Hypertension is the single most commonly treated chronic disease. Left untreated or undertreated, hypertension predisposes to heart disease, kidney failure, and stroke. In only half of those affected is blood pressure controlled. A clearer understanding of why certain individuals become hypertensive could lead to more specific and effective therapies. The origins of hypertension are complex, but a feature common to virtually all forms is a pressure-natriuresis relationship that achieves a balance between intake and output of Na⁺. Although a sustained increase in the kidney’s reabsorption of Na⁺ underlies much of the need to generate a higher natriuretic pressure, 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⁺ reabsorption 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.

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) 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 attributed to a functional deficiency in NKCC2. Lifeiton and others showed that Bartter syndrome results from mutations in NKCC2 itself (type 1) or in its regulators for a total of 5 types.

Loss-of-function mutations in KCNJ1, which encodes for the K⁺ channel renal outer medullary K⁺ (ROMK), result in Bartter syndrome type 2. Because the tubular concentration of K⁺ in TAL is low relative to the concentrations of Na⁺ and Cl⁻, the availability of K⁺ for occupancy on the cotransporter is rate limiting for its activation. On the other hand, when recycling of K⁺ is impeded, Na⁺ uptake in TAL declines (an increase in K⁺ recycling would increase Na⁺ uptake). In addition, K⁺ returning to the lumen via ROMK results in a more positively charged milieu that promotes the departure of Ca²⁺ and Mg²⁺ through paracellular routes. Thus, as NKCC2 becomes more active, urinary excretion of these cations decreases. When NKCC2 activity is decreased as in the Bartter syndrome, Ca²⁺ excretion is increased.

Cl⁻ carried into the cell by NKCC2 exits through the Cl⁻ channels CLCNAK and CLCNKB with facilitation by BSND (Bartter syndrome, infantile, with sensorineural deafness). Mutations in CLCNKB and BSND result in, respectively, types 3 and 4 Bartter syndrome. Thus, clearing the cell of the additional Cl⁻ is a determinant of NKCC2 functional activity and potentially level of blood pressure.

The Ca²⁺ sensing receptor (CASR) on the basolateral surface in TAL binds to inhibitory NKCC2. This then results in a more negatively charged lumen (there is less recycling of K⁺), which facilitates excretion of Ca²⁺, serving as a means for correcting or preventing a state of hypercalcemia. CASR is also expressed in other sites in kidney and in nonrenal sites including the parathyroid gland, where a gain-of-function mutation in CASR can result in not only Bartter syndrome (type 5) but also hypoparathyroidism.

The scope of the regulation of TAL activities, however, goes much beyond what can be appreciated from studying Bartter syndrome. For example, TAL function is highly integrated into the dynamic events of the renal medulla, where regulation is multifaceted. NO, which inhibits NKCC2, is a principal conveyor of influence here, as demonstrated in a series of studies. Other bioactive mediators include endothelin, superoxide, angiotensin II, vasopressin, and prostaglandins. An excellent and comprehensive review of the renal medulla and blood pressure by Cowley was published in Hypertension in 2008.

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 common than in whites.22 Hypertension in blacks is more common than in whites.22 In children, long before onset is delayed, plasma aldosterone levels and aldosterone excretion rates are lower in blacks than in whites.26 The onset is delayed, plasma aldosterone levels and aldosterone excretion rates are lower in blacks than in whites.26 The results of ROMK inhibits NKCC2 because of less K+ in solution, thereby favoring its excretion (as opposed to its paracellular uptake). CLCNKB and BSND are required for maintaining efflux of Cl− and are rate limiting for the optimal function of the cotransporter. Activation of CASR by extracellular Ca++ has an effect to inhibit indirectly the cotransporter. ROMK indicates K+ channel; CLCNKB, Cl− channel; BSND, Bartter syndrome, infantile, with sensorineural deafness; also known as barttin; and CASR, Ca++-sensing receptor.

