Sodium Reabsorption in the Thick Ascending Limb in Relation to Blood Pressure: A Clinical Perspective

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Regulation of TAL Function, Normally and in Bartter Syndrome

Approximately one quarter of the filtered load of Na\(^+\), is taken up in TAL, in part by way of the paracellular route (along an electric gradient) and in part by the Na\(^+\),K\(^+\),2Cl\(^-\) cotransporter (NKCC2) residing at the apical surface (Figure)\(^4\) where uptake is electroneutral. One can begin to appreciate the complexity of its regulation from the number of genes that, when mutated, result in Bartter syndrome, a severe salt-losing nephropathy. Approximate one quarter of the filtered load of Na\(^+\) is taken up in TAL, in part by way of the paracellular route (along an electric gradient) and in part by the Na\(^+\),K\(^+\),2Cl\(^-\) cotransporter (NKCC2) residing at the apical surface (Figure)\(^4\) where uptake is electroneutral. One can begin to appreciate the complexity of its regulation from the number of genes that, when mutated, result in Bartter syndrome, a severe salt-losing nephropathy accompanied by low blood pressure\(^5\) attributed to a functional hyperparathyroidism.5 Although a sustained increase in the kidney’s reabsorption of Na\(^+\) underlies much of the need to generate a higher natriuretic pressure,\(^2\) the specific nephron regions where the more active uptake occurs are not known. In the discussion that follows, we review findings from human studies that suggest that variations in Na\(^+\) uptake by the thick ascending limb (TAL) of the loop of Henle influence an individual’s chronic level of blood pressure, as well as risk for hypertension. The intent of this review is to encourage consideration of TAL in the scheme of renal events that can affect blood pressure and influence a propensity for becoming hypertensive.

Race Differences in TAL Function?

The existence of a more actively functioning TAL in large population groups is suggested from studies comparing
blacks with whites. Hypertension in blacks is more common22 and clinically a more serious disorder23 than in whites. Blood pressure in blacks is typically salt sensitive, which differs what is observed in blacks, including lower levels of blood pressure and aldosterone and lower urinary excretion rates of K\textsuperscript{+} and Ca\textsuperscript{2+}.24,25 In children, long before the onset is delayed, plasma aldosterone levels and aldosterone hypertension is likely to occur, and maybe in part why its secretion rates in blacks could result from or be significantly lower in blacks than in whites.26 There have been instances, however, where the diets in the 2 race groups contained the same amount of K\textsuperscript{+}, and blacks still excreted less urinary K\textsuperscript{+}. Turban et al33 analyzed data from the DASH (Dietary Approaches to Stop Hypertension) study,34 a clinical trial of the effect on blood pressure of a diet containing low-fat dairy products, as well as fruits and vegetables (75 blacks and 46 whites participated). At completion of the 8-week DASH dietary intervention that included a fixed intake of K\textsuperscript{+}, urinary K\textsuperscript{+} excretion rates were significantly lower in blacks (2465±592 mg/d; P<0.001). The percentage of dietary K\textsuperscript{+} excreted in urine was also significantly less in blacks (50±18%) than in whites (69±17%; P<0.001), suggesting greater excretion of K\textsuperscript{+} by nonrenal routes in blacks. Palacios et al35 made similar observations in adolescent girls studied under highly controlled and carefully monitored inpatient conditions. They also measured fecal and sweat K\textsuperscript{+} but could not detect a race difference in their excretion. Thus, although in blacks dietary intake of K\textsuperscript{+} appears to be definitely less than that for whites, the lower urinary excretion rates of K\textsuperscript{+} in blacks may also reflect a difference in K\textsuperscript{+} handling.

