

Central Role for ENaC in Development of Hypertension

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Na⁺ reabsorption by the epithelial Na⁺ channel (ENaC) in cortical collecting duct provides the final renal adjustment to Na⁺ balance, there being no further downstream Na⁺ transport system. This fact coupled with the responsiveness of ENaC to aldosterone, which conveys stimulation inversely proportional to the state of Na⁺ balance, places ENaC in a pivotal position to influence the risk for hypertension. Although several molecular variants of ENaC subunits have been identified, there has been no consistent demonstration of an association of any of the variants with hypertension. More compelling is the notion that ENaC activity does not fully adjust to an increase in Na⁺ reabsorption occurring elsewhere in the nephron, there being overstimulation by inappropriately elevated aldosterone levels. Additional evidence that the maintenance of hypertension can be dependent on ENaC is derived from the observed responses to the treatment of hypertensive individuals with inhibitors of ENaC. Described is a clinical trial in which black hypertensive individuals who did not fully respond to more traditional therapy were given amiloride, spironolactone, a combination of the two drugs, or placebo. Treatment with either of the active inhibitors of ENaC resulted in a substantial improvement in BP. In conclusion, evidence to date is supportive of the concept that an increase in Na⁺ transport by ENaC may be a common and requisite component of salt-dependent forms of hypertension.

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An increase in Na⁺ and water retention is required for the development of most forms of hypertension as was proposed by Guyton years ago (1) and to this day has never really been refuted. In instances in which the mechanism for the hypertension is known, in which an identified gene mutation defines it, generally speaking an increase in the function of the epithelial Na⁺ channel (ENaC) leads to the Na⁺ retention and elevation in BP (2). The bigger question and the issue addressed in the current review is the extent to which ENaC participates in the development of common forms of hypertension. A strong case can be made for the fact that ENaC is pivotally positioned, both anatomically and physiologically, to convey a dominant influence on the prevalence of hypertension. Studies of hypertension in relation to ENaC and its major regulator, aldosterone, are reviewed here, and a general mechanism for development of hypertension is formulated. Finally, a clinical trial of the effectiveness of ENaC inhibition to lower BP in patients with hypertension is described.

Common Molecular Variations in ENaC and Risk for Hypertension

The discovery of mutations in β - and γ -subunits of ENaC to explain Liddle syndrome (3,4), a severe form of low-renin

hypertension (5), was soon followed by a search for common genetic variants in ENaC subunits that affect susceptibility in less rare forms of hypertension. Of course variants were identified, and they were almost universally more common in black individuals, which fit nicely with the higher prevalence of low-renin hypertension in black individuals. After a number of years of study by several investigative groups, however, it remains unestablished that a given variant influences the risk for hypertension.

The first molecular variant to show an association with hypertension was T594M in the C-terminus of β -ENaC (Figure 1) in a study of black individuals who lived in London (6) (the variant is rare in white individuals). Frequencies of the variant were found to be 8.3% in hypertensive individuals and 2.4% in normotensive individuals. In addition, plasma renin activity was lower in carriers of T594M. Subsequent studies by others have for the most part failed to replicate the same relationship to hypertension (7,8). In another study, seven molecular variants, all in β -ENaC, with overall allele frequencies of 44% in black individuals and 1% in white individuals, showed no association with hypertension and showed no effect on function using two different *in vitro* techniques (7). In our own laboratory, we found a G442V variant in the extracellular loop (Figure 1) in β -ENaC (again occurring almost exclusively in black individuals) significantly associated with an index of ENaC activity, the urinary aldosterone/K⁺ ratio, in normotensive young people, but it did not associate with hypertension in a study of adults (8). We also found that the A663T variant in

