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PERSPECTIVES IN BASIC SCIENCE

Liddle syndrome: An autosomal dominant form of human hypertension

DAVID G. WARNOCK

Departments of Medicine and Physiology & Biophysics and Nephrology Research and Training Center, University of Alabama at Birmingham, UAB Station, Birmingham, Alabama, USA

Liddle syndrome is a rare form of autosomal dominant hypertension with early penetrance and impressive cardiovascular sequelae. The basic features of this syndrome were described in a large kindred from Alabama by Grant Liddle and co-workers in 1963 [1]. In addition to severe hypertension, many of the patients had overt hypokalemia. Despite having the clinical presentation typical of primary aldosteronism, the actual rates of aldosterone excretion were markedly suppressed, accounting for the descriptive term "pseudoaldosteronism." Liddle's original publication is a classic description of clinical investigation; spironolactone, a mineralocorticoid antagonist did not have any discernible effect, while triamterene normalized the blood pressure, reversed the renal potassium wasting and corrected the hypokalemia as long as the subjects restricted their dietary salt intake. Liddle correctly interpreted these findings as indicating an intrinsic renal defect in the regulation of salt absorption rather than the effects on the distal tubule of some unidentified mineralocorticoid other than aldosterone [1]. This interpretation has been strengthened by the finding that renal transplantation also corrects most of the abnormalities in Liddle syndrome [2, 3].

Liddle syndrome is an extreme example of low renin, volume-expanded hypertension. Tremendous progress has been made in understanding unusual forms of low renin hypertension at the level of discrete genetic mutations [4]. We now have much better knowledge of the underlying pathophysiology of glucocorticoid-remediable aldosteronism [5], Liddle syndrome (pseudoaldosteronism) [6], and the apparent mineralocorticoid excess syndrome [7]. The functional effects of the specific mutations described in each of these syndromes have been confirmed with expression studies in various systems. In general, inappropriate renal Na^+ retention with subsequent volume expansion, low plasma renin activity and hypertension are the consequences of unusual or inappropriate mineralocorticoid excess, or in the case of "pseudoaldosteronism," apparent mineralocorticoid excess that results from constitutive activation of the amiloride-sensitive epithelial Na^+ channel (ENaC) in the terminal nephron segments.

The original pedigree described by Liddle, Bledsoe and Coppage [1] was Caucasian. Other pedigrees, including Japanese [8–10], Hispanic [11], and African-American [12] families have been described. These cases may not have presented with severe hypertension at an early age, but in retrospect, after extensive (and expensive) diagnostic work-ups for secondary hypertension, these patients were found to have pseudoaldosteronism with long-standing hypokalemia and hypertension.

The original index case ("GS") developed renal failure in 1989, and underwent successful renal transplation (cadaver donor) at the University of Alabama at Birmingham. The subsequent improvement in her condition [2] confirmed Liddle et al's theory that pseudoaldosteronism is "a disorder in which the renal tubules transport ions with such abnormal facility that the end result simulates that of a mineralocorticoid excess" [1]. A previous report from Paris described a pair of sisters with Liddle syndrome who developed renal insufficiency, presumably from hypertension; the older sister underwent renal transplantation at the age of 23, and her hypokalemia and severe hypertension were corrected [3]. These cases are notable because the incidence of severe renal insufficiency in Liddle syndrome is distinctly unusual [2]. Cardiovascular and cerebrovascular complications of hypertension are much more common findings, and the usual cause of death in undiagnosed or untreated patients.

GENETIC LINKAGE STUDIES AND MUTATION ANALYSES

The size of the original pedigree and careful clinical assessment of the members at risk [2] demonstrated that Liddle syndrome is an autosomal dominant disorder, and also provided the material required for genetic linkage analysis that permitted the identification the causal mutation in the beta subunit of the amiloride-sensitive Na^+ channel [6]. The subunits of the epithelial sodium channel (ENaC) complex have been defined by expression cloning in the *Xenopus* oocyte expression system, were recognized as important candidate genes for the cause of Liddle syndrome at the time of the initial description of the structure and function of the channel subunits by Canessa and Rossier [13, 14]. The ENaC complex is composed of three homologous subunits, each of which has two membrane spanning regions and a large extracellular domain with glycosylation sites and cysteine-rich regions. The N′- and C′-terminal domains of each subunit are located in the cytoplasm, but comprise relatively minor amounts of the protein

