Salt handling in the distal nephron: lessons learned from inherited human disorders

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Inherited salt-losing renal tubular disorders with secondary hypokalemia (alternatively referred to as Bartter-like syndrome) are an outstanding example of the successful integration of basic science (renal physiology, pharmacology, genetics) and clinical disciplines (endocrinology, nephrology, pediatrics, and neonatology). To illustrate the importance of thorough clinical observation and profound knowledge of renal physiology for the understanding of renal tubular salt transport disorders, we will start our review with the description of a patient suffering from life-threatening salt wasting. As will be pointed out in the course of this review, unique clinical characteristics of the affected child in combination with detailed knowledge of distal tubular salt transport mechanisms paved the way to the clarification of the hitherto unknown genetic defect underlying this patient’s disorder.

CASE REPORT

A child was prematurely born to consanguineous parents of Arabic origin at 28 wk of gestation. The parents had already given birth to two healthy children; another child, however, had died as a premature infant, and there were two former miscarriages. The birth weight of this child was 1,250 g (~0.3 SDS). The pregnancy was complicated by severe maternal polyhydramnios. Polyhydramnios was likely of fetal renal tubulopathy. Blood volume depletion was accompanied by low plasma Na concentration, indicating additional renal salt wasting. The child developed metabolic alkalosis, hypokalemia, and hypomagnesemia. Plasma renin activity exceeded the upper measurable limit. Intractable vomiting and abdominal distention required total parenteral nutrition.

In view of the prenatal manifestation, as well as postnatal profound saluretic polyuria and the finding of markedly increased renal and extrarenal prostaglandin E formation, the diagnosis of a disorder of the loop of Henle, referred to as hyperprostaglandin E syndrome (also referred to as antenatal hyperprostaglandin E syndrome; Bartter syndrome), was considered. Treatment with cyclooxygenase inhibitors was initiated 10 days after birth with beneficial effects on fluid and electrolyte balance. The infant still required repeated amniocenteses during the last 6 wk of gestation. Total volume of amniotic drainage was ~15 liters. Within 72 h after birth, the child was noted to have isosthenuric polyuria with urinary outputs exceeding 25 ml·kg⁻¹·h⁻¹ (normal 2–6). Consequently, the preterm infant lost nearly 20% of its birth weight. Extracellular fluid (ECF) volume depletion was accompanied by low plasma Na⁺ and Cl⁻ concentrations, indicating additional renal salt wasting. The child developed metabolic alkalosis, hypokalemia, and hypomagnesemia. Plasma renin activity exceeded the upper measurable limit. Intractable vomiting and abdominal distention required total parenteral nutrition.

In view of the prenatal manifestation, as well as postnatal profound saluretic polyuria and the finding of markedly increased renal and extrarenal prostaglandin E formation, the diagnosis of a disorder of the loop of Henle, referred to as hyperprostaglandin E syndrome (also referred to as antenatal Bartter syndrome), was considered. Treatment with cyclooxygenase inhibitors was initiated 10 days after birth with beneficial effects on fluid and electrolyte balance. The infant still required supplementation of KCl, NaCl, and magnesium sulfate, albeit to a lesser extent with 15, 4, and 0.8 mmol·kg⁻¹·day⁻¹, respectively (normal 1, 3, and 0.25). Glomerular filtration was moderately impaired with calculated glomerular filtration rate (GFR; Schwartz formula) of 57 ml·min⁻¹·(1.73 m²)⁻¹ at the age of 9 mo (mean GFR at 6–12 mo being 103 ml·min⁻¹·(1.73 m²)⁻¹). In addition to renal symptoms, the child exhibited gross motor retardation and bilateral sensorineural deafness as revealed by electric re-
sponse audiometry of the brain stem. Surprisingly, nephrocalcinosis, a classical feature of hyperprostaglandin E syndrome, was not detected in repeated ultrasound examinations of the patient’s kidneys.