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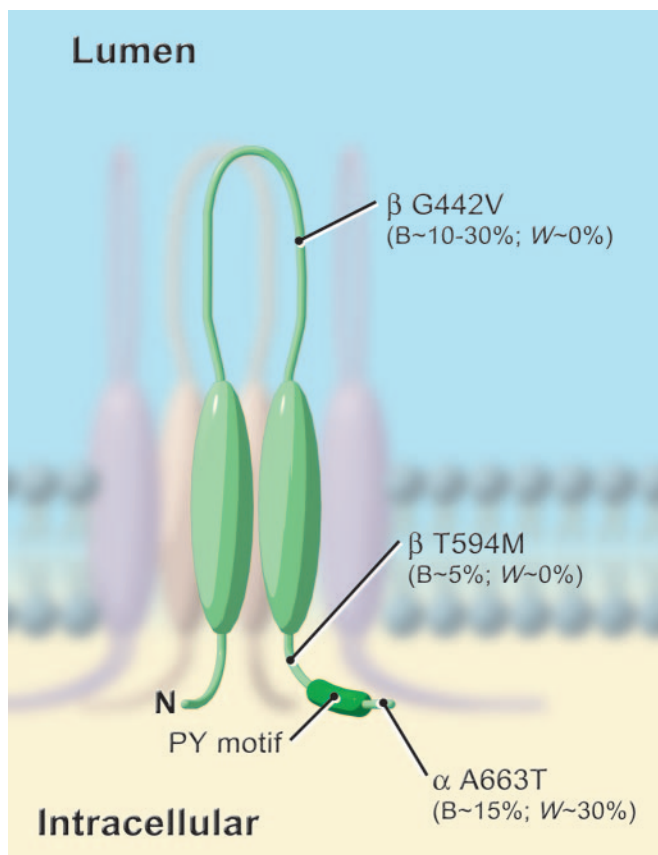


Figure 1. The locations along the subunits of representative molecular variants that have shown significant associations with BP (α -A663T, β -T594M) or an index of epithelial Na^+ channel (ENaC) activity (β -G442V). Allele frequencies are in brackets; B, black; W, white. Illustration by Josh Gramling—Gramling Medical Illustration.

α -ENaC near the PY motif on the carboxyl terminus (Figure 1), the only variant that was more frequent in white than in black individuals (30 versus 15%), associated with being normotensive as opposed to hypertensive in separate studies of white and black individuals (8). As an example of how difficult it is to find meaningful associations, Kleyman's laboratory, using a *Xenopus* oocyte expression system, found that the amiloride-sensitive current was increased not with the A but with the T allele at position 663 (9), the opposite of what our clinical studies would have predicted. Thus, the evidence is still mostly missing that common variations in ENaC influence the risk for hypertension. The higher frequency of variants in black individuals can be explained by the greater genetic diversity among black individuals (10).

Several explanations can be proposed for why the association studies of ENaC variants are so often inconclusive. To begin with, a modification that increases the activity of ENaC would not result in Na^+ retention if at the same time there were an appropriate downward adjustment in aldosterone secretion. Thus, a variant that affected function *in vitro* would in general fail to affect the *in vivo* expression, unless of course aldosterone secretion does not adjust appropriately (below). There obvi-

ously are many pressor and depressor influences that exist apart from ENaC that make it additionally difficult ever to detect associations using the phenotype hypertension.

The inconclusiveness of association studies, however, does not diminish the unlikelihood that all ENaC are equal. Its complexity and the multiplicity of factors that regulate it would seemingly lend ENaC vulnerable to genetic modification. Future studies are more likely to succeed in showing significant associations if the gene's net influence can be studied in contrast to the effect of an isolated variation. This can be accomplished through use of a series of single-nucleotide polymorphisms in linkage disequilibrium and with construction of haplotypes that are representative of the entire gene. The influence of the combined variation of a given ENaC subunit or of a regulatory protein, for example Nedd4-2, can be better appreciated.

Adaptations of ENaC to Increases in Na^+ Reabsorption

The key to whether a salt-dependent form of hypertension ensues or not may depend on the fidelity of the adjustments that take place at the level of ENaC. An example of where ENaC adjusts to increased Na^+ reabsorption (resulting in there being no increase in BP) and evidence of when it may not adjust (with increased risk for hypertension) are presented.

