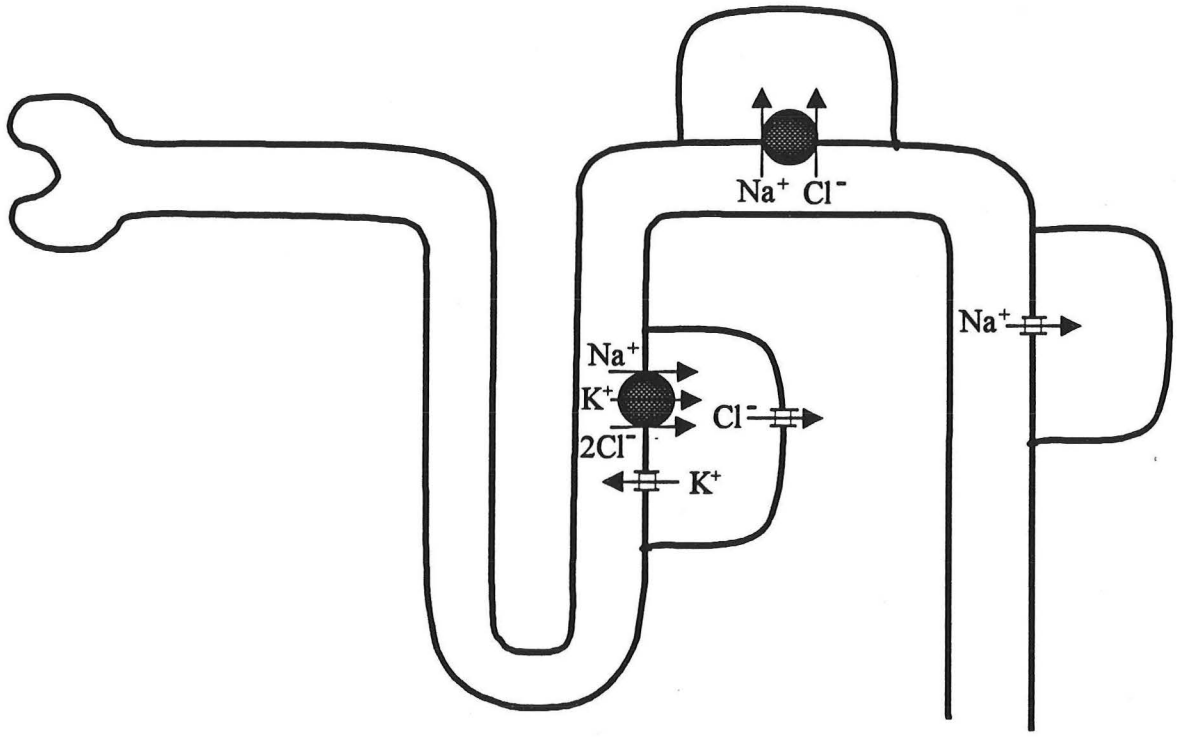
GENETIC RENAL DYSKALEMIAS: OLD SYNDROMES, NEW GENES

Robert J. Alpern, M.D.



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Research Interests: pH regulated cell signaling pathways
Regulation of renal Na^+/H^+ antiporter, NHE3
Regulation of renal Na^+/citrate cotransporter, NaDC-1
Role of endothelins in regulation of blood pressure and fluid and electrolyte composition, and in renal disease

LIDDLE'S SYNDROME

G.S. was a 16-year-old girl referred with a provisional diagnosis of primary aldosteronism. Blood pressure was consistently elevated at 180/120, serum K^+ was 2.6 mEq/l, and serum HCO_3^- was 30 mEq/l. The patient's younger brother, C.S., had a blood pressure of 200/100, serum K^+ of 2.7 mEq/l, and serum HCO_3^- of 29 mEq/l.

This patient was reported by Grant Liddle in a classic paper in the Transactions of the Association of American Professors (1). Before describing Liddle's work up of this patient, I would like to provide a brief review of the renal mechanisms responsible for hypokalemia.

RENAL HYPOKALEMIA

Potassium is freely filtered in the glomerulus and then is reabsorbed in the proximal tubule and the thick ascending limb, such that very small amounts of K^+ remain in the lumen at the beginning of the cortical collecting duct. The cortical collecting duct then secretes K^+ into the luminal fluid. The amount of K^+ secretion in the cortical collecting duct determines urinary K^+ excretion.

The cortical collecting duct is composed of principal cells and intercalated cells. Intercalated cells are responsible for transport of H^+ and HCO_3^-, and will not be discussed further. Principal cells absorb Na^+ and secrete K^+. Figure 1 shows the mechanisms responsible for Na^+ and K^+ transport in the principal cell of the cortical collecting duct. The major energy for transport is provided by the Na/K ATPase on the basolateral membrane. This transporter transports 3 Na^+ from the cell into the interstitial fluid in exchange for 2 K^+ entering the cell. This generates a low cell Na^+ concentration and a high cell K^+ concentration which provides the driving forces for movement across channels on the apical membrane.

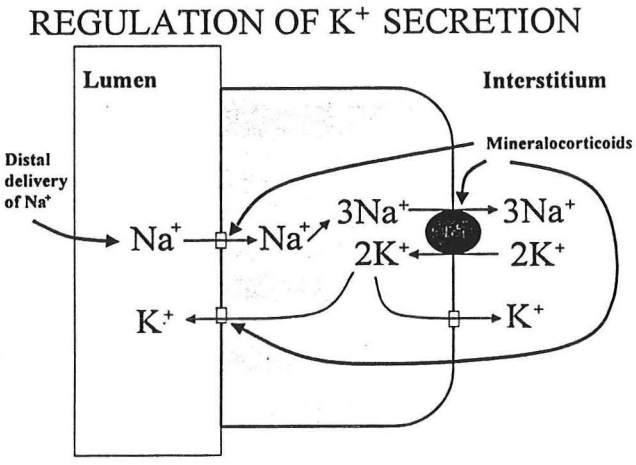


Figure 1

The rate of K^+ secretion in the cortical collecting duct is regulated by two principal factors, mineralocorticoid levels and distal delivery of Na^+. Mineralocorticoids stimulate this process by three mechanisms, shown in Figure 1. First, mineralocorticoids directly increase the rate of Na^+ entry from the luminal fluid into the cell by increasing the apical membrane Na^+ conductance. Second, mineralocorticoids increase the activity of the Na/K ATPase. Lastly, mineralocorticoids directly increase K^+ efflux from the cell into the luminal fluid by regulating the apical membrane K^+ channel. Distal delivery of Na^+ refers here to the delivery to the cortical collecting duct. Increased distal delivery of Na^+ leads to a higher luminal concentration of Na^+ which enhances the rate of Na^+ movement from lumen to cell, raises cell Na^+

concentration, and secondarily enhances the rate of the Na/K ATPase. This leads to a higher cell K^+ concentration which drives K^+ into the luminal fluid across the apical membrane K^+ channel. In addition, Na^+ transport from lumen to cell across the Na^+ channel generates a lumen-negative voltage which also serves to drive K^+ secretion into the luminal fluid.