**Differences in Electrolyte Excretion**

Racial differences in TAL function can be suggested from excretion rates of certain cations. Although urinary excretion rates of Na+ are similar in blacks and whites,26,28 urinary excretion rates of K+ are lower in blacks by 20–40%.26,28–31 under steady state conditions where K+ excretion (urinary and fecal) equals K+ intake. The disparity in K+ excretion between race groups would seem to largely reflect differences in dietary K+. Indeed, 24-hour dietary recalls from the Third National Health and Nutrition Examination Survey found that dietary K+ was ~20% lower in blacks than in whites. There have been instances, however, where the diets in the 2 race groups contained the same amount of K+, and blacks still excreted less urinary K+. Turban et al33 analyzed data from the DASH (Dietary Approaches to Stop Hypertension) study,44 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+, urinary K+ excretion rates were significantly lower in blacks (2465±992 mg/d; P<0.001) than in whites (3584±962 mg/d; P.<0.001). The percentage of dietary K+ excreted in urine was also significantly lower in blacks (50±18%) than in whites (69±17%; P.<0.001), suggesting greater excretion of K+ 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+ but could not detect a race difference in their excretion. Thus, although in blacks dietary intake of K+ appears to be definitely less than that for whites, the lower urinary excretion rates of K+ in blacks may also reflect a difference in K+ handling.

Race differences in the kidney disposition of K+ can be appreciated more easily by observing the kinetics of K+. Luft et al26 studied responses to graded increments in Na+ intake (10 to 1500 mmol/d) in blacks and in whites and where K+ intake was set at 80 mmol/d. At the lower intakes of Na+, there was a net accumulation of K+ in both race groups—the maximal amount reached was ~200 mmol in blacks and 100 mmol in whites. As the intake of Na+ reached higher levels, a kaliuresis ensued (earlier in whites than in blacks). At the highest intake of Na+, the net loss of K+ was in the range of 400 mmol in whites and <100 mmol in blacks. Thus, over a wide range of Na+ intakes, the fractional excretion of K+ was consistently less in blacks. One explanation would be greater K+ uptake by NKCC2. A concurrently enhanced uptake of Na+ would result in less aldosterone targeting ENaC, leading to even less K+ secretion.

K+ is a stimulus of aldosterone secretion, and reduced secretion rates in blacks could result from or be significantly influenced by a diet containing less K+. Urinary excretion

![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 of Cl− from what is observed in whites.24,25 In children, long before onset is delayed, plasma aldosterone levels and aldosterone excretion rates are lower in blacks than in whites.26 The results of ROMK inhibits NKCC2 because of less K+ in solution, thereby favoring its excretion (as opposed to its paracellular uptake). CLCNKB and BSND are required for maintaining efflux of Cl− and are rate limiting for the optimal function of the cotransporter. Activation of CASR by extracellular Ca++ has an effect to inhibit indirectly the cotransporter. ROMK indicates K+ channel; CLCNKB, Cl− channel; BSND, Bartter syndrome, infantile, with sensorineural deafness; also known as barttin; and CASR, Ca++-sensing receptor.]

**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+ Excretion</th>
<th>Urinary Ca++ Excretion</th>
<th>Urinary Mg++ Excretion</th>
<th>Urine Osmolality</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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TAL indicates thick ascending limb; BP, blood pressure.
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; \(P<0.0001\)).\(^{37}\) 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\(^+\) levels as well.\(^{38,39}\)

TAL is a principal site for reclaiming the divalent cations, Ca\(^{2+}\) and Mg\(^{2+}\).\(^{40-45}\) A more active NKCC2 would render the tubular lumen more positively charged, thereby favoring their paracellular uptake. Blacks have been shown repeatedly to have lower urinary excretion rates of Ca\(^{2+}\) than whites\(^{44,45}\); Mg\(^{2+}\) excretion rates are also lower in blacks.\(^{33}\) The race difference in Ca\(^{2+}\) excretion remains when dietary Ca\(^{2+}\) is kept constant in the 2 groups,\(^{44,45}\) and gastrointestinal absorption of Ca\(^{2+}\) is actually higher in blacks than in whites.\(^{46}\) Although not the only site for Ca\(^{2+}\) and Mg\(^{2+}\) reabsorption, their lower excretion rates at the least suggest a higher level of NKCC2 activity in TAL.