Increased Na^+ Reabsorption in Proximal Nephron Accompanied by an Appropriate Decrease in ENaC Activity: Studies in Normotensive Black and White Individuals

We tested the hypothesis that black individuals, a group at high risk for developing hypertension, have a greater level of ENaC activity when compared with white individuals, a group at lower risk. Participants were from a cohort of young people (mean age 17 yr), all of whom were normotensive (but inclusive of prehypertensives); thus, we avoided the confounding influences associated with hypertension and in particular its treatment. Black individuals of the cohort had consistently demonstrated lower aldosterone levels than the white individuals (Figure 2) (11), suggesting to us that greater Na^+ retention was suppressing the renin-angiotensin-aldosterone axis in black individuals. Black and white individuals were treated for 1 wk with a 5-mg/d dose of amiloride. The BP response was used to gauge ENaC activity (12). Measurements of BP were made after an overnight hospitalization in the General Clinical Research Center so as to minimize differences between the conditions at baseline and the posttreatment period. The results indeed were surprising to us. In response to amiloride, systolic and diastolic BP decreased in white but not in black individuals, with the differences between groups significant for both systolic ($P = 0.034$) and diastolic ($P = 0.010$) BP. Black individuals seemed to have less ENaC activity to inhibit. In a cross-sectional sampling of participants from the same cohort, the urinary excretion of K^+ was significantly lower in black than in white individuals (3.2 ± 0.1 versus 3.8 ± 0.1 mmol/mmol creatinine; $P < 0.0001$), and the serum K^+ concentration was significantly higher in black than in white individuals (4.35 ± 0.05 versus 4.21 ± 0.03 [SE] mmol/L; $P = 0.012$), both findings indicating less K^+

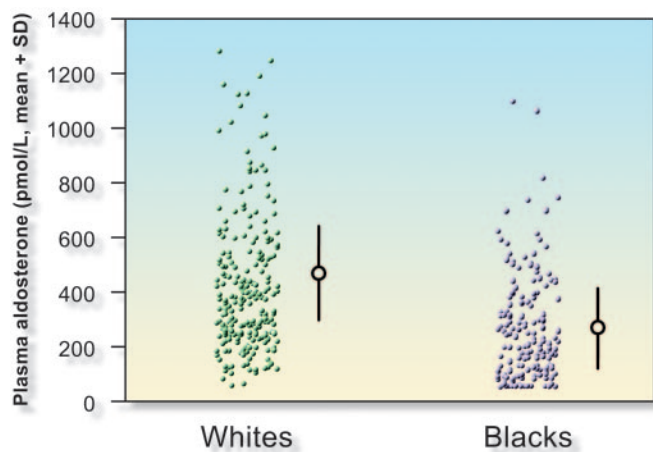


Figure 2. Plasma aldosterone concentrations in white and black school-aged children (pmol/L, mean \pm SD). The difference between groups was significant at $P < 0.001$. Illustration by Josh Gramling—Gramling Medical Illustration.

secretion in black individuals and consistent with less ENaC activity.

We concluded that the lower aldosterone levels in black individuals was the primary event and that ENaC activity was secondarily reduced—the opposite of what we had conjectured previously. We deduced from the findings that increased Na^+ reabsorption was occurring in another nephron region that led to suppressed aldosterone secretion and in turn a reciprocal decrement in Na^+ reabsorption by ENaC. Na^+ transport by ENaC adjusted appropriately to the increase in Na^+ reabsorption because the BP in black and white individuals were similar.

“Normal” Aldosterone Levels Lead to an Inappropriate Level of ENaC Activity

Unlike what we observed in young people without hypertension, ENaC may not adjust to an increase in Na^+ reabsorption in adults with the same facility. In a study of normotensive individuals from the Framingham Offspring Study cohort ($n = 1688$; mean age 55 yr), Vasan *et al.* (13) found a significant relationship between normal aldosterone levels and the BP 4 yr later. Specifically, there was a 16% increase in risk for an increase in BP and a 17% increase in risk for actually becoming hypertensive with each quartile increment in serum aldosterone level. The authors suggested that the regulation of aldosterone secretion developed along the lines that improved survival during an early ancestral period, when there was a scarcity of salt and water. There was no similarly compelling need for a full downward adjustment in aldosterone secretion to accommodate the higher intakes of Na^+ that typify modern-day diets (Figure 3). Thus, many people, some more than others, may be exposed to an excess of aldosterone with the potential for an inappropriately increased reabsorption of Na^+ by ENaC.