Differences in Electrolyte Excretion

Racial differences in TAL function can be suggested from excretion rates of certain cations. Although urinary excretion rates of Na\textsuperscript{+} are similar in blacks and whites26,28 urinary excretion rates of K\textsuperscript{+} are lower in blacks by 20–40%.26,28–31 under steady state conditions where K\textsuperscript{+} excretion (urinary and fecal) equals K\textsuperscript{+} intake. The disparity in K\textsuperscript{+} excretion between race groups would seem to largely if not totally reflect differences in dietary K\textsuperscript{+}. Indeed, 24-hour dietary recalls from the Third National Health and Nutrition Examination Survey found that dietary K\textsuperscript{+} was ≈20% lower in blacks than in whites.32 There have been instances, however, where the diets in the 2 race groups contained the same amount of K\textsuperscript{+}, and blacks still excreted less urinary K\textsuperscript{+}. Table 1. Directional Comparison of Individuals With Increased TAL Activity, Individuals Who Are Black, and Individuals With Bartter Syndrome

<table>
<thead>
<tr>
<th>Variable</th>
<th>BP</th>
<th>Renin</th>
<th>Aldosterone</th>
<th>Urinary K\textsuperscript{+} Excretion</th>
<th>Urinary Ca\textsuperscript{2+} Excretion</th>
<th>Urinary Mg\textsuperscript{2+} Excretion</th>
<th>Urine Osmolarity</th>
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<tr>
<td>↑ TAL (predicted)</td>
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<td>Blacks (in comparison to whites)</td>
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<tr>
<td>Bartter syndrome</td>
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</table>

TAL indicates thick ascending limb; BP, blood pressure.

Figure. Schematic depiction of a TAL tubular cell uptake of ions by the NKCC2. Loss-of-function mutations in the genes for NKCC2, ROMK, CLCNKB, or BSND account for, respectively, types 1 to 4 Bartter syndrome. A gain-of-function mutation in the CASR gene results in type 5 Bartter syndrome. A deficiency in ROMK inhibits NKCC2 because of less K\textsuperscript{+} available to the cotransporter (K\textsuperscript{+} availability is rate limiting); less luminal K\textsuperscript{+} results in a more negative charge that keeps Ca\textsuperscript{2+} in solution, thereby favoring its excretion (as opposed to its paracellular reabsorption by the NKCC2. ROMK indicates K\textsuperscript{+} channel; CLCNKB, Cl\textsuperscript{-} channel; BSND, Bartter syndrome, infantile, with sensorineural deafness; also known as barttin; and CASR, Ca\textsuperscript{2+}-sensing receptor.
rates of aldosterone can be adjusted for dietary K⁺ by measuring K⁺ excretion over the same collection period if urinary K⁺ reflects dietary K⁺.

In a study of 351 white and 170 black children with multiple overnight urine collections (on average, 5 per subject at intervals of 6 months), K⁺ excretion accounted for 20% of the variation in aldosterone excretion (Na⁺ excretion accounted for 10%). After adjusting for urinary excretion of K⁺ and Na⁺, the aldosterone excretion rate remained lower in blacks than in whites (35% lower; P<0.0001). Plasma aldosterone levels cannot be adjusted as easily for variations in diet, but the lower levels of aldosterone would be expected to at least partially result from less stimulation by angiotensin II (levels of plasma renin activity being lower), with less of an effect of angiotensin II and K⁺ stimulation by angiotensin II (levels of plasma renin activity being lower).

The greater uptake of Na⁺ and Mg⁺⁺ reabsorption, their lower excretion rates at the least suggest a higher level of NKCC2 activity in TAL.

**Increased NKCC2 Activity and a Decrease in Tubuloglomerular Feedback: A Proposed Mechanism for Salt-Sensitive Hypertension in Blacks**

Aviv et al. undertook a review of previously reported clinical studies, which then led them to place TAL, at least theoretically, in a pivotal position for contributing to the heightened susceptibility of blacks to hypertension. A mechanism whereby an increase in NKCC2 activity leads to an expansion of the extracellular volume or an alternative mechanism whereby no expansion occurs was proposed. Either would result in a decrease in urine K⁺ excretion and increased Na⁺ reabsorption and greater water conservation. An increase in Na⁺ uptake by NKCC2 would enhance tubuloglomerular feedback (less Na⁺ reaching the macula densa) leading to glomerular hypertension (by reducing afferent arteriolar tone) and, in turn, the delivery of more Na⁺ to the proximal tubule, resulting in an increase in the absolute amount of Na⁺ reabsorbed here and downstream in TAL. Glomerular hypertension would also result in an increase in colloid osmotic pressure in the peritubular arterioles to further promote Na⁺ reabsorption. Of course an increase in NKCC2 activity that increased blood pressure would be met by an adjustment consisting of reduced Na⁺ reabsorption in the proximal tubule, and suppression of the renin-angiotensin-aldosterone system would further help to restore Na⁺ balance.

