that makes 20-HETE, cosegregates with the development of hypertension in an F₂ cross of Dahl S and Lewis rats. Finally, induction of renal production of 20-HETE with clofibrate prevents the development of hypertension in Dahl S rats and inhibition of renal 20-HETE formation produces hypertension in Lewis rats fed a high salt diet. These results implicate the CYP4A2 locus as a candidate gene that contributes to the alterations in renal function and the development of hypertension in Dahl S rats. Am J Hypertens 1997;10:63S–67S © 1997 American Journal of Hypertension, Ltd.

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In the early 1960s, Dr. Lewis K. Dahl developed through selective breeding, a strain of rats whose blood pressure rapidly increased when salt intake was elevated. Subsequently, inbred strains were independently developed by Dr. John P. Rapp and Dr. J. Iwai. Mean arterial pressure typically increases about 20 mm Hg on the first day that Dahl salt-sensitive (Dahl S) rats are switched to a high salt diet, and pressure reaches a plateau of about 170 mm Hg after 2 weeks. The increase in arterial pressure is associated with sodium retention and total body weight increases about 7% when the rats are fed a high salt diet. Initially, the increase in blood pressure appears to be due to an increase in cardiac output in the absence of peripheral vasodilation, and later it is maintained by a rise in total peripheral resistance. The rise in arterial pressure after exposure to a high salt diet can be prevented by maintaining extracellular fluid volume constant or by preventing sodium retention using diuretics.

Dahl S rats exhibit many phenotypic traits associated with salt-sensitive hypertension especially in African Americans. Specifically, Dahl S rats are salt sensitive, insulin resistant, and hyperlipidemic.
They have a low renin form of hypertension that is refractory to treatment with converting enzyme inhibitors, but is effectively treated with diuretics. Moreover, Dahl S rats rapidly develop proteinuria and glomerulosclerosis during the development of hypertension. In this regard, they resemble hypertensive African Americans in whom the incidence of end-stage renal disease is 16 times higher than that seen in white hypertensive patients.

**EVIDENCE THAT AN ABNORMALITY IN RENAL FUNCTION UNDERLIES THE DEVELOPMENT OF HYPERTENSION IN DAHL S RATS**

The strongest evidence supporting the role of the kidney in the development of hypertension in Dahl S rats has been derived from renal transplantation studies. Transplantation of a kidney from a hypertensive Dahl S rat to a normotensive Dahl salt-resistant (Dahl R) rat increases arterial pressure. Vise versa, transplantation of a kidney from a Dahl R rat to an S rat lowers pressure. These observations have provided the rationale for a large number of studies to identify the abnormalities in renal function that underlie the development of hypertension in Dahl S rats.

Numerous investigators have now identified a common abnormality in renal function that precedes the development of hypertension in Dahl S rats. As presented in Figure 1 (top), the pressure natriuresis relationship is blunted and shifted to the right in Dahl S compared to Dahl R rats. For any given neural and hormonal background to the kidney, Dahl S rats require a higher renal perfusion pressure to achieve the same rate of sodium excretion as normotensive controls. Glomerular filtration rate is about 25% lower in inbred Dahl S/Jr rats compared to normotensive rats; therefore, part of the resetting of the pressure-natriuresis relationship in this strain can be explained by a decrease in the filtered load of sodium. However, fractional excretion of sodium is also reduced in Dahl S as compared to R rats for any given level of renal perfusion pressure, indicating that tubular reabsorption is also elevated in these rats.

Renal micropuncture studies have been performed to identify the nephron segments in which tubular reabsorption is enhanced. At the same level of renal perfusion pressure, Cl⁻ reabsorption is markedly enhanced in the thick ascending limb of the loop of Henle (TALH). Interestingly, Cl⁻ reabsorption in other nephron segments, such as the proximal tubule and the cortical collecting duct, is reduced in Dahl S rats compared to normotensive controls, perhaps to compensate for the elevation in Cl⁻ transport in the TALH. This original observation has now been confirmed by several investigators. In these studies, the loop of Henle of Dahl S and R rats was perfused in vivo, and the Cl⁻ transport was determined. As presented in Figure 1 (bottom), Cl⁻ transport is about 30% percent greater in the loop of Henle of Dahl S compared to that seen in Dahl R rats.