ENaC’s Pivotal Position Lends Itself to a “Two-Hit Model” for Development of Hypertension

If there is increased Na^+ reabsorption in a proximal region of the nephron, then a downstream site must respond with an

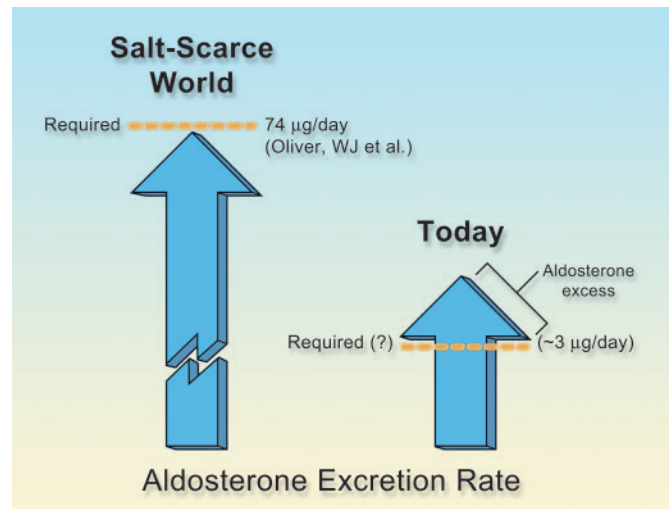


Figure 3. Aldosterone excretion rates in the Yanomamo Indians (21), who consumed an extremely low Na^+ diet, probably typical of the early ancestral period, when regulation of aldosterone secretion evolved to optimize the conservation of Na^+ for purposes of survival (Salt-Scarce World), and in individuals who consume a diet replete with Na^+ (Today). There presumably was no similar survival advantage for aldosterone secretion to decrease to where there would be an avoidance of an excess of Na^+ retention. Under low-salt conditions, excretion of aldosterone matches the amount required for Na^+ and water homeostasis. Today, with diets containing an abundance of salt, aldosterone excretion may exceed what is required, leading to an inappropriately elevated level of ENaC activity. The “required” aldosterone excretion rate when diets are replete with Na^+ was arbitrarily set at 3 $\mu\text{g}/\text{d}$. Illustration by Josh Gramling—Gramling Medical Illustration.

equivalent in magnitude reduction in Na^+ reabsorption if normal Na^+ and volume homeostasis are to be maintained. A principal downstream site is ENaC, with aldosterone delivering the level of stimulation to ENaC in keeping with the prevailing state of Na^+ balance. If ENaC fails to decrease reciprocally its activity and because there is no additional downstream site to adjust to the increase in Na^+ reabsorption by ENaC, then net Na^+ retention will take place. Thus, a scenario can be proposed, one requiring “two hits”: An increase in Na^+ reabsorption at a nephron site proximal to ENaC or possibly at ENaC itself (hit 1) together with a failure on the part of aldosterone secretion to decrease fully (hit 2), leaving ENaC operating at an inappropriately increased level. The orchestration of the kidney’s functions of course is more complex and fluid than such a simple scheme implies (Figure 4). For example, the pressure natriuresis that develops in response to the increase in BP would work toward restoring Na^+ homeostasis followed by normalization of BP. Whether a hypertensive state ensues would also depend on the pressure-natriuretic responsiveness of the kidney and thus the possible requisite for a third hit. Nonetheless, ENaC, because of its distal location in the nephron and because of its regulation being driven by aldosterone, would seem to be at the

center of the development of commonly encountered forms of hypertension. Proof of the concept will rest with demonstration of a combination of molecular or genetic variations acting together to increase risk for hypertension. That such a pairing of genes can affect BP was suggested in an association study in which individuals with a *HindIII* restriction site on the Y-chromosome were at increased risk for hypertension if they were also carriers of a variant in the aldosterone synthase gene (14).