Bochud et al. used the clearance of endogenous Li⁺ to estimate Na⁺ reabsorption in proximal and downstream nephron segments. They showed, first of all, that reabsorption was heritable. In addition, proximal reabsorption was found to be greater in South African blacks than in Belgian whites. The evidence for greater proximal tubular reabsorption of
Na\(^+\) in blacks could be explained by a more active TAL, resulting in less tubuloglomerular feedback, followed by greater glomerular filtration with delivery of more Na\(^+\) to the proximal tubule that then increases absolute amounts of Na\(^+\) reabsorption, as was proposed by Aviv et al.\(^{54}\)

**Shortcomings of Clinical Studies**

A role for TAL in regulating blood pressure in humans is difficult to establish due in part to limitations inherent to clinical assessments. In the case where TAL function was increased, the increment in retained Na\(^+\) required to raise blood pressure would probably be small, only enough over time to supersede any adjustments occurring elsewhere, either proximally or distally. In the case of the latter, for example, ENaC in the collecting duct reabsorbs only a small fraction, possibly as little as 2%, of the filtered load of Na\(^+\). Any increase in Na\(^+\) reabsorption in TAL would need only to exceed this small fractional range to potentially affect blood pressure. If one could directly probe the function of NKCC2, which one cannot, the level of activity that was enough to result in Na\(^+\) retention and an increase in blood pressure might not be detectable. On the other hand, a sustained effect that resulted in a chronically developed state might easily be recognized. This could take the form, in the case of a more active TAL, of a higher urine concentration or higher blood pressure.

Recent advances in imaging the human kidney hold promise for intrarenal quantitative measurements. 23Na MRI was used by Maril et al\(^{57}\) to determine the contribution of NKCC2 to the renal medullary osmotic gradient. Using this noninvasive approach, these investigators demonstrated that water deprivation resulted in a 25% increase in the gradient within 12 hours. Conceivably, such techniques could delineate race differences both basally and after certain manipulations, such as the administration of furosemide.

**Genetic Studies**

The genetically derived Dahl salt-sensitive rat and Milan rat, widely used models of hypertension, have a more active TAL than their respective controls.\(^{20,38,50}\) For the rare mendelian forms of hypertension, the single gene mutations in every instance localize the increase in Na\(^+\) reabsorption to either the collecting duct\(^{60,61}\) or the distal convoluted tubule,\(^{62}\) but never to TAL. The only known monogenic blood pressure disorders of which expression takes place in TAL results in hypotension (collectively, Barter syndrome), thus suggesting a role for variations in TAL to lower rather than raise blood pressure, to mitigate risk rather than increase risk for hypertension. A search for rare genetic variants is currently considered a promising means for delineating genetic contributions to complex diseases.\(^{63}\) When members of the Framingham Heart Study were screened for rare mutations in 2 genes, SLC12A1 (NKCC2) and KCNJ1 (ROMK), which, in the homozygous state, produce Barter syndrome, significant associations of these mutations in the heterozygous state with a lower blood pressure were observed.\(^{64}\) In a second study, common variants in KCNJ1 were also shown to associate with a lower blood pressure.\(^{65}\) These studies limited to these 2 genes together with the severe loss of function in Barter syndrome imply a role of TAL variants to diminish the likelihood for hypertension.

An association study with common variants of CASR showed a significantly positive association with both blood pressure and urinary Ca\(^++\) excretion in blacks but not in whites.\(^{66}\) In a recent genome-wide meta-analysis (data integrated from separate cohorts of whites and Indian Asians),\(^{67}\) serum Ca\(^++\) concentration associated with single nucleotide polymorphisms in and around CASR (P = 6 × 10\(^{-37}\)) for the most significant single nucleotide polymorphism, demonstrating that common variations in the receptor may indeed affect function. Illustrating the extent to which CASR can influence Na\(^+\) homeostasis, Ca\(^++\) infused into healthy men, raising serum Ca\(^++\) levels by 25%, seemingly enough to increase binding of Ca\(^++\) to CASR and inhibit NKCC2,\(^{12}\) increasing excretion of Na\(^+\) by 150%.\(^{68}\) Although it was reported early on that diets higher in Ca\(^++\) were accompanied by lower blood pressures,\(^{69}\) subsequent studies demonstrated little if any clinically significant improvement in blood pressure after enrichment of diets with Ca\(^++\).\(^{70,71}\) To our knowledge, however, an effect of dietary Ca\(^++\) on blood pressure has not been studied extensively in blacks, a population group that could, if enhanced natriuresis is mechanistically involved, be more responsive to dietary manipulations of Ca\(^++\).