Figure 2

K^+ SECRETION IN THE CCD

	Determinants		
	Mineralocorticoid	**Distal Delivery**	**K^+ Secretion**
EAV Increased	Decreased	Increased	No Δ
EAV decreased	Increased	Decreased	No Δ
Primary increase in mineralocorticoid	Increased	Increased	Increased
Primary increase in distal delivery	Increased	Increased	Increased

Almost all causes of renal hypokalemia, hypokalemia generated by the kidney, can be attributed to abnormal regulation of distal delivery of sodium and/or mineralocorticoids. Figure 2 shows how these two determinants interact to regulate renal K^+ excretion. Decreases in effective arterial volume lead to an increase in aldosterone levels and a decrease in distal delivery of Na^+ (due to enhanced proximal Na^+ reabsorption), which stimulates and inhibits K^+ secretion, respectively. On the other hand, an increased effective arterial volume suppresses aldosterone secretion but increases distal delivery of sodium (due to decreased proximal Na^+ reabsorption), inhibiting and stimulating distal K^+ secretion, respectively. The net result is that changes in effective arterial volume although having significant effects on the determinants of K^+ secretion, generally do not affect cortical collecting duct K^+ secretion or K^+ excretion unless the changes are extreme.

As shown in Figure 2, defects in renal K^+ excretion occur when changes in distal delivery of Na^+ and mineralocorticoids are not accompanied by changes of the other determinant in the opposite direction. This generally occurs in two settings. First, this is seen with a **primary increase in mineralocorticoid levels**. This refers to an increase in mineralocorticoid levels that is not due to a contracted effective arterial volume. Increased mineralocorticoid levels stimulate Na^+ absorption in the cortical collecting duct, which leads to volume expansion and hypertension. The expanded effective arterial volume then inhibits proximal Na^+ absorption, leading to enhanced distal delivery of Na^+. The combination of high mineralocorticoid levels and high distal delivery of Na^+ leads to increased cortical collecting duct K^+ secretion and hypokalemia.

Conversely, a **primary increase in distal delivery of Na^+** refers to an increase in distal delivery of Na^+ that is not secondary to an expanded effective arterial volume. A good example of this occurs with diuretics that inhibit Na^+ absorption proximal to the cortical collecting duct. Inhibition of

proximal Na^+ absorption leads to increased distal delivery of Na^+, and excretion of Na^+. Loss of Na^+ leads to a contracted effective arterial volume which then increases aldosterone levels. The net result is an increase in distal delivery of Na^+ and an increase in mineralocorticoid levels, the combination of which leads to increased cortical collecting duct K^+ secretion and hypokalemia.

In general, these two groups of causes of renal hypokalemia can be distinguished based on assessment of the effective arterial volume and blood pressure. Primary increases in mineralocorticoid levels are associated with an expanded effective arterial volume and hypertension. Primary increases in distal delivery of Na^+ are associated with a contracted effective arterial volume and low to normal blood pressure.

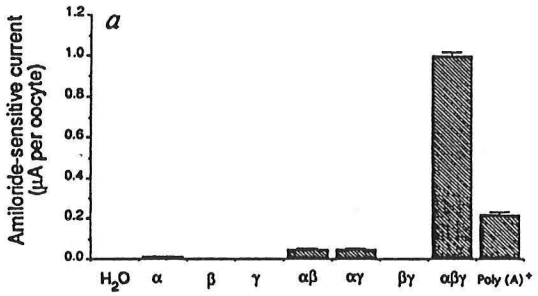
LIDDLE'S WORK-UP

On reviewing Liddle's original patient, she was noted to have a hypokalemic alkalosis associated with hypertension. This suggests a primary increase in mineralocorticoid levels. However, in his careful work-up he found that rates of aldosterone secretion were low (1). In addition, there was no response to SV-9055, an inhibitor of aldosterone secretion. Based on this, he concluded that while there may be an increase in mineralocorticoid activity, it was not mediated by increased levels of aldosterone. He next found, however, no response to spironolactone, a mineralocorticoid receptor antagonist. This implied that the abnormality in this patient had to be distal to the mineralocorticoid receptor. Lastly, he found that triamterene, a direct inhibitor of cortical collecting duct Na^+ and K^+ transport, normalized the blood pressure and the serum K^+. Subsequent studies have shown that triamterene directly inhibits the apical membrane Na^+ channel of the cortical collecting duct (2). Thus, the defect in Liddle's patient involved enhanced Na^+-dependent K^+ secretion in the cortical collecting duct, but was distal to the mineralocorticoid receptor. The fact that this abnormality was a renal abnormality was confirmed when patient G.S. lost her kidney and received a kidney transplant (3). With the kidney transplant, both blood pressure and serum K^+ were normal. This experiment of nature confirms that the abnormality is renal.

EPITHELIAL Na^+ CHANNEL: ENaC

A candidate gene for Liddle's syndrome is that of the epithelial Na^+ channel. In 1993, an epithelial Na^+ channel was cloned by expression cloning in *Xenopus* oocytes (4,5). Pools of cDNA (complementary DNA) were used to transcribe cRNA (complementary RNA) which was then injected into *Xenopus* oocytes. Oocytes were then screened for an amiloride-sensitive Na^+ current and positive clones selected. Canessa et al named this channel ENaC

ENaC EXPRESSION IN *XENOPUS* OOCYTES



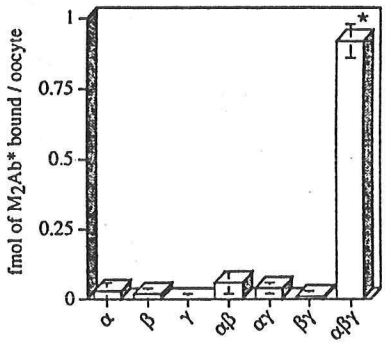
Canessa CM, et al. *Nature* 367:464, 1994

Figure 3

(Epithelial Na^+ Channel) (4). This procedure eventually led to the identification and cloning of three subunits of ENaC which have been named α, β, and γ (4,6). As can be seen in Figure 3, expression of the α subunit alone leads to a small Na^+ current. Injection of β or γ with the α subunit leads to an increased current, and injection of α, β, and γ leads to a very large amiloride-sensitive Na^+ current. Injection of β, γ, or β and γ, in the absence of α leads to no current. These studies suggest that the α subunit is required, whereas the magnitude of the current is significantly enhanced by the presence of β and γ. This channel is distinct from the voltage gated Na^+ channel of neurons in that it is not voltage gated, is inhibited by amiloride, is insensitive to tetrodatoxin, has a low single channel conductance, and a long open and closed time.

Subsequent studies have examined the expression of α, β, and γ ENaC on the plasma membrane of the *Xenopus* oocyte (7). As can be seen in Figure 4, expression of α, β, or γ alone leads to very little plasma membrane expression. Similarly, expression of αβ, αγ, and βγ leads to little plasma membrane expression. However, expression of α, β, and γ together leads to significant plasma membrane expression.

CELL SURFACE EXPRESSION
OF ENaC IN *XENOPUS* OOCYTES

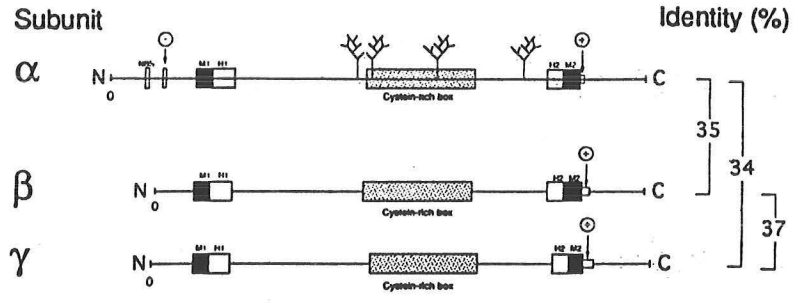


Firsov et al. *PNAS* 93:15370, 1996

Figure 4

Figure 5 shows a schematic representation of the α, β, and γ subunits of ENaC. All three subunits consist of short N-terminal and C-terminal intracellular domains, two transmembrane domains, and a large extracellular domain which contains a cysteine rich box. α, β, and γ subunits are approximately 35% identical with each other, and thus likely evolved from a similar gene.