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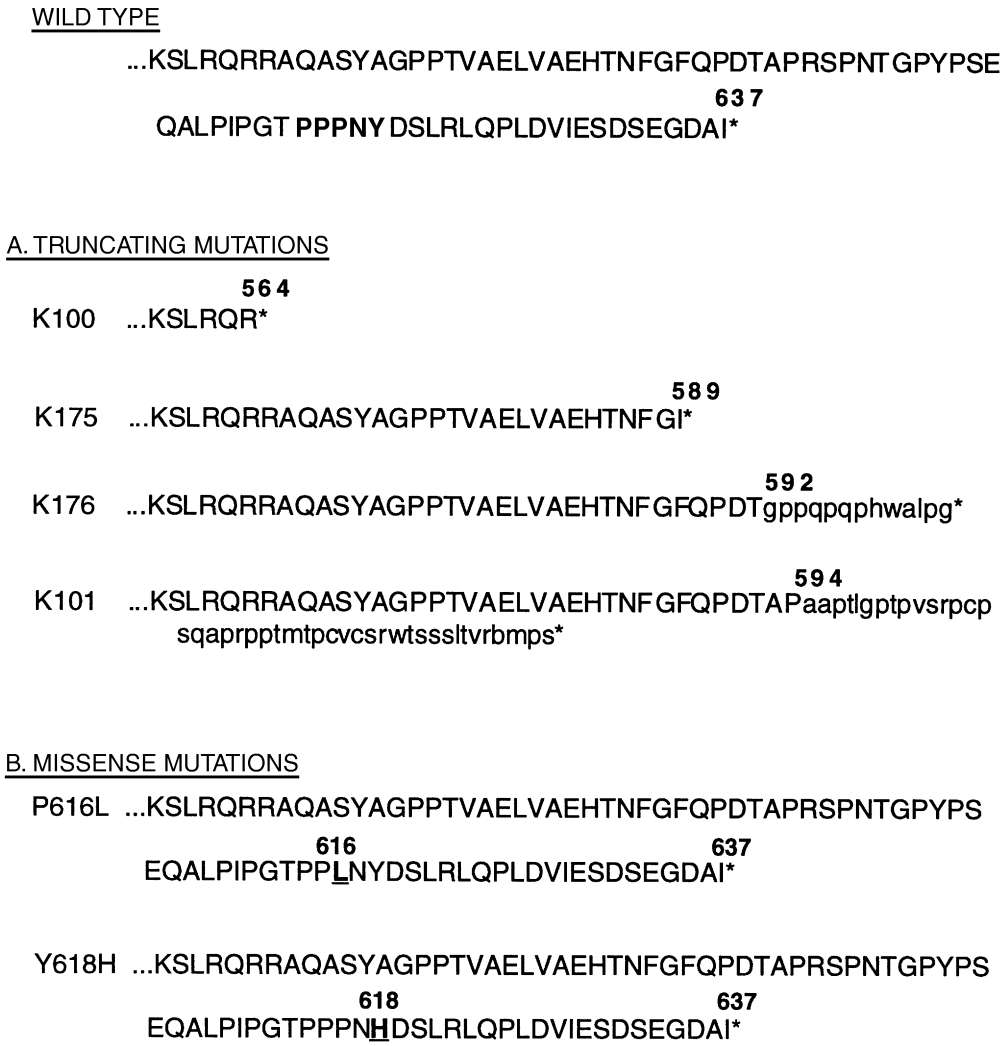

Fig. 1. Cytoplasmic domains of the mutated beta subunits in Liddle syndrome. The wild-type sequence is shown at the top; each of the subsequent listings represent distinct pedigrees. The outlined PPPNY sequence in the wild-type represents a Nedd4 binding domain [22], which is deleted in each of the affected pedigrees described in the original publication [6]. (*A*) Truncating mutations. Pedigrees K100 (the original pedigree described by Liddle) and K175 have premature terminations of the beta subunit (βR564X, and βF589IX) due to mutations which introduce stop codons into the translated sequence. Pedigrees K176 and K101 have frame-shifts with abnormal protein sequences followed by new stop codons. (*B*) Missense mutations. Point mutations are illustrated (underlined) which change the critical PPPNY domain of the beta subunit [10, 21, 46].

chains. The actual stoichiometry of the ENaC complex has not yet been determined. Each ENaC complex is assumed to contain at least one of each of the three currently defined subunits, but the actual ratio of subunits needs to be defined. These issues are thoroughly reviewed in the recent Homer Smith Lecture by Bernard Rossier [15].