Descriptions of inherited hypochloremic alkalosis with secondary hypokalemia date back to the 1960s (95), and later, a defect in renal salt conservation as an underlying cause was hypothesized (4). In the following two decades, a plethora of data derived from perfusion studies in isolated nephron segments established the mode and stoichiometry of transepithelial ion transport (34). Specialized molecules were proposed to accomplish Na⁺-Cl⁻ entry from the tubular lumen into the cell and further exit through the basolateral membrane. Inhibitors of these pathways were identified. Among those were the high-ceiling diuretics and thiazides, which had already been established in clinical practice (13, 64). The functional description of ion transport proteins was a prerequisite that allowed cloning of the molecules by expression and homology-based cloning strategies. These efforts led to the identification of the cation-chloride cotransporters NCCT (28) and NKCC2 (27), the renal outer medullary K⁺ (ROMK) channel (46), and the CLC chloride channels (53). Cloning of these molecules, in turn, provided the candidate genes for the identification of inactivating mutations leading to syndromes of tubular salt wasting (47, 107–110).

A second line of research focused on a minority of patients excluded for linkage to these candidate genes. By subsequent genome-wide linkage strategy, novel proteins were discovered, and their physiological role as channel subunits or regulators was established (8, 20, 122). Heterologous expression of cloned molecules allowed for the characterization of their electrophysiological and biochemical properties and their putative interaction with accessory proteins. An additional approach to study the in vivo function by generation of genetically engineered mice deficient in the respective genes turned out to be more delicate because the phenotype of these animals did not exactly resemble the corresponding human disease (73, 100, 114). Therefore, clinicians in immediate care of the patients have the chance to complete the knowledge about the physiological role of distinct renal proteins in humans.

This review has been written by pediatricians and details their 30 years of experience in diagnosis and treatment of more than 100 patients suffering from the different forms of salt-wasting tubulopathies (SLTs), with a special emphasis on its most severe form, the hyperprostaglandin E syndrome (104).

FROM THE FIRST CLINICAL OBSERVATION TO THE MOLECULAR DECIPHERMENT OF RENAL SALT WASTING

In 1957, two pediatricians described a 2-mo-old African American infant with congenital hypokalemic alkalosis, failure to thrive, dehydration, and hyposthenuria, who finally died at the age of 7.5 mo (93). Some years later, two African American patients with normotensive hyperaldosteronism, hyperplasia of the juxtaglomerular apparatus, metabolic alkalosis and severe renal K⁺ wasting were characterized by the endocrinologist Frederic Bartter (5). Other features of this syndrome included increased activity of the renin-angiotensin system and a relative vascular resistance to the pressor effect of exogenous ANG II. After these original reports, hundreds of Bartter-like syndrome (BS) cases have been described. Although all shared the finding of hypokalemia and hypochloremic alkalosis, patients differed with regard to age of onset, severity of symptoms, degree of growth retardation, urinary concentration capacity, magnitude of urinary K⁺ and prostaglandin excretion, presence of hypomagnesemia, and extent of urinary Ca²⁺ excretion.

Gitelman and colleagues (32) pointed to the susceptibility to carpopedal spasms and tetany in three BS cases. Tetany was attributed to low plasma Mg²⁺ levels, secondary to impaired renal conservation of Mg²⁺. Further examination of these patients also revealed low urinary Ca²⁺ excretion (92). The dissociation of renal Ca²⁺ and Mg²⁺ handling was regarded to be pathognomonic for Gitelman syndrome (GS) (7). Interestingly, both patients in Bartter’s original report displayed positive Chvostek’s sign and carpopedal spasms. Indeed, in a recent discussion of the original Bartter paper in Milestones in Nephrology, one of the coauthors, John R. Gill, conceded that the majority of patients seen by both endocrinologists perfectly matched the later description of Gitelman (6).

Phenotypic homogeneity of BS was questioned more seriously when the pediatricians Fanconi (21) and McCredie (77) found high urinary Ca²⁺ excretion and medullary nephrocalcinosis in infants initially suspected for BS. Descriptions of this variant in the literature became more frequent in the 1980s, most likely because advances in neonatal medicine resulted in higher survival rates of extremely preterm born babies. The neonatologist Ohlsson (83) defined the antenatal history with maternal polyhydramnios, which likely predisposed to premature birth.