ENaC SUBUNITS



Canessa CM, et al. *Nature* 367:466, 1994

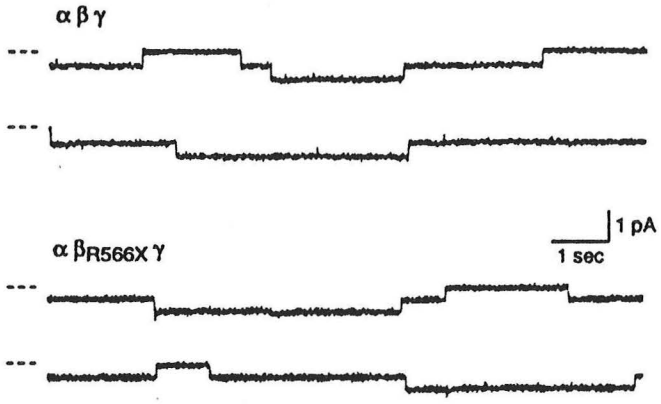
Figure 5

ENaC MUTATIONS IN LIDDLE'S SYNDROME

Once ENaC was cloned, investigators quickly examined whether mutations in ENaC were responsible for Liddle's syndrome. Five kindreds were found in which C-terminal deletions of 45-75 amino acids in the β subunit of ENaC were responsible for Liddle's syndrome (8). Thus, a C-terminal deletion in βENaC is able to lead to increased ENaC activity. One kindred was then reported with a C-terminal deletion of 76 amino acids in γENaC (9), suggesting that a similar deletion in γENaC could lead to activation of ENaC. Lastly, two kindreds were reported with missense mutations of β ENaC. Hansson et al reported a mutation converting proline 616 to leucine (10), and Tamura et al reported a mutation converting tyrosine 618 to histidine (11).

Taken together, these results suggested that the carboxy terminal portions of the β and γ subunits served as negative regulators of ENaC. Deletion of these portions of the molecule serve to increase the ENaC activity. The missense mutations at proline 616 and tyrosine 618, serve to further narrow down the key region within the carboxyterminus. Figure 6 shows a patch clamp tracing comparing wild type ENaC with ENaC including a truncated β subunit (12). As can be seen, ENaC has a small conductance with slow opening and closing. Truncation of the β subunit does not result in changes in single channel conductance or open probability of the channel. In addition, there is no effect on ion selectivity or affinity of the channel for amiloride. These results, confirmed by others suggests that the mutations in Liddle's syndrome do not affect the intrinsic properties of the channel, but rather lead to an increased number of channels in the membrane (12,13).

LIDDLE'S MUTATION DOES NOT MODIFY ENaC CHANNEL PROPERTIES
(Cell attached patch in Xenopus oocytes)



Snyder et al. *Cell* 83:969, 1995

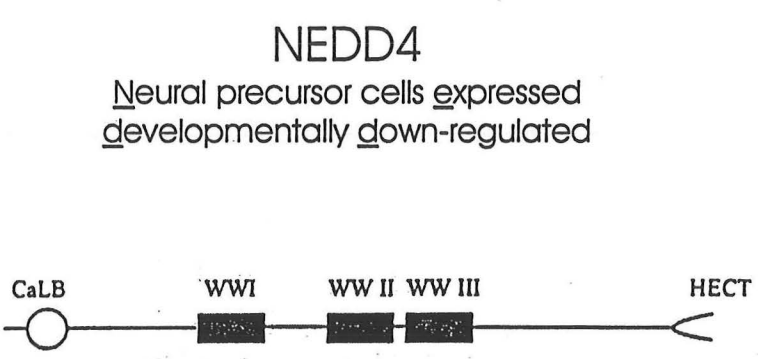
Figure 6

This conclusion has been confirmed in two studies (7,12), in which tags have been added to the expressed ENaC proteins, and the binding of antibodies directed against the tags used to study apical membrane expression. In both studies, C-terminal deletions in the β subunit led to an increased number of plasma membrane channels (7,12).

A number of studies have focused on determining the mechanisms by which mutations in the carboxyterminus of β and γ subunits increase ENaC activity. Review of the carboxyterminal sequences shows a conserved sequence, PPPXY, present in α, β, and γ ENaC subunits. These

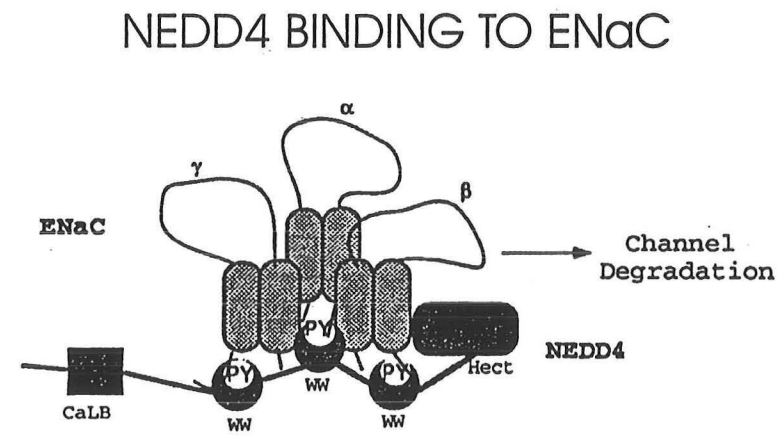
sequences are conserved between rat, human, and *Xenopus* ENaC. In addition, this sequence includes both of the missense mutations discussed above (10,11). Based on these results it was postulated that the sequence played a key role in inhibition of ENaC, likely signaling removal of ENaC from the plasma membrane. This conclusion was confirmed in a systematic mutagenesis study (14).

To address this, Staub et al (15) used a yeast two hybrid system to identify proteins that bound to this sequence of PPPXY, referred to as a PY domain. Using a sequence including the PY domain of βENaC, they cloned a previously identified cDNA, referred to as NEDD4 (Neural precursor cells expressed developmentally down-regulated). Figure 7 shows a schematic of this protein. It includes three WW domains, which have been shown in Yes-associated protein and dystrophin to bind to similar PY domains (16,17). In addition, NEDD4 includes a calcium-dependent lipid binding domain shown as CaLB and a Hect domain. The Hect domain mediates ubiquitination of proteins which generally leads to their eventual proteolysis in proteosomes. This offers a potential explanation as to how NEDD4 could lead to a decrease in the number of ENaC channels on the plasma membrane. NEDD4 binds to the α, β, and γ subunits of ENaC, and this binding is eliminated by deletion of a carboxy terminus. In addition, mutation of proline 616 or tyrosine 618, the missense mutations identified above in Liddle's syndrome, inhibits binding of NEDD4 (15).