**Differences in Water Conservation**

Blacks have a more recent ancestral history of living in sub-Saharan desert regions where a highly developed ability to reclaim Na\(^+\) was life sustaining. Bankir et al\(^{47}\) has suggested that a climatic adaptation even more relevant to survival was a means for conserving water in that survival is placed in jeopardy more quickly in the absence of water than in the absence of salt. Using data collected in earlier clinical studies, Bankir and her collaborators\(^{47,48}\) showed that blacks indeed concentrate urine to a greater extent than whites. Higher levels of vasopressin in blacks that could account for the race difference have been observed, but only in blacks who were hypertensive,\(^{49-51}\) and not in blacks who were normotensive,\(^{32,50}\) even when challenged with water deprivation.\(^{52}\) If not vasopressin dependent, the possibility remains that the renal medullary osmotic gradient that water in the collecting duct is reabsorbed against is greater in blacks. The gradient is partially a product of TAL activity in that Na\(^+\) reabsorbed here adds to the osmotic differential that draws water from the collecting duct; thus, one explanation for the more concentrated urine in blacks could be a more active TAL. For blacks to maintain a state of greater water conservation by this mechanism, vasopressin secretion must not fully “adjust” (secondarily decrease), which indeed may be the case, since lower levels of vasopressin have not been described in blacks.

**Differences in Response to Acute Perturbations of TAL Function**

A role for TAL in determining race differences in water conservation and urinary excretion of cations was studied by comparing the response in blacks and whites to complete inhibition of NKCC2 with a single dose of furosemide, 40 mg IV.\(^{32}\) At baseline and with ad libitum intake of water, urine concentrations and volumes were lower in blacks, but when water intake was restricted to a constant intravenous infusion, the race difference in water conservation disappeared. The baseline race differences in urinary excretion of K\(^+\) (44% lower in blacks) and Ca\(^{2+}\) (22% lower in blacks) were reduced by half in response to inhibition of NKCC2. Although a clear delineation of a mechanism for why blacks conserve water better than whites could not be determined with certainty, changes in cation excretion in response to furosemide were consistent with there being greater TAL activity in blacks.

Weder et al\(^{53}\) used a water-loading protocol to assess free water generation in cortical TAL, as well as distal convoluted tubule. These nphron regions are impermeable to water, and the tubular fluid becomes increasingly dilute because of removal of ions by NKCC2. The water loading suppresses the levels of endogenous vasopressin, and the final urine concentration is a gauge of the reabsorption of ions by NKCC2. Under these conditions, blacks produced a less dilute urine than whites, consistent with their having a lower level of NKCC2 activity in cortical TAL. The greater uptake of Na\(^+\) in medullary TAL (suggested by furosemide study) and reduced uptake in cortical TAL (revealed in water-loading study) would be complimentary for purposes of optimizing water conservation.

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

Aviv et al\(^{54,55}\) 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 urinary 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 hyperfiltration (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 hyperfiltration 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\(^{56}\) used the clearance of endogenous Li\(^+\) to estimate Na\(^+\) reabsorption in proximal and downstream nphron 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, even though 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.²⁰,³⁸,⁵⁹ For the rare mendelian forms of hypertension, the single gene mutations in every instance localize the increase in Na\(^+\) reabsorption to either the collecting duct⁶⁰,⁶¹ 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 = \(6\times10^{-37}\)) 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\(^++\).
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 Ca²⁺. 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**

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