**FACTORS CONTRIBUTING TO THE ELEVATED Cl⁻ TRANSPORT IN THE LOOP OF HENLE OF DAHL S RATS**

Despite the importance of the TALH in the regulation of sodium excretion, very little is known about the factors that regulate transport in this segment. Vasopressin stimulates and prostaglandin E₂ inhibits sodium transport in this segment, and there is one paper suggesting that a deficiency in the renal synthesis of prostaglandin E₂ may contribute to the blunted pressure natriuresis in Dahl S rats. There is also evi-
dence suggesting a role for nitric oxide. In this regard, increasing NO synthesis with L-arginine normalizes the pressure natriuresis response\(^1^7\) and prevents the development hypertension in Dahl S rats.\(^2^6\) However, L-arginine does not correct the underlying defect in Cl\(^-\) transport in the TALH.

Recently, Escalante et al\(^2^7\) and Carroll et al\(^2^8\) reported that the TALH metabolizes arachidonic acid (AA) to a novel metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE), through a cytochrome P450 enzyme of the 4A family (P4504A). They further demonstrated that 20-HETE serves as an endogenous inhibitor of Na\(^+\), K\(^+\), 2Cl\(^-\).\(^2^7\) Subsequently, we studied the renal metabolism of arachidonic acid in Dahl S and R rats and found that the production of 20-HETE was markedly reduced in the outer medulla of Dahl S rats compared to Dahl R rats.\(^2^9\) This observation led us to propose that a deficiency in the renal production of 20-HETE may contribute to the elevation in Cl\(^-\) transport in the TALH and the development of hypertension in Dahl S rats.

**FIVE LINES OF EVIDENCE IMPLICATING A ROLE FOR 20-HETE IN THE DEVELOPMENT OF HYPERTENSION IN DAHL S RATS**

Recently, we have confirmed that renal metabolism of arachidonic acid is altered in Dahl S rats compared to another normotensive strain.\(^3^0\) In these studies (Figure 2), the production of 20-HETE was lower in the outer medulla of Dahl S rats compared to normotensive Lewis rats and that this was associated with a three-to fourfold reduction in the levels of the P4504A enzyme that catalyzes the formation of 20-HETE.

To determine whether this abnormality in the renal metabolism of arachidonic acid by P4504A enzymes contributes to the development of hypertension in Dahl S rats, a genetic cosegregation study was performed.\(^3^0\) The results of this study are presented in Figure 3. We identified a genetic marker spanning a repeated element in intron 11 of the P4504A2 gene, mapped this locus to rat chromosome 5, and used it to genotype 151 F\(_2\) rats derived from a cross of Dahl S and Lewis rats. The P4504A2 genotype was found to cosegregate with blood pressure in this population. Systolic blood pressure averaged 201 ± 6 mm Hg in rats with the SS genotype, 192 ± 4 mm Hg in heterozygotes, and 169 ± 3 mm Hg in rats with the LL genotype. These results suggest that P4504A2 is a viable candidate gene for the development of hypertension in Dahl S rats.

To determine whether the abnormality in 20-HETE production in the outer medulla contributes to the elevation in Cl\(^-\) transport in the TALH of Dahl S rats, in vivo tubular perfusion experiments were performed.\(^2^3\) We found that 77 ± 2% of the Cl\(^-\) load was reabsorbed in the loop of Henle of Dahl S rats versus only 57 ± 3% in Dahl R rats. Addition of 20-HETE to the perfusate normalized loop Cl\(^-\) transport in Dahl S rats, but it had little effect in Dahl R rats. Addition of an inhibitor of the production of 20-HETE to the perfusate, 17-octadecynoic acid (17-ODYA), increased Cl\(^-\) reabsorption in the loop of Henle of Dahl R rats to the same level as that seen in Dahl S rats, but it had no effect on loop Cl\(^-\) transport in Dahl S rats. These results indicate that endogenously formed 20-HETE regulates loop Cl\(^-\) transport in the rat, and suggest that a deficiency in the formation of this substance contributes to the elevation in Cl\(^-\) transport in Dahl S rats.