Immediately after birth, profound polyuria put these type of patients at high risk for life-threatening dehydration (61). Contraction of ECF volume was accompanied by markedly elevated renal and extrarenal PGE₂ production. Treatment with PG synthesis inhibitors effectively reduced polyuria, ameliorated hypokalemia, and improved growth. To emphasize the obviously critical role of PGE₂ in the pathogenesis of this distinct tubular disorder, Seyberth (102) coined the term hyperprostaglandin E syndrome (HPS).

Early reports postulated that the primary defect in “congenital hypokalemic alkalosis” was an unresponsiveness of the vascular bed to ANG II (5). In the 1970s, a defect in renal Na⁺-Cl⁻ conservation was suggested as the possible culprit. This assumption was largely based on studies of free water clearance and distal fractional Cl⁻ reabsorption and the observation that affected subjects exhibited impaired renal concentrating and diluting capacities as well as decreased distal Cl⁻ reabsorption (30). However, efforts to define the affected tubular segment were hampered by the fact that compensatory activation of salt-conserving mechanisms in nephron segments different from that affected by the primary genetic defect enabled partial correction of urinary salt wasting (10, 54, 80, 112). Controversial results might have also been attributed to the selection of studied patients, which, at that time, were not separated between BS (and GS) on one, and HPS on the other side. This difference became more evident when the patients’ symptoms were compared with the pharmacological effects of diuretics and, subsequently, when the patients were exposed to diuretics with a well-defined action on the tubule. Patients with BS/GS syndrome displayed a thiazide-like phenotype with Mg²⁺ wasting associated with hypocalciuria and a normal
increase in solute excretion upon furosemide administration (16, 113) but an impaired response upon chlorothiazide administration (86, 113). Conversely, patients with polyuric HPS were insensitive to loop diuretics such as furosemide and displayed an exaggerated salt loss with thiazides (58). These results provided clear evidence that the basic defect in BS/GS localized to the distal convoluted tubule (DCT), while HPS, the furosemide-like SLT, was due to defective salt transport in the thick ascending limb of Henle’s loop (TAL). Definition of two distinct tubular sites affected in BS/GS and HPS, respectively, was essential for further selection of candidate genes and, finally, identification of inactivating mutations in the ion transport machinery of the renal tubule.

Characterization of the human genes encoding the tubular solute transporters and subsequent identification of loss-of-function mutations was the outstanding contribution of Simon and Lifton (107–110) from Yale University. Remarkably, their initial interest was directed toward the identification of genes relevant for monogenic forms of hypertension. Increased salt reabsorption in the renal tubule due to overactive epithelial Na⁺ channel (ENaC) was previously identified in Liddle’s syndrome by the same group (41, 42). Although within the next two years, no further genes could be associated with inherited forms of hypertension, Simon and collaborators could establish four genes of the tubular apparatus responsible for the opposite phenotype of renal salt wasting, thereby deciphering the molecular pathogenesis of BS/GS and HPS (107–110). Other groups corroborated their results and elaborated a correlation between the genotypes and phenotypes (Table 1; 47, 62, 65, 75, 118).

MECHANISMS OF TRANSEPIHELIAL SALT REABSORPTION IN THE DISTAL NEPHRON

Description of salt reabsorption pathways and quantitation of the contribution of a specific tubular segment to total luminal Na⁺-Cl⁻ uptake were mainly established by experiments in rodents using micropuncture and microperfusion techniques.