Staub et al. *EMBO J* 15:2371, 1996

Figure 7



Staub et al. *EMBO J* 15:2371, 1996

Figure 8

Figure 8 shows a model in which NEDD4 binds to the α, β, and γ subunits of ENaC using its 3 WW domains. This would then allow the Hect domain to ubiquitinate ENaC leading to endocytosis and channel degradation. Mutations in the carboxyterminus of the β or γ subunit would prevent NEDD4 binding and increase the number of ENaC remaining on the plasma membrane.

Studies in *Xenopus* oocytes have also suggested a role for clathrin mediated endocytosis as a mechanism of ENaC down regulation (18). Inhibition of

clathrin mediated endocytosis by expression of dominant negative dynamin together with ENaC led to increased ENaC activity. However, when dominant negative dynamin was coexpressed with ENaC composed of truncated β and γ subunits, it was without effect on ENaC activity. This suggests that wild type ENaC is retrieved from the membrane by clathrin mediated endocytosis, and that the Liddle's mutation inhibits this process.

LIDDLE'S SYNDROME AS A PARADIGM FOR ESSENTIAL HYPERTENSION

Liddle's syndrome represents an example of a genetic condition where a single mutation resulting in an increase in renal Na^+ absorption is able to cause hypertension. Essential hypertension is believed to be more polygenic in nature. Based on the paradigm seen in Liddle's syndrome it is possible that mutations in many different genes which effect regulation of ENaC or other Na^+ transport mechanisms could predispose to hypertension. Thus, inactivating mutations in pathways which inhibit ENaC could predispose to hypertension and activating mutations in pathways which activate ENaC could predispose to hypertension. While any single mutation may not in itself cause hypertension, a number of such mutations could summate into a predisposition. This may provide a molecular basis for essential hypertension. Further studies are required to examine this.

BARTTER'S SYNDROME

C.J. was a 5-year-old black boy who presented with complaints of tetany and dwarfism. History was positive for polydipsia (10-12 glasses of fluid daily) and constipation. At 4 days old he was hospitalized for dehydration and convulsions. On physical exam, he was short and appeared dehydrated with blood pressures ranging from 95/52 to 120/80. He had muscle spasms and a positive Chvostek's sign. Serum K^+ was 2.2 mEq/l and total CO_2 was 34 mEq/l. Serum Ca^{2+} was normal.

This patient was first reported by Bartter and collaborators in a classic article in the American Journal of Medicine (19). His evaluation of this patient revealed a normal blood pressure, and hypokalemic alkalosis. Angiotensin and aldosterone levels were elevated. A renal biopsy showed hyperplasia of the renal juxtaglomerular apparatus. Blockade of the mineralocorticoid receptor with spironolactone caused the serum K^+ to rise. Lastly, infusion of angiotensin II led to a subnormal increase in blood pressure.

Subsequent studies have demonstrated that resistance to angiotensin II in this disorder and in a number of other disorders is attributable to volume contraction. Goodman et al showed that Bartter's patients were resistant to the hypertensive effects of angiotensin II and norepinephrine (20). Albumin infusion corrected both (20). In addition, Goodman et al showed that albumin infusion lowered plasma renin levels and aldosterone secretion, lowered urinary K^+, and partially corrected hypokalemia (20). These results suggest that many of the defects in Bartter's syndrome are at least partially due to volume contraction and are corrected by volume expansion.

Based on this, it can be concluded that Bartter's patients have a salt wasting defect which is present proximal to the cortical collecting duct. This leads to a primary increase in distal delivery of Na^+ to

the cortical collecting duct, volume contraction, and secondary hyperaldosteronism. The combination of high distal delivery of sodium and high mineralocorticoid levels leads to a hypokalemic alkalosis.

BARTTER'S VS. GITELMAN'S SYNDROME

Subsequently, Gitelman et al reported a patient who was in many respects similar to Bartter's patients, but had hypomagnesemia (21). Figure 9 shows a comparison of these syndromes developed by Bettinelli et al (22). In general, both Bartter's and Gitelman's patients have a hypokalemic alkalosis which is associated with relative volume depletion. However, a number of significant differences exist. First, Bartter's syndrome is a more severe disorder which frequently occurs during fetal life. Bartter's syndrome is associated with polyhydramnios and premature delivery, whereas Gitelman's syndrome is not. Diagnosis of Bartter's syndrome is frequently made before the age of one, and is always made before the age of six years old, while Gitelman's syndrome is rarely diagnosed before the age of six. Bartter's syndrome is associated with a significant defect in urinary concentration, whereas Gitelman's syndrome is not. Hypomagnesemia is present in all patients with Gitelman's syndrome, whereas it is only present in some patients with Bartter's syndrome.

Figure 9

BARTTER'S VS GITELMAN'S

	Bartter's (N=18)	**Gitelman's (n=16)**
$[K^+]$, mEq/l	2.5	2.7
$[HCO_3^-]$, mEq/l	33.5	29.2
Hypomagnesemia	7/18	16/16
$[Na^+]$, mEq/l	130	138
$(Ca^{2+}/Creatinine)_{Urinary}$	1.41 (all>0.4)	0.06 (All<0.1)
Urine osmolality	406	885
Polyhydramnios	8/18	0/16
Premature delivery	7/18	0/16
Age at diagnosis <1 year of age <6 years of age	 12/18 18/18	 0/16 2/16
Polydipsia, polyuria, or volume contraction	16/18	1/16
Febrile seizures or tetanic episodes	0/18	12/16

Bettinelli et al, *J Pediatrics* 120:38, 1992

The most significant difference between Bartter's and Gitelman's syndromes resides in calcium excretion. All Bartter's patients are hypercalciuric, while all Gitelman's patients are hypocalciuric. This one difference provides the basis for choosing candidate genes in these disorders. Thus, one can compare the defects in Bartter's and Gitelman's syndromes with the effects of diuretics known to act proximal to the cortical collecting duct. As shown in Figure 10, loop diuretics such as furosemide lead to a hypokalemic alkalosis, hypercalciuria, impaired urinary concentration, and marked volume depletion, all of which are characteristics of patients with Bartter's syndrome. This suggests that Bartter's syndrome may be due to a defect in thick ascending limb NaCl absorption. Thiazide diuretics are also associated with hypokalemic alkalosis, but lead to hypocalciuria, normal urinary concentration, and more mild volume depletion, all characteristics of Gitelman's syndrome. This raises the question of whether Gitelman's syndrome can be attributed to inhibition of NaCl absorption in the thiazide-sensitive segment of the nephron, the distal convoluted tubule.

Figure 10
DIFFERENTIAL EFFECTS OF LOOP AND THIAZIDE DIURETICS

	Loop Diuretics	Thiazide Diuretics
Plasma $[K^+]$	Low	Low
Plasma $[HCO_3^-]$	High	High
Urinary Ca^{2+}	High	Low
Urinary concentration	Impaired	Normal
Volume depletion	Marked	Mild

Na^+ TRANSPORT ALONG THE NEPHRON

In order to discuss the causes of Bartter's and Gitelman's syndromes we must first discuss the mechanisms of Na^+ transport along the nephron. Figure 11A shows a generalized cell model which applies to Na^+ absorption in each of the segments of the nephron. A basolateral membrane Na/K ATPase lowers cell Na^+ concentration which provides the driving force for Na^+ entry from the lumen into the cell. Each segment of the nephron possesses a distinct mechanism for apical or luminal membrane Na^+ entry. As discussed earlier, in the principal cell of the cortical collecting duct, the apical membrane mechanism is a Na^+ channel encoded by α, β, and γ ENaC (Figure 1).