**CYP450 Enzymes and 20-Hydroxyeicosatetraenoic Acid**

In animal models of hypertension, 20-hydroxyeicosatetraenoic acid (20-HETE) can lower blood pressure by inhibiting NKCC2.\(^{72}\) The Dahl salt-sensitive rat, for example, may have increased NKCC2 activity at least in part because of reduced levels of 20-HETE.\(^{20,73}\) Salt-sensitive human hypertension has been associated with a decrease in urinary excretion of 20-HETE after furosemide administration,\(^{74}\) suggesting that a 20-HETE deficiency state with less inhibition of NKCC2 contributes to salt sensitivity of blood pressure. 20-HETE is synthesized from arachidonic acid by cytochrome CYP450 enzymes\(^{75}\) (specifically, the CYP 4A and 4F families of enzymes synthesize 20-HETE). A variant nucleotide in the gene for CYP4A11, T8590C, which has known reduced catalytic activity, was found to associate with hypertension in subjects from 2 independent population groups.\(^{76}\) Additional studies have also now found significant associations with T8590C.\(^{77,78}\) Hypertensive blacks from the African American Study of Kidney Disease (AASKD) showed an association of the 8590CC genotype with a higher systolic blood pressure.\(^{79}\) 20-HETE also increases vascular tone and can potentially raise blood pressure.\(^{80}\) A variant in CYP4F2, an additional enzyme for 20-HETE synthesis, was associated with increases in both 20-HETE and blood pressure,\(^{81}\) demonstrating the less than always straightforward effects of 20-HETE on blood pressure. The genetic variation in the enzymes that lead to different levels of 20-HETE would seem to be extremely promising determinants of hypertension risk. There is a strong likelihood that much of their blood pressure effects are mediated through ion transport in TAL.

Association studies with candidate genes can potentially identify, for example, variations in ion transport systems that alter Na\(^+\) reabsorption to where blood pressure is affected.
active ENaC or to an elevated level of aldosterone. 82–84 aldosterone system, reduce its uptake of Naagas a consequence of the renin-angiotensin-is increased, the distal nephron, primarily ENaC in the accordingly.

Indeed, it was shown recently that activation of the Na\(^+\)-Cl\(^-\) cotransporter required participation by ENaC or another Na\(^+\) reabsorption site.\(^85\) Variations in proximal tubular function could also take part in establishing the arterial pressure necessary to achieve the natriuresis for maintaining Na\(^+\) balance.\(^86\) When searching for a role for TAL to increase blood pressure, one might miss it unless one also considers the distal nephron or proximal tubule. At the same time, greater Na\(^+\) reabsorption elsewhere could require a more active TAL before manifesting in an increase in blood pressure.

**Perspectives**

TAL has been shown to be a site for a variety of monogenic forms of hypertension, collectively known as Bartter syndrome. Variations in Na\(^+\) uptake in TAL hold the potential to similarly influence Na\(^+\) reabsorption and, in turn, blood pressure. A comparison of race groups suggests that TAL can also operate at a higher level in blacks, a population group prone to develop a more severe type of hypertension. Clinical studies of TAL might be best served by inclusion of blacks. Future studies should encompass additional translational research, applying what has been learned from animal models to studies of clinical hypertension and vice versa. For example, generation of models that incorporate functional variants of CASR in TAL to look for blood pressure responses to differences in dietary calcium. Knowing that TAL participated in the development of hypertension more commonly than currently realized could have a practical bearing, by providing, for example, the impetus for designing loop diuretics that are more lifestyle compatible or by making possible the selection of certain groups based on genotype and race who would benefit from diets enriched in Ca\(^{2+}\). If TAL is shown to participate in affecting hypertension risk (either by lowering it or increasing it), preventive strategies and treatment paradigms for many individuals could change.

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**Disclosures**

None.

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