While the genetic linkage results clearly implicated the beta subunit as the locus of interest, the most striking finding was the demonstration of premature stop codons and frame-shifts in four independent pedigrees [6]. The identification of these mutations in the coding domain of the beta subunit was guided by analogous mutations previously described in homologous gene products that are expressed in *Caenorhabditis elegans*, which appear to be involved in mechanotransduction [16–18]. The mutations described in *C. elegans* were in the second transmembrane region, and the original mutations described in Liddle syndrome was immediately adjacent in the cytosolic tail of the beta subunit [6]. Description of these specific mutations has established Liddle syndrome as a cardinal example of familial hypertension in which a physiologically plausible candidate gene has been functionally linked to the underlying pathophysiology of the disease.

The deduced amino acid sequence of the beta subunit, and the location of its currently described mutations [6] are presented in Figure 1. It is curious that all of the defined mutations in pedigrees with Liddle syndrome fall within a very narrow range of 30 amino acids of the cytosolic tail of the beta subunit. An analogous mutation to that in the original pedigree (βR564X) has been described in the gamma subunit [19]. Although suggested by expression studies in the oocyte system [20], causal truncating mutations in the cytosolic tail of the alpha subunit have not yet been described in any human subjects.

Missense mutations in the proline-tyrosine motif (PPPXY) of the beta subunit have also been described which constitutively activate ENaC activity [10, 21]. These missense mutations are also

illustrated in Figure 1, and confirm the importance of this region previously suggested by *in vitro* expression studies [20] as well as studies of the apparent interaction of these regions with alpha spectrin (for the alpha subunit) and the Nedd4 protein for all three subunits [22, 23]. The latter protein appears to play a role in the assembly, insertion and/or or retrieval of the ENaC complex membrane in the plasma membrane.

EXPRESSION STUDIES

Oocyte expression studies have defined the alpha subunit as the most critical entity in the channel complex which by itself can express amiloride-sensitive sodium channel activity [13, 24–26]. Kleyman and co-workers have recently identified an 8 amino acid stretch in the extracellular loop of the alpha subunit that appears to be intimately involved in the amiloride-binding site [27]. Schild et al [28] have recently used site directed mutagenesis to define a short region (pre-M2 segment) that immediately precedes the second membrane spanning region of all of the defined members of the ENaC gene family that strikingly affects cation permeation and amiloride sensitivity. These pre-M2 segments are hypothesized to be involved in the transmembrane ion permeation pathway [28]. The beta and gamma subunits appear to stabilize the expression of alpha subunit [13, 24, 25], and also appear to participate in the ion permeation pathway and to convey amiloride sensitivity [28]. It would not be surprising if additional mutations were discovered in the cytosolic and pre-M2 domains of the beta and gamma subunits, judging from their role in the expression of ENaC activity. Because of its critical role, truncating mutations in the alpha subunit have been associated with complete non-functioning of the channel complex (pseudohypoaldosteronism, type I [29]). Resistance to inhibition by amiloride and triamterene could also result from mutations in the extracellular loops of the subunits, but these have yet to be described in human subjects.

CELLULAR PHENOTYPE

We have extended earlier studies [30] of red cell Na^+ fluxes in Liddle syndrome, and have described, in affected members of the original pedigree, constitutive activation of the amiloride-sensitive Na^+ channel in B lymphocytes [31]. While it is not yet established that the same factors regulate the amiloride-sensitive sodium channel in the B cell as in the principal of the collecting tubule, it has been demonstrated with whole cell patch clamp studies that the amiloride-sensitive sodium channels in both systems are pharmacologically and functionally quite similar [32, 33]. At present, specific mutations in the beta subunit have been identified in a number distinct kindreds (Fig. 1). While continued surveys of affected kindreds such as described by Gadallah, Abreo and Work [12] may ultimately define the entire set of relevant mutations in pseudoaldosteronism, the functional screening of the relevant cellular phenotype by electrophysiologic or fluorescence-based techniques may also be an important approach to define the extent of constitutive activation of epithelial sodium channels in low-renin hypertension.