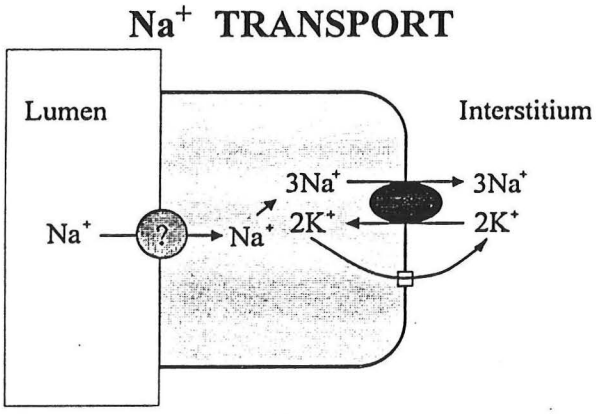


Figure 11A

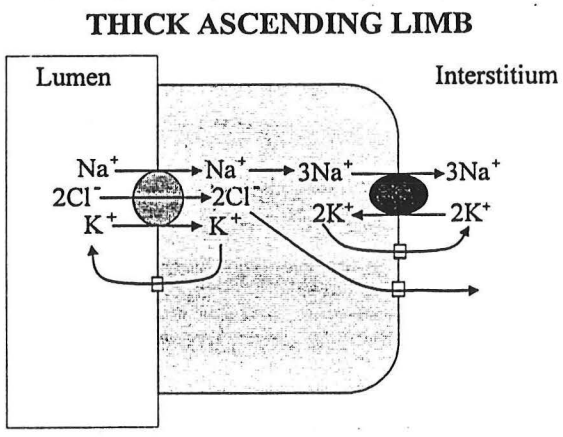
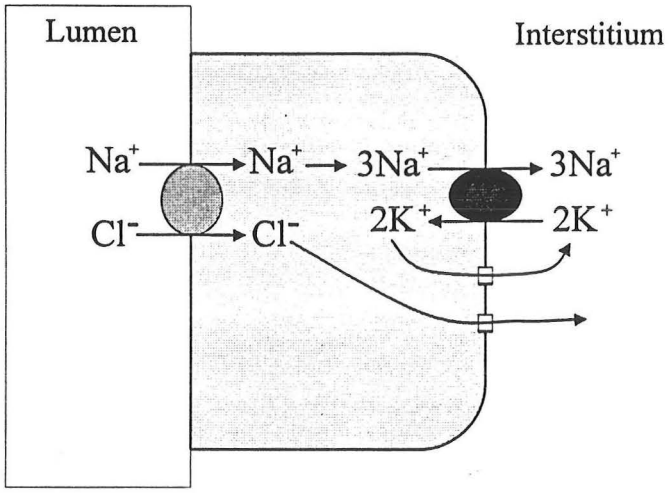


Figure 11B

In the thick ascending limb, Na^+ enters the cell on an electroneutral Na/K/2Cl cotransporter (Figure 11B). Na^+ which enters the cell on this transporter exits on the Na/K ATPase. K^+ which enters the cell on the apical Na/K/2Cl transporter recycles back across the apical membrane. The two Cl^- which enter the cell on the Na/K/2Cl transporter, exit on the basolateral membrane Cl^- conductance. The net result of transport is that each turnover of the Na/K/2Cl cotransporter leads to absorption of 2 Cl^- and 1 Na^+ ion. Because the number of Cl^- ions is greater than the number of Na^+ ions, transport is electrogenic and generates a lumen-positive voltage.

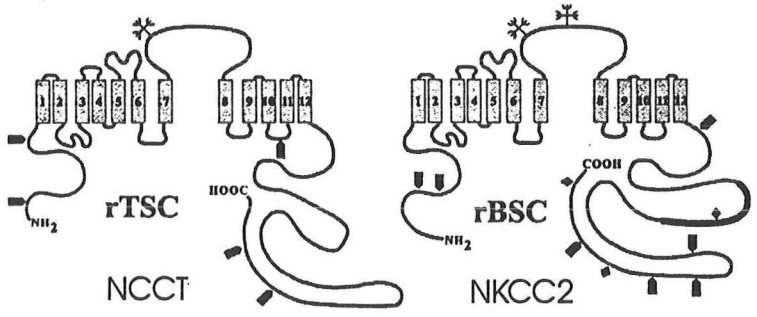
DISTAL CONVOLUTED TUBULE



In the distal convoluted tubule NaCl absorption is mediated by an electroneutral Na/Cl cotransporter which carries one Na^+ and one Cl^- (Figure 11C). The Na^+ which enters on the cotransporter exits on the Na/K ATPase, while the Cl^- which enters on the cotransporter exits on the basolateral membrane Cl^- channel. The net result is that transport is electroneutral.

Figure 11C

The apical membrane Na/Cl cotransporter and Na/K/2Cl cotransporter have been cloned (23,24), and are referred to by various names. Figure 12 shows the general structure of these transporters which are similar to each other. On the left is shown the Na/Cl cotransporter which has also been referred to as TSC (thiazide-sensitive cotransporter) or NCCT (Na/Cl cotransporter). On the right is shown the Na/K/2Cl cotransporter which has been referred to BSC (bumetanide-sensitive cotransporter) or NKCC2 (Na/K/Cl/Cl cotransporter, second isoform). NCCT has been localized to the apical membrane of the distal convoluted tubule (25-27), and NKCC2 has been localized to the apical membrane of the thick ascending limb (28).

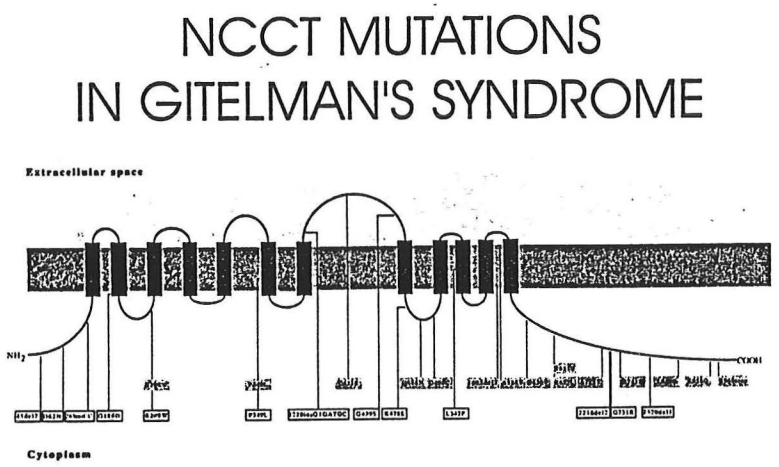


Gamba et al. *JBC* 269:17713, 1994

Figure 12

GENETIC DEFECT IN GITELMAN'S SYNDROME

As noted above, based on the observation that hypocalciuria is present in Gitelman's and in patients receiving thiazide diuretics, a candidate gene for Gitelman's syndrome is the thiazide-sensitive NaCl cotransporter encoded by the gene, NCCT. Indeed, in all Gitelman's families studied thus far, mutations have been found in NCCT (29-32). Figure 13 shows the location of the various mutations identified thus far. As can be seen, mutations are located throughout the gene with no predisposition to involvement of any specific region of the cDNA. Thus, as predicted Gitelman's syndrome is similar to thiazide diuretic ingestion in that both are associated with inhibition of the apical membrane Na/Cl cotransporter.