Clinical Trial of ENaC Inhibition

To test further whether ENaC is integral to the pathophysiology of hypertension, one can remove its influence through targeted drug therapy. We had early anecdotal evidence that small doses of spironolactone, an aldosterone receptor antagonist, normalized BP when added to the existing antihypertensive therapy of black individuals with suppressed plasma renin activity (15). A study of larger numbers of subjects by Calhoun *et al.* (16) showed that regardless of ethnicity, spironolactone was effective in otherwise treatment-resistant hypertensive individuals. We recently reported on a prospective, randomized, double-blind, placebo-controlled clinical trial of ENaC inhibition in hypertension (17). Enrollment was limited to black individuals because of their known greater predilection to retain Na^+ (18).

Study Design and Procedures

The study used a two-by-two factorial design with four treatment groups: Amiloride (10 mg/d), a direct inhibitor of ENaC; spironolactone (25 mg/d); the combination of both drugs; and placebo. Patients ($n = 98$) were between 18 and 75 yr of age (mean age 46 yr), with a systolic BP >140 and/or a diastolic BP >90 mmHg while receiving hydrochlorothiazide (minimum dose of 25 mg) or furosemide (minimum dose of 40 mg) or

equivalent doses of similar diuretics and amlodipine 5 or 10 mg or equivalent doses of a similar calcium channel blocker. Other drugs could be used (*e.g.*, β blockers), but triamterene, angiotensin-converting enzyme inhibitors, and/or angiotensin receptor blockers were discontinued for 1 mo before the study was started because of the added risk for hyperkalemia. Exclusion criteria were an elevated plasma renin activity and evidence of a secondary form of hypertension. BP measurements were made at baseline and at weeks 1, 3, 5, 7, and 9 of active treatment. The study was designed with the primary end points being the changes from baseline in systolic and diastolic BP.

Outcomes

The reductions in systolic and diastolic BP (mmHg; mean \pm SEM) in responses to the additional treatment were, respectively, 9.8 ± 1.6 and 3.4 ± 1.0 for amiloride and 4.6 ± 1.6 and 1.8 ± 1.0 for spironolactone. Amiloride significantly reduced both systolic and diastolic BP ($P < 0.001$). Spironolactone produced a significant reduction in systolic BP ($P = 0.006$) and a marginally significant reduction in diastolic BP ($P = 0.07$). Figure 5 shows the adjusted mean changes from baseline in BP. The largest drop in BP occurred at 3 wk, but it continued to decrease until week 7, at which point values stabilized for each of the treatment groups. The amiloride-spironolactone combination group showed the greatest reduction in BP followed by amiloride alone and spironolactone alone. We observed, however, no significant interaction of amiloride with spironolactone.

The BP-lowering response was greater for amiloride than for spironolactone, but a response similar to that seen with amiloride might have occurred had a larger dose of spironolactone been used. The superior response to amiloride suggests that it was indeed ENaC inhibition that led to the reduction in BP rather than another aldosterone response site for sodium reabsorption (19,20). The results of targeting ENaC for inhibition are