The previous studies of cellular physiology clearly demonstrate activated sodium channel activity in non-epithelial tissues [30, 31]. It seems certain that expression of ENaC-like activity is not, therefore, limited to classical epithelial tissues. We have recently demonstrated that the beta ENaC subunit mRNA can be demonstrated in lymphocytes by reverse transcriptase/polymerase chain reaction, and that lymphocytes from the original Liddle's pedigree demonstrate an equal mixture of wild-type (WT) and mutated (βR564X) messages, consistent with autosomal dominant inheritance [34]. It has not yet been possible to define the sequence of the putative alpha subunit expressed in lymphocytes, presumably due to extremely low message abundance. Recently, several other variants of the alpha subunit have been described [35–38]. Thus, there may be a number of members of the ENaC gene family, and their expression is not limited to epithelial systems.

MOLECULAR PATHOPHYSIOLOGY

The identification of specific Nedd4 and alpha spectrin binding domains in the cytosolic region of the ENaC subunits [22, 23], and the analysis of these domains by site directed mutagenesis [20] suggest that interactions with cytoskeletal elements may control the expression of ENaC complexes at the membrane surface. The well described interactions of ENaC with actin and other cytoskeletal elements are notable in this regard [39]. Therefore, some sort of membrane retrieval mechanism may be important in regulating ENaC activity. It appears that this process is effected by interactions of the PPPXY sequence in the cytosolic tails of the ENaC subunits with the cytosolic retrieval mechanism [20, 22, 23]. It is notable that all of the mutations described in Liddle syndrome which are associated with constitutive activation of ENaC activity, either truncate the beta or gamma subunits before the critical PPPXY domain, or cause missense mutations within this domain (Fig. 1).

None of the previous expression studies have definitively distinguished between constitutive activation due to increase open probability of individual channels, or increased number of channels expressed at the membrane surface. Rossier and colleagues [24] have recently examined this issue in the oocyte expression system using ENaC constructs to which a defined epitope (FLAG®, Kodak; DYKDDDDK) was added to the external loop of each subunit. These additions did not affect channel activity, but permitted immunoprecipitation and surface labeling. With this approach, surface expression of the ENaC complex could be quantitated by binding of iodianted monoclonal antibodies to the FLAG epitope on the surface of intact oocytes.

There was nearly a fivefold difference in amiloride-sensitive Na^+ current when ENaC was expressed in oocytes with the originally described beta subunit mutation, as was previously described [40]. While there was a 1.9-fold increase in surface binding of the ENaC complex when the βR564X construct was compared to the wild-type (WT), there was a much larger increase ($>$5-fold) when the slopes of the current/binding site regressions were compared (Fig. 2). Therefore, the major effect of the originally described truncating mutation was to increase the conductance of the individual ENaC complex (such as the open probability), rather than to increase the number of channels in the plasma membrane. Thus, the membrane retrieval theory can account for some of the increased constitutive activation observed with the truncating beta mutation, but a far greater effect is observed when the current per binding site is considered.

Additional insights into the regulation of amiloride-sensitive sodium channel activity has been obtained from complexes reconstituted in lipid bilayers [41], and from the B lymphocyte system [42]. Sodium channel activity can be described with a dual regulation model, including reciprocating pathways for protein