Mastroianni et al. *Am J Hum Gen* 59:1019, 1996
Simon et al. *Nature Genetics* 12:24, 1996

Figure 13

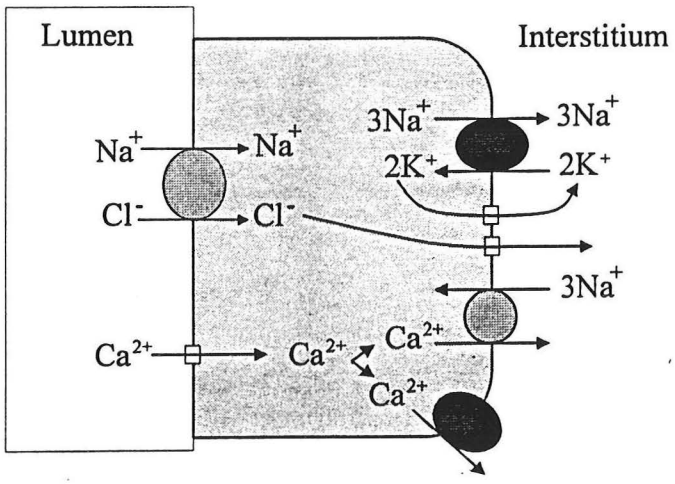


Figure 14

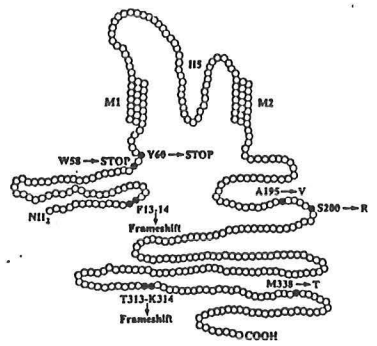
The mechanism of hypocalciuria in this condition is shown in Figure 14. Inhibition of Na/Cl cotransport leads to decreases in cell Na^+ and cell Cl^- concentration. Because the cell has a large basolateral membrane Cl^- conductance, decreases in cell Cl^- concentration lead to hyperpolarization of the cell (33). This secondarily leads to activation of an apical membrane Ca^{2+} channel which enhances cell Ca^{2+} entry and increases cell Ca^{2+} concentration (33). Calcium then exits on a basolateral membrane Ca^{2+} ATPase and on a Na/Ca exchanger. The rate of the Na/Ca exchanger is also enhanced by the decrease in cell Na^+ concentration.

THE GENETIC BASIS OF BARTTER'S SYNDROME

As noted above, the presence of hypercalciuria in patients with Bartter's syndrome is similar to the effect of furosemide and other loop diuretics, and suggests that Bartter's syndrome may be mediated by defects in thick ascending limb Na^+ absorption. Indeed, the first mutations that were found involved NKCC2, the apical membrane Na/K/2Cl cotransporter that is directly inhibited by furosemide, bumetanide, and other loop diuretics. Mutations that were found included frame shift mutations, premature stop codons, and nonconservative missense mutations at various locations along the protein (34,35). Again, no specific location was found for these inactivating mutations.

While it is interesting that mutations in NKCC2 were found in Bartter's syndrome, it was also noted that a number of families did not have mutations in this transport protein. As can be seen in Figure 11B, NaCl absorption in the thick ascending limb also requires activity of the apical membrane K^+ channel and the basolateral membrane Cl^- channel. Indeed, families have also been identified in which Bartter's syndrome is mediated by mutations in the apical membrane K^+ channel encoded by ROMK (36-38). ROMK has been localized to the apical membranes of the thick ascending limb and the K^+ secreting principal cell of the cortical collecting duct (39). Figure 15A and B show the locations of ROMK mutations in Bartter's. This finding confirms the previous speculation that ROMK indeed encodes the apical membrane K^+ channel that mediates K^+ recycling required for NaCl absorption in the thick ascending limb.

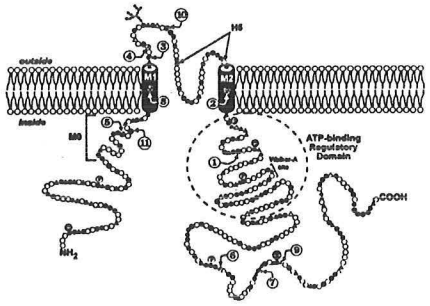
ROMK MUTATIONS IN BARTTER'S SYNDROME



Simon et al. *Nature Genetics* 14:152, 1996

Figure 15A

ROMK MUTATIONS IN BARTTER'S SYNDROME



International Collaborative Group. *Human Mol Gen* 6:17, 1997

Figure 15B

The gene encoding the basolateral membrane Cl^- channel of the thick ascending limb was not clear. Two Cl^- channel genes, CLCNKA and CLCNKB are both expressed in the thick ascending limb (40,41). Indeed, families have been found in which Bartter's syndrome is attributable to mutations in CLCNKB (Figure 16) (42). No mutations have been found in CLCNKA. This provides convincing evidence that CLCNKB encodes the basolateral membrane Cl^- channel mediating Cl^- absorption in thick ascending limb.