Additional experiments were performed to determined whether altered levels of 20-HETE in the kidney could change arterial pressure in rats. Clofibrate and other fibrate lipid-lowering agents induce the expression of enzymes of the P4504A family in the kid-
ney. In Dahl S rats treated with clofibrate, the expression of P4504A2 protein in the kidney was markedly elevated, and the production of 20-HETE in the renal cortex and outer medulla increased. Induction of the renal formation of 20-HETE prevented the development of hypertension in Dahl S rats fed an 8% salt diet for 4 weeks (Figure 4, top). Moreover, the effects of clofibrate were reversible. Interestingly, clofibrate does not lower blood pressure in Dahl S rats with established hypertension. This may be due to the extensive glomerulosclerosis in hypertensive Dahl S rats. At this point, hypertension may be sustained due to the loss of renal mass. Clofibrate also had no effect on blood pressure in normotensive Dahl R rats exposed to a high salt diet, ruling out a nonspecific effect of the drug.

Experiments have also been performed to determine whether a reduction in the formation of 20-HETE in the kidney is sufficient to induce hypertension in normotensive rats. In these experiments, 17-ODYA was chronically infused directly into the renal medullary interstitium of normotensive Lewis rats. Lewis rats were studied because they were the control strain in the cosegregation analysis. Renal medullary infusion of 17-ODYA (400 picomoles/min; Figure 4) increased mean arterial pressure from 115 ± 2 to 142 ± 2 mm Hg over a 5-day period in Lewis rats fed an 8% salt diet. Infusion of vehicle alone had no effect on arterial pressure. Renal interstitial infusion of 17-ODYA reduced the metabolism of AA to 20-HETE by 70% in the outer medulla to a level normally seen in Dahl S rats. The synthesis of 20-HETE in the renal cortex was not altered by 17-ODYA, confirming that its effects were limited to the renal medulla. These results indicate that a reduction in the synthesis of 20-HETE in the outer medulla of the kidney can induce hypertension in a normotensive strain of rat.

CONCLUSION

There are now five lines of evidence that a deficiency in the production of 20-HETE in the renal outer medulla of Dahl S rats contributes to the elevation in Cl⁻ transport in the TALH and the development of hypertension in Dahl S rats. Further studies focusing on the mechanisms by which 20-HETE regulates loop Cl⁻ transport and identification of the specific genetic abnormality responsible for the altered P4504A2 ex-

FIGURE 3. Systolic blood pressure of male rats of the F2 (Dahl S X Lewis) generation sorted by P4504A2 genotype. Pressure was monitored using the tail-cuff method. The number at bottom of each column indicates the number of animals with the corresponding genotype. *Significant difference from the corresponding value in F2 rats with an LL genotype. Data redrawn with permission from reference 30.

FIGURE 4. (Top) Effect of clofibrate (80 mg/day, n = 19) or vehicle (20 nmol/L Na2CO3, n = 17) on mean arterial pressure in Dahl salt-sensitive rats fed a high salt diet (8% NaCl) for 4 weeks. Graph was replotted with permission from reference 31. (Bottom) Effect of a renal medullary interstitial infusion of 17-ODYA (400 picomoles/min, n = 6) or vehicle (1% albumin in 0.9% NaCl, n = 5) on mean arterial pressure in conscious Lewis rats fed a high salt diet (8% NaCl). Control values represent the average of three consecutive days of blood pressure recording while saline was infused through the interstitial catheter. Data replotted with permission from reference 33.
pression in the kidneys of Dahl S rats seem warranted. Finally, it would be interesting to determine whether the renal excretion of 20-HETE is altered in salt-sensitive hypertensive patients and whether genetic abnormalities in P4504A genes are linked to the development of hypertension in humans.

REFERENCES