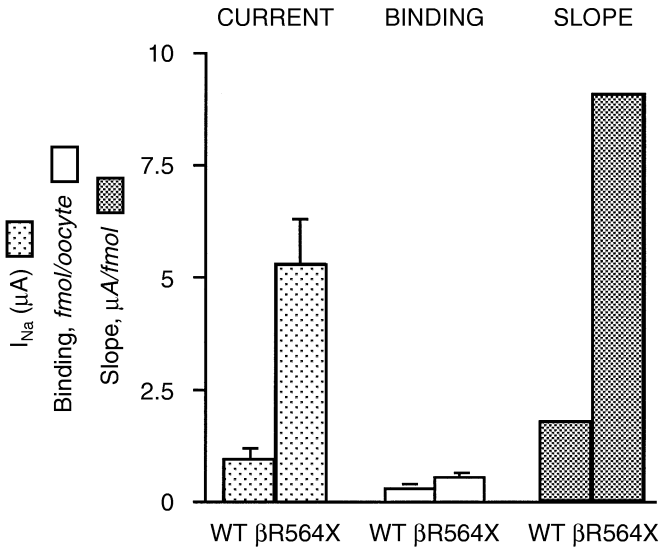

Fig. 2. Effects of a beta subunit truncating mutation (βR564X) on the amiloride-sensitive sodium current, surface binding, and current per binding site for wild type (WT) and the mutation described in the original Liddle pedigree (βR564X). Amiloride-sensitive current (μM) per oocyte was measured with a two electrode technique at −100 mV. Binding sites (fmol/oocyte) were determined in individual oocytes with an ^{125}I-labeled monoclonal antibody to the FLAG epitope. The slope (μA/fmol), which is a measure of amiloride-sensitive current normalized for surface expression of ENaC was obtained from the regression of current versus binding sites for at least 15 oocytes injected with the WT or βR564X cRNA along with alpha and gamma cRNA. Each of the ENaC subunits incorporated the FLAG epitope into the extracellular loop. Modified from reference [24]. Symbols are: (▨) I_{Na} μA; (■) slope μA/fmol; (□) binding fmol/oocyte.

kinase A (PKA) and protein kinase C (PKC) in B cells and the oocyte expression system [33, 43]. Constitutive activation of sodium channel activity with the βR564X mutation may reflect enhanced sensitivity of the channel complex and its associated proteins [39] to the activating effects mediated by PKA. Inhibition of PKA with a competitive inhibitor of cAMP binding to the regulator site [44] reduced the constitutive activated sodium current in lymphocytes from affected patients to basal levels of activity [42]. Of interest, addition of the missing terminal ten amino acids of the beta subunit also restored the current to basal levels in both the B cell [42] and reconstituted channel system. [41]. This specific peptide (IESDSEGDAI) has a net negative charge and is deleted by the truncating mutations shown in Figure 1A. It was hypothesized that this peptide interacts with some target for PKA-mediated phosphorylation on the channel complex (subunits or associate cytoskeletal elements), and affects the activity of the complex by altering its phosphorylation state [41, 45]. This explanation may not be relevant for the missense mutations shown in Figure 1B since the terminal peptide has not been conserved. In addition, truncation of the terminal ten amino acids of the beta subunit does not activate the rat ENaC system expressed in oocytes [20], while the same truncation does appear to be activating when human ENaC subunits are expressed in oocytes [26]. While the effects of the terminal peptide are somewhat controversial, it does appear certain that originally described mutation (βR564X) effects constitutive activation of the channel complex *per se* [24], and that this activation appears to represent an enhanced sensitivity to the activating effects (via PKA) of normal endogenous cAMP levels [33, 42]. Further studies are needed to define the phosphorylated components of the channel complex and/or its associated cytoskeletal elements, and the mechanism by which the PKA-mediated phosphorylation causes constitutive activation of amiloride-sensitive sodium channel activity. A T594 M mutation has been described in the beta subunit of some African-Americans which appears to increase the cAMP-sensitivity on sodium channel activity in B lymphocytes [46]. There was not any obvious relationship of this polymorphism to hypertension or plasma renin levels in these individuals, while there was an increased plasma aldosterone level in these subjects. Nevertheless, this study raises the possibility that polymorphisms that exist in the general population may affect the sensitivity of ENaC to regulator influences [46, 47].

Table 1. Urinary steroid profiles in mendelian forms of low renin hypertension

	Liddle syndrome	GRA	AME
Aldosterone	⇓⇓	↑	↓
TH-Aldo	⇓⇓	↑	↓
18-OH-TH-Aldo	⇓⇓	↑	↓
18-OH F	–	⇑⇑	–
TH-F	nl	nl	⇑
TH-E	nl	nl	⇓
TH-F/TH-E	nl	nl	⇑⇑

Urinary steroid profiles are performed with gas chromatography/mass spectroscopy [58] by the Quest Diagnostic Lab (San Juan Capistrano, CA, USA). Abbreviations are: GRA, glucocorticoid remedial aldosteronism; AME, apparent mineralocorticoid excess; nl, normal; –, not usually detected; TH-aldo, tetrahydro-aldosterone; 18-OH-TH-Aldo, 18-OH tetrahydro-aldosterone; 18-OH F, 18-OH cortisol; TH-F, tetrahydro-cortisol; TH-E, tetrahydro-cortisone; TH-F/TH-E, ratio of TH-F to TH-E.