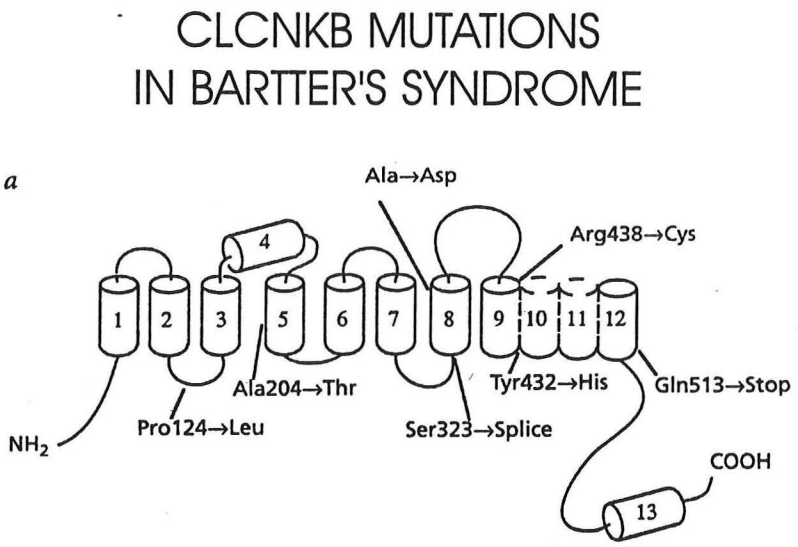


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Figure 16

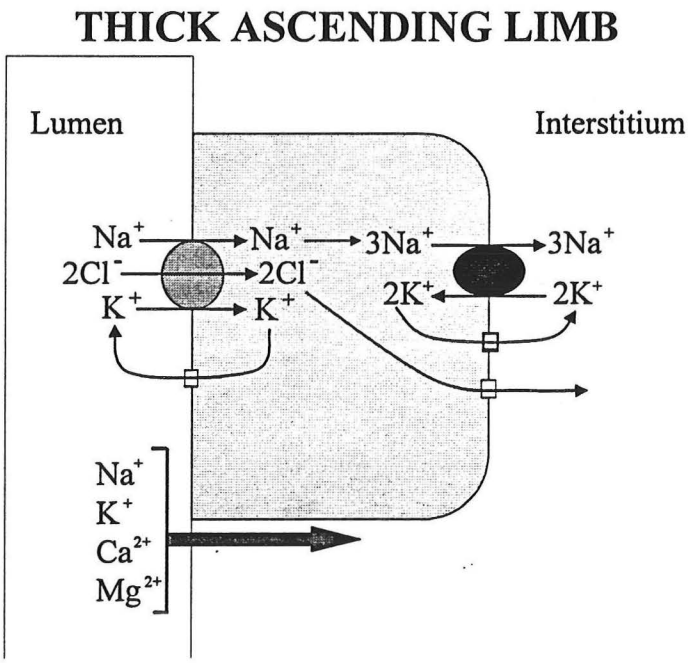
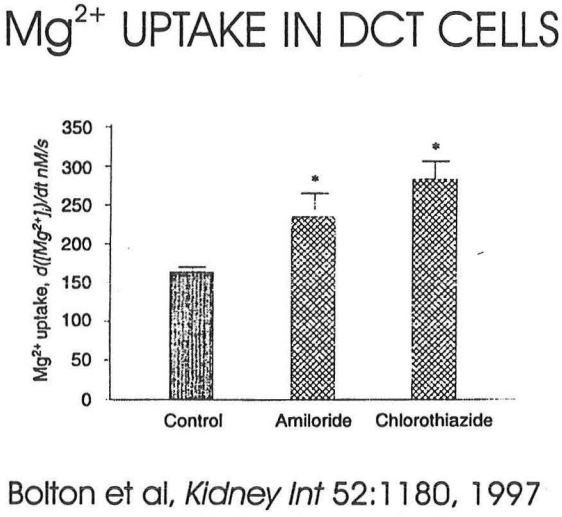


Figure 17

As described above, patients with Bartter's syndrome are hypercalciuric. Again, this is very easy to understand given the mutations described above. Inactivating mutations involving either the Na/K/2Cl cotransporter, the apical membrane K^+ channel, or the basolateral membrane Cl^- channel will inhibit NaCl absorption in the thick ascending limb which will inhibit the generation of the lumen-positive voltage. As shown in Figure 17, this lumen positive voltage drives the passive paracellular absorption of a number of cations including Na^+, K^+, Ca^{2+}, and Mg^{2+}. Thus, inactivating mutations which inhibit NaCl absorption will inhibit passive absorption of Ca^{2+} and lead to hypercalciuria. Quantitatively, this is the segment where the largest amount of Ca^{2+} is reabsorbed.

HYPOMAGNESEMIA IN BARTTER'S AND GITELMAN'S SYNDROME

As described above, Gitelman's syndrome is invariably accompanied by hypomagnesemia, while Bartter's syndrome is sometimes associated with hypomagnesemia. While differences in Ca^{2+} handling are easily explained physiologically, the differences observed in Mg^{2+} handling are more difficult to comprehend. Inhibition of NaCl absorption in the thick ascending limb in Bartter's syndrome should lead to the inhibition of the lumen positive voltage which should inhibit Mg^{2+} absorption. Because this mechanism is responsible for the majority of tubular Mg^{2+} reabsorption, one would expect that Bartter's syndrome would be associated with significant hypermagnesuria and hypomagnesemia. Thus, while it is not surprising that some patients with Bartter's are hypomagnesemic, it is surprising that most are not.



Mg^{2+} absorption in the distal convoluted tubule is similar to Ca^{2+} absorption. An apical membrane Mg^{2+} channel is believed to provide a pathway for Mg^{2+} entry, and Mg^{2+} exit on the basolateral membrane is believed to be mediated by either a Mg^{2+} ATPase or a Na/Mg^{2+} exchanger. In isolated DCT (distal convoluted cells), application of amiloride or chlorothiazide enhance Mg^{2+} uptake (Figure 18) (43). Both of these diuretics hyperpolarize these cells, and thus results are similar to the effects on Ca^{2+} uptake in this segment. Since Gitelman's syndrome is similar to the effect of thiazides, it would be expected that Mg^{2+} transport would be stimulated in Gitelman's syndrome and that patients would be hypomagesuric and certainly not hypomagnesemic.

Figure 18

Thus, the presence of hypomagnesemia in all patients with Gitelman's syndrome remains unexplained. While in the past it was believed that the defect in Mg^{2+} could be primary, the demonstration of mutations in NCCT has eliminated this possibility.

PROSTAGLANDINS IN BARTTER'S SYNDROME

Prostaglandins have been proposed to play an important role in Bartter's syndrome. Prostaglandin excretion is increased in Bartter's syndrome, and Indomethacin improves the salt wasting as well as the hypokalemic alkalosis in these patients. However, it is clear that overproduction of prostaglandins is secondary rather than primary in Bartter's syndrome (44). This conclusion is based on observations that: 1) increased prostaglandin excretion is variable in Bartter's syndrome; 2) Indomethacin improves but does not cure Bartter's syndrome; and 3) many states with low effective arterial volume have high prostaglandin excretion.

PROSTAGLANDINS IN BARTTER'S

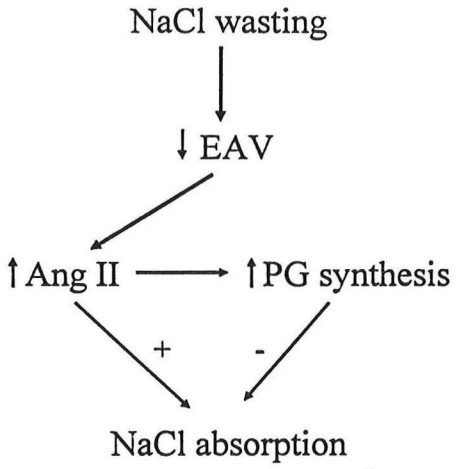


Figure 19 shows the mechanism of and role of increased prostaglandin excretion in Bartter's syndrome. NaCl wasting leads to a low effective arterial volume which leads to increased generation of angiotensin II. Increased angiotensin II directly stimulates NaCl absorption but increases prostaglandin synthesis which inhibits NaCl absorption along the nephron. This prostaglandin induced inhibition of NaCl absorption exacerbates the defect already present in Bartter's patients. Thus, Indomethacin by inhibiting this secondary increase in prostaglandin synthesis ameliorates the syndrome.