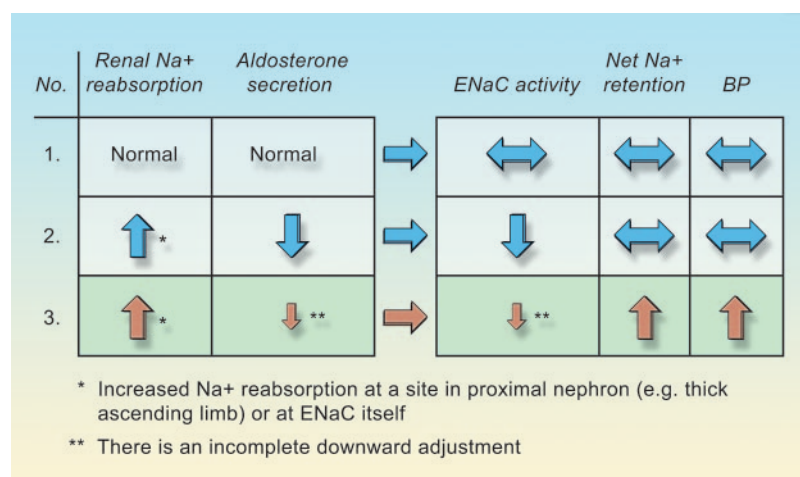


Figure 4. An ENaC-mediated mechanism for the development of hypertension. In the normotensive state, there is no increase in Na^+ reabsorption (example 1), or there is an increase in Na^+ reabsorption that is accompanied by an appropriate downward adjustment in aldosterone secretion (example 2). An increase in BP occurs when there is an increase in Na^+ reabsorption, with incomplete suppression of aldosterone secretion leading to incomplete suppression of ENaC activity (example 3). Because of the pivotal position of ENaC within the nephron, there being no downstream Na^+ reabsorptive site to adjust reciprocally to the increase in Na^+ reabsorption by ENaC, an increase in BP can ensue. Illustration by Josh Gramling—Gramling Medical Illustration.

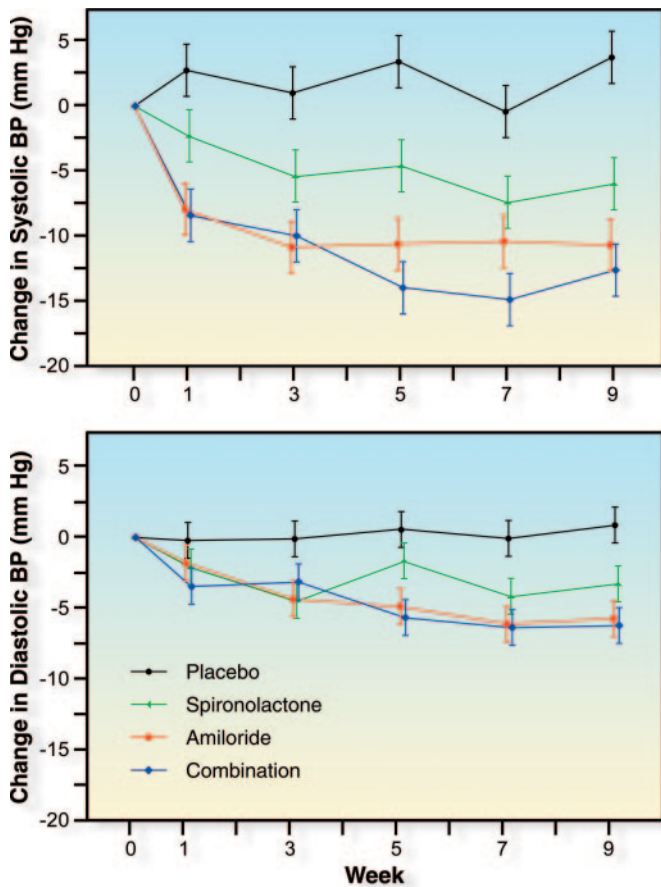


Figure 5. Changes from baseline in systolic and diastolic BP. The decrease in systolic BP was significant for all of the treatment groups when compared with placebo: $P < 0.001$ for amiloride, $P < 0.010$ for spironolactone, and $P < 0.001$ for the combination of amiloride and spironolactone. The decrease in diastolic BP was significant for the amiloride-treated group ($P = 0.003$) and for the combination of amiloride and spironolactone ($P = 0.002$) when compared with placebo. Illustration by Josh Gramling—Gramling Medical Illustration.

consistent with a significant role for ENaC in the maintenance if not the development of common forms of hypertension.

Conclusion

Evidence has been presented that taken together places ENaC in a central position for creating the Na^+ retention that is necessary to achieve a state of hypertension. It would seem a mistake in considering mechanisms for hypertension to think that a given Na^+ reabsorptive site acts alone—the evidence is compelling for consideration of ENaC as the additional requisite participant.

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