CLINICAL DIAGNOSIS OF LOW RENIN HYPERTENSION

At present, Liddle syndrome joins the ranks with glucocorticoid-remediable aldosteronism (GRA) as Mendelian forms of severe hypertension marked by early penetrance and substantial target organ damage. Glucocorticoid-remediable aldosteronism is another rare, autosomal dominant form of familial hypertension for which the causal genetic defect has recently been identified by Lifton and co-workers [5, 48]. This syndrome is caused by a chimeric gene duplication arising from unequal crossing-over, such that the regulatory sequences of steroid 11 β-hydroxylase control the expression of the coding sequences of aldosterone synthase. The chimeric gene product is expressed, under the control of ACTH, in both the zona glomerulosa and zona fasciculata [49]. Since the chimeric gene product is expresses throughout the adrenal cortex, the production of 18-hydroxy cortisol and aldosterone metabolites is increased and can be suppressed by dexamethasone suppression of ACTH secretion.

A number of cross-over points have been described in a number of different pedigrees, but the cross-over point in a given pedigree is conserved and contained within the intron 2 to intron 4 stretch of the parental genes. The possibility of a susceptibility allele for GRA of Irish origin is suspected [50], and the syndrome has not yet been described in African-Americans. While the severity of hypertension, early penetrance, and autosomal dominant inheritance [50] are similar to Liddle syndrome [2], these disorders can be readily distinguished by the pattern of urinary steroid excretion

with increased 18-OH-cortisol and 18-oxocortisol in GRA, and suppressed aldosterone and aldosterone metabolite excretion in Liddle syndrome.

The apparent mineralocorticoid excess syndrome (AME) is another rare Mendelian form of hypertension that is most often inherited as an autosomal recessive disorder [7, 51]. Mutations in this disorder disrupt the 11-β-OH steroid dehydrogenase, type II gene which uni-directionally metabolizes cortisol to cortisone, and prevents cortisol from expressing mineralocorticoid effects in target tissues. With these mutations, or with exogenous inhibitors of this enzyme (such as licorice [52]) a fairly typical picture of mineralocorticoid excess is seen, including hypertension and potassium wasting with metabolic alkalosis. Once again, the definitive clinical diagnosis is made by analysis of the urinary steroid profiles since there is increased excretion of reduced cortisol metabolites compared to reduced cortisone metabolites. In addition, the mineralocorticoid excess and resulting volume expansion suppresses plasma renin activity and aldosterone secretion.

The incidence of Liddle syndrome [12] and GRA [50] may be greater that originally appreciated, and an appropriate level of clinical suspicion must be maintained. Analysis of urinary steroid profiles is worthwhile, and represents a cost-effective approach to the work-up of secondary hypertension. While low-renin hypertension in the adult is relatively common, and the great majority of these individuals will not have one of the currently described Mendelian causes of hypertension [4], the finding of low renin hypertension in a child, especially with a provocative family history warrants a consideration of the monogenic causes of hypertension [53]. Overt hypertension may be detected in affected patients with either Liddle syndrome and GRA without overt hypokalemia or renal potassium wasting [2, 50]. Therefore, the clinical adage that unexplained hypokalemia in a hypertensive patient is an indication for working-up secondary causes of hypertension is not all inclusive. Patients will be missed who are not overtly hypokalemic but do have a secondary cause of their hypertension. The true prevalence of Liddle syndrome and GRA in low-renin hypertension remains to be defined, but only account for a minority of such cases. Clinicians can sharpen their diagnostic skills by recognizing the importance of a strong family history of severe hypertension with target organ damage, and gaining a working familiarity with the analysis of urinary steroid excretion patterns as they pertain to Liddle syndrome, GRA and the Apparent Mineralocorticoid Excess syndrome (Table 1). In the more common forms of low renin hypertension for which a genetic cause is not yet defined, the measured aldosterone levels and excretion are relatively normal despite suppression of plasma renin activity, suggesting a state of relative mineralocorticoid excess [54]. Bilateral adrenal hyperplasia or adrenal adenomas would present with suppression of plasma renin activity, but the plasma levels and urinary excretion of aldosterone and its metabolites would be distinctly elevated. In fact, plasma aldosterone levels may be normal even though the 24-hour urinary excretion of aldosterone metabolites is distinctly elevated in some patients with adrenal adenomas (personal observation).