Figure 19

WORK UP OF THE HYPOKALEMIC PATIENT

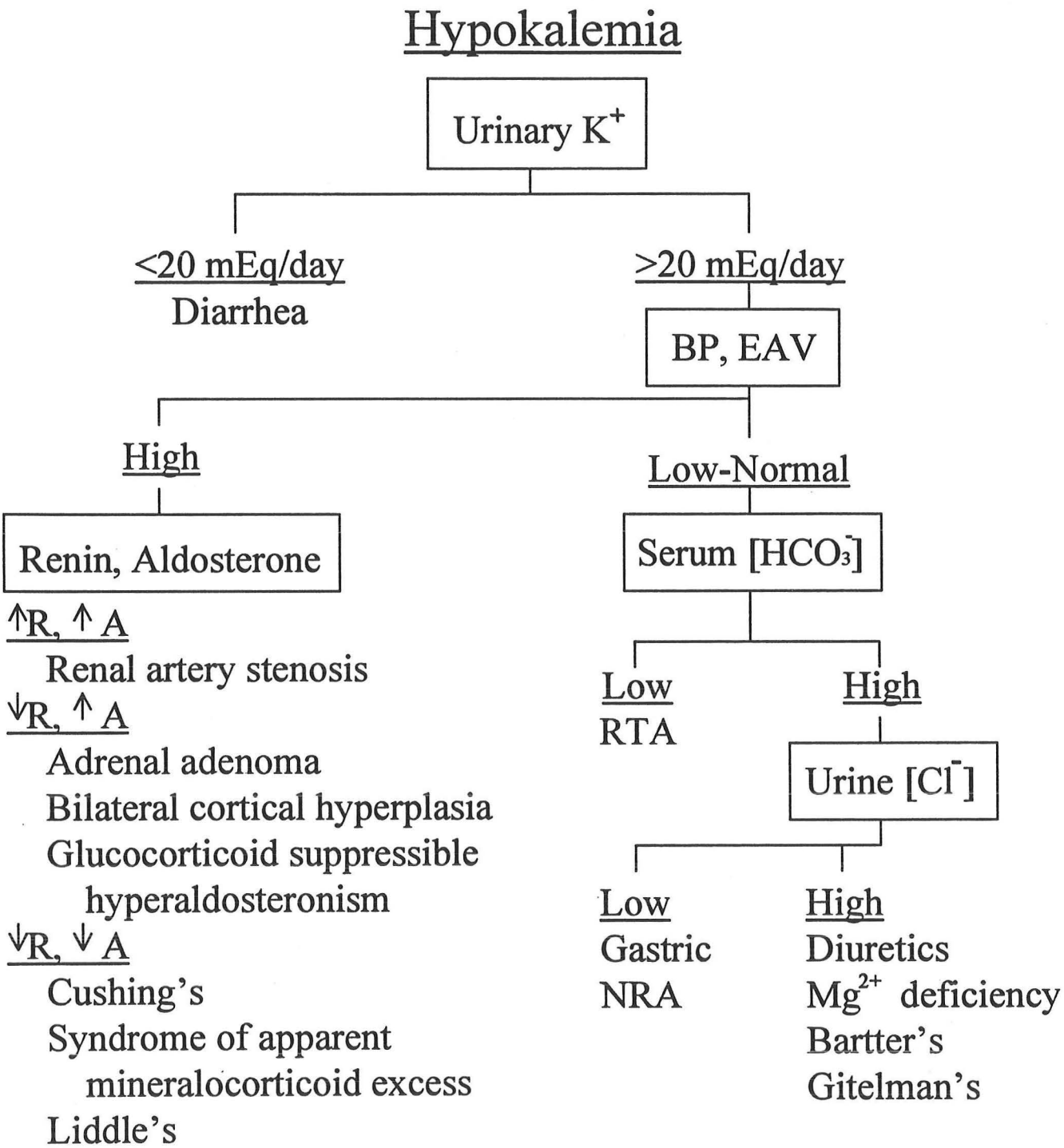
Figure 20 gives a schematic representation of the work up of a patient with hypokalemia. The distinction between renal and extrarenal K^+ wasting can generally be made by obtaining a history of gastrointestinal disturbances, or by measuring urinary K^+ excretion. A urinary K^+ excretion in excess of 20 mEq/day in the presence of hypokalemia suggests renal K^+ wasting. On the other hand, excretion of less than 20 mEq K^+ per day suggests extrarenal K^+ losses, which most frequently are due to diarrhea.

If hypokalemia is renal in origin, the next step is to assess the effective arterial volume and blood pressure. If these are judged to be high, the patient's hypokalemia is due to a primary increase in mineralocorticoid "effect." The numerous syndromes which can cause this are best discriminated by the measurement of renin and aldosterone levels. A high renin and high aldosterone level indicates renal artery stenosis. A low renin and high aldosterone level indicates primary hyperaldosteronism. This can be due to an adrenal adenoma or bilateral cortical hyperplasia. A familial cause of primary hyperaldosteronism is glucocorticoid suppressible hyperaldosteronism.

A low renin and a low aldosterone indicate a non-aldosterone mineralocorticoid effect. This is most frequently due to Cushing's syndrome. It can also be due to the syndrome of apparent mineralocorticoid excess which is due to a genetic defect in 11β-hydroxylase. This enzyme normally inactivates cortisol in mineralocorticoid target cells, preventing cortisol from interacting with the mineralocorticoid receptor. In the absence of this enzyme, cortisol binds to the mineralocorticoid receptor and results in a hypermineralocorticoid state. Lastly, Liddle's syndrome should be considered in this group. While there is no increase in circulating mineralocorticoids, the activating mutation in the Na^+ channel leads to an apparent mineralocorticoid effect.

It should be noted that if renin and aldosterone levels are both high, the physician needs to be certain that effective arterial volume is indeed increased. A low effective arterial volume can lead to high

Figure 20


Hypokalemia
Urinary K^+
<20 mEq/day
Diarrhea
>20 mEq/day
BP, EAV
High
Renin, Aldosterone
↑R, ↑A
Renal artery stenosis
↓R, ↑A
Adrenal adenoma
Bilateral cortical hyperplasia
Glucocorticoid suppressible hyperaldosteronism
↓R, ↓A
Cushing's
Syndrome of apparent mineralocorticoid excess
Liddle's
Low-Normal
Serum [HCO_3^-]
Low
RTA
High
Urine [Cl^-]
Low
Gastric
NRA
High
Diuretics
Mg^{2+} deficiency
Bartter's
Gitelman's

renin and high aldosterone levels. If there is any uncertainty, a saline suppression test should be performed in which the patient receives a large infusion of normal saline over a few hours and renin and aldosterone levels are measured.

If effective arterial volume is deemed to be low to normal, the next step is to examine the serum HCO_3^- concentration. A low serum HCO_3^- concentration in the plasma indicates renal tubular acidosis. In hypokalemic patients this can be due to hypokalemic distal RTA or to proximal RTA.

If the serum HCO_3^- concentration is high, the next step is to examine the urinary Cl^-. A low urinary Cl^- indicates that K^+ is being dragged out by a non-Cl^- anion such as HCO_3^- in vomiting or other non-reabsorbable anions such as penicillins or ketoanions. A high urinary Cl^- indicates a primary defect in renal NaCl absorption which could be due to ingestion of diuretics, Mg^{2+} deficiency, Bartter's syndrome, or Gitelman's syndrome.

SUMMARY

Liddle's, Bartter's, and Gitelman's syndromes represent interesting lessons in the evolution of our understanding of physiology and medicine. Observations made by these astute clinicians taught us much about how the nephron functions. Subsequent clinicians and physiologists were able to predict the likely locations of the defects in these patients, but much uncertainty existed until the genes encoding the specific transporters were defined. This then allowed geneticists to precisely locate the mutations responsible for these syndromes.

However, a number of questions remain. While all patients with Gitelman's syndrome have been found to have mutations in NCCT, some families with Bartter's syndrome do not have mutations in NKCC2, ROMK, or CLCNKB. Another interesting question is that of the mechanism of hypokalemia in patients with Bartter's syndrome due to mutations in ROMK. ROMK is also believed to encode the apical membrane K^+ channel of the cortical collecting duct principal cell. If this is the only channel capable of K^+ secretion, one would predict that mutations in ROMK should inhibit the ascending limb salt absorption but not lead to hypokalemia. The finding that these patients indeed have hypokalemia tells us that a simple model with ROMK as the only cortical collecting duct apical membrane K^+ channel is likely not true. Lastly, our present understanding of renal Mg^{2+} handling would not have predicted the changes seen in Bartter's and Gitelman's. Future studies will need to resolve this discrepancy.

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