Approximately 25% of the filtered solute load is reabsorbed in the TAL. Transmembrane transport of Na⁺, K⁺, and Cl⁻ from the lumen into the epithelial cell is mediated by the Na⁺-K⁺-2Cl⁻ symporter type 2 (NKCC2) (Fig. 1A). NKCC2 is driven by the steep electrochemical gradient for Na⁺ established by the basolateral ATP-dependent Na⁺ pump. This Na⁺ gradient provides the energy for “uphill” transport of K⁺ and Cl⁻ into the cell. K⁺ channels (ROMK) in the luminal membrane allow for apical K⁺ recycling, whereas two highly homologous Cl⁻ channels (ClC-K) act in parallel as basolateral exit mechanisms for Cl⁻. Operation of both Cl⁻ channels is dependent on an additional protein, the β-subunit barttin. Furosemide acts via a direct inhibition of the Na⁺-K⁺-2Cl⁻ symporter, probably by attachment to its central hydrophobic domain (117).

In the DCT, ~5% of the filtered load of Na⁺ is reabsorbed. As in the TAL, transepithelial Na⁺-Cl⁻ transport requires the activity of the basolateral Na⁺ pump (Fig. 1B). The energy provided by the electrochemical gradient for Na⁺ is utilized by a Na⁺-Cl⁻ cotransporter (NCCT) in the luminal membrane which moves Cl⁻ into the epithelial cell against its electrochemical gradient. Cl⁻ then passively exits through the basolateral membrane via a Cl⁻ channel (ClC-Kb). Thiazide diuretics inhibit the Na⁺-Cl⁻ cotransporter, perhaps by competing for the Cl⁻ binding site (79).

It is important to note that in contrast to the proximal tubule or collecting duct, in the TAL and DCT segments, trancellular Na⁺ reabsorption is directly coupled to Cl⁻ reabsorption. Therefore, disorders of salt reabsorption in the TAL and DCT affect both Na⁺ and Cl⁻ homeostasis. With the exception of Na⁺-K⁺-ATPase, mutations in all proteins, which have been mentioned here, were identified to cause SLTs with secondary hypokalemia. Despite this genetic heterogeneity, the historical separation into two major phenotypes still holds true, with disorders of the TAL, the furosemide-like SLTs, on the one side and disorders of the DCT, the thiazide-like SLTs, on the other. Because ion transport mechanisms are tightly coupled to each other, loss-of-function mutations affecting one module lead to the breakdown of the complete transport process in the respective tubular segment. Nevertheless, careful clinical evaluation revealed some distinctive features (Fig. 2), which are restricted to the defect of distinct proteins, and will be discussed in the following section in more detail.

DISORDERS OF THE LOOP OF HENLE, THE FUROSEMIDE-LIKE SLTs

The low incidence of salt-losing tubular disorders with hypokalemia, as well as its phenotypic and genetic variability, often confused investigators in search of the underlying defect and of a clinically applicable classification of the disease. Recent advances in molecular medicine, which allowed for genetic testing, made it possible to define patients on the basis of their genotypes. Evaluation of genetically defined patients, in turn, helped to determine the function of a single protein in the integrative process of tubular salt reabsorption in humans.

Table 1. Genes and gene products involved in the etiology of inherited salt-losing tubulopathies and their localization along the nephron

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Product</th>
<th>Disease</th>
<th>Affected Tubular Segment</th>
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<tbody>
<tr>
<td>SLC12A1</td>
<td>NKCC2</td>
<td>HPS</td>
<td>TAL</td>
</tr>
<tr>
<td>KCNJ1</td>
<td>ROMK</td>
<td>HPS</td>
<td>TAL</td>
</tr>
<tr>
<td>BSN1</td>
<td>Barttin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLCNKA + CLCNKB</td>
<td>CIC-Ka + CIC-Kb</td>
<td>HPS + SND</td>
<td>tAL + TAL + DCT</td>
</tr>
<tr>
<td>CLCNKB</td>
<td>CIC-Kb</td>
<td>BS</td>
<td>DCT/TAL</td>
</tr>
<tr>
<td>SLC12A3</td>
<td>NCCT</td>
<td>BS (GS)</td>
<td>DCT</td>
</tr>
</tbody>
</table>