TREATMENT OF LIDDLE SYNDROME

While the monogenic forms of human hypertension are quite rare compared to the usual form of low renin hypertension seen in African Americans [47, 55], several relevant therapeutic insights have emerged from the studies of the Mendelian forms of human hypertension. Treatment of Liddle syndrome has increased our appreciation of the use of K^+-sparing diuretics. While spironolactone is not useful in Liddle syndrome [1], it could be considered in low renin hypertension since the aldosterone excretion rates are "normal" despite suppression of plasma renin activity [47, 54]. In Liddle syndrome, there appear to be some pedigrees that are more responsive to amiloride and others in whom triamterene is more effective [45]. In every instance, the utility of amiloride and triamterene are enhanced by dietary salt restriction. This clinical observation is understandable in light of the known competition between these agents and Na^+ at the level of the ENaC conducting pore [56].

Low cost preparations of amiloride and triamterene are available that contain either agent in combination with a thiazide diuretic, but the amount of thiazide is excessive compared to the amount of K^+ sparing diuretic. While these combinations are useful in treating the underlying Na^+ retention, the thiazide component may worsen renal K^+ wasting in susceptible patients, especially if dietary salt intake continues to be excessive. Rather than fixed dose combinations, it may be necessary to specifically increasing the dosage of triamterene or amiloride as well as reducing dietary salt intake in patients receiving thiazides or furosemide for their effective volume expansion.

While amiloride or triamterene alone may be adequate to control the hypertension in patients with Liddle syndrome, older patients may not have adequate blood pressure control despite resolution of their K^+ wasting with these agents. Continued use of the K^+ sparing agents is worthwhile, but the hypertension must be treated aggressively with vasodilators and/or beta blockers to minimize cardiovascular and cerebrovascular morbidity.

Since the causal mutations are conserved within pedigrees with Liddle syndrome, it is possible to use genetic screening at a young age of children at risk. While pre-natal diagnosis does not seem worthwhile, and any such genetic screening raises serious legal and ethical issues [53], genetic counseling is useful in this autosomal dominant condition if one of the parents is known to be affected. The utility of this approach is to enhance and target surveillance of at-risk subjects at an early age since each child of an affected parent has a 50% chance of inheriting the mutated allele from the affected parent. It should be kept in mind that there were a few subjects described in the original pedigree [2] who were not overtly hypertensive or hypokalemic despite being subsequently identified as carrying the causal mutation [6]. Continued surveillance of these subjects is necessary.

Can any of these principles be applied to treatment of the much more common form of low renin hypertension in which the genetic cause(s) have yet to be identified? The utility of dietary salt restriction, and a more aggressive use of the K^+ sparing diuretics would seem worthwhile. It is probably important to coordinate the administration of the K^+ sparing diuretics with the pattern of dietary salt intake. Bedtime dosing with amiloride or triamterene is not recommended when the bulk of the dietary salt intake and excretion occurs during waking hours. It is possible that there are subsets of patients who may respond to amiloride but not to triamterene, and others that would best be treated with triamterene rather than amiloride. Therefore, both agents should be tried in an individual patient before abandoning the use of K^+

sparing diuretics. These agents would seem preferable to spironolactone because of its anti-androgen side effects (gynecomastia, benign prostatic hypertrophy, etc.), but spironolactone is certainly recognized as a component of the therapy of primary aldosteronism [57]. If renal function is normal, and dietary K^+ intake is not excessive, there should not an be untoward incidence of hyperkalemia in patients with low renin hypertension who are treated with K^+ sparing diuretics, but monitoring of the serum electrolytes is worthwhile.

Finally, it is important to remember that there are well described examples of patients who are hypertensive and have Liddle syndrome [2] or GRA [50], but who are not overtly hypokalemic. This observation greatly weakens the use of hypokalemia as screening test for secondary and/or endocrinologic causes of human hypertension. Despite the lack of overt potassium wasting, an argument can be made for the use of K^+-sparing diuretics in these patients, and by inference in patients with low renin hypertension without a defined genetic cause if an underlying cause of the enhanced renal Na^+ absorption is dysregulation of ENaC activity, especially when dietary salt intake is increased [47].

Reprint requests to David G. Warnock, M.D., Division of Nephrology, University of Alabama at Birmingham, UAB Station, Birmingham, Alabama 35294-0007, USA.
E-mail: dwarnock@nrtc.dom.uab.edu

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