NKCC2, Na⁺-K⁺-2Cl⁻ symporter type 2; ROMK, renal outer medullary K⁺ channel; CIC-Ka and CIC-Kb, renal Cl⁻ channels Ka and Kb; NCCT, Na⁺-Cl⁻ cotransporter; HPS, hyperprostaglandin E syndrome; SND, sensorineural deafness; BS, Bartter syndrome; GS, Gitelman syndrome; TAL, thick ascending limb of Henle’s loop; tAL, thin ascending limb of Henle’s loop.
Disruption of Na\(^+\)-Cl\(^-\) reabsorption in the TAL due to inactivating mutations in NKCC2 causes a severe disorder with antenatal onset. Within the 2nd trimenon, fetal polyuria leads to increasing maternal polyhydramnios. Cl\(^-\) concentration in the amniotic fluid is elevated up to 118 mM (74, 85). Untreated, premature delivery occurs around 32 wk of gestation. The most striking abnormality of the newborns is profound polyuria. With adequate fluid replacement, daily urinary outputs can easily exceed half of the body weight of the newborn (20 ml/kg/1). Despite both ECF volume depletion and presence of high vasopressin levels, the urine still has an osmolality hardly approaching that of plasma, indicating a virtually complete concentrating defect (103) (Fig. 2).

A gross deficit in the ability to concentrate urine is not unexpected in view of the unique location of the NKCC2 protein at the luminal surface of the TAL cells. Absence of the transcellular Na\(^+\)-Cl\(^-\) transport via NKCC2 is also expected to abolish the lumen-positive transepithelial voltage that enables paracellular reabsorption of Na\(^+\). The combined absence of transcellular and paracellular transport of salt across the TAL cells prevents the establishment of the cortico-medullary osmotic gradient necessary for urine concentration. In turn, as salt reabsorption in the TAL segment is also critical for urine dilution, patients' urine osmolality typically did not decrease below 160 mosmol/kgH\(_2\)O. Moderately preserved ability to dilute urine might be explained by an adaptive increase of DCT salt reabsorption, which functions as the most distal portion of the diluting segment (87). This moderate hyposthenuria clearly separates NKCC2-deficient patients from polyuric patients with nephrogenic diabetes insipidus, who often display urine osmolalities below 100 mosmol/kgH\(_2\)O.

In addition to iso- or hyposthenuric polyuria, newborns lose large amounts of Na\(^+\)-Cl\(^-\) through the urine, causing hyponatremia (120 mM) and hypochloremia (90 mM). Within days or a few weeks, urinary Na\(^+\) is decreasing and urinary K\(^+\) is increasing (“Na\(^+\)-K\(^+\)-switch”) (88). Increased Na\(^+\) delivery to the distal tubule, particularly when combined with activation of renin-angiotensin-aldosterone system (RAAS), enhances excretion of K\(^+\) and H\(^+\), causing hypokalemic alkalosis. Interestingly, RAAS activation is present both prenatally and postnatally. Therefore, increased aldosterone levels in the amniotic fluid had been proposed as a diagnostic marker for the disorder (106).

Control of renin secretion is closely dependent on the function of salt-reabsorbing molecules. Macula densa (MD) cells possess the same solute transporter equipment as TAL epithelial cells. NKCC2 mediates Cl\(^-\) entry and, by this, provides a sensing mechanism for Cl\(^-\) concentration in the fluid of the adjacent lumen. When NKCC2 is defective, MD cells transduce a signal falsely indicating a very low amount of Cl\(^-\) in the lumen and activate, via MAP-kinases and cyclooxygenase (COX-2), renin release from juxtaglomerular cells (90, 127).

An impaired MD chloride-sensing mechanism also disrupts tubuloglomerular feedback (TGF). Pharmacological inhibition of NKCC2 is known to prevent luminal Na\(^+\)-Cl\(^-\) from affecting glomerular vascular tone (125). The absence of compensatory TGF-mediated decrease of GFR aggravates tubular salt and fluid wasting.
Within the first months of life, nearly all patients develop medullary nephrocalcinosis in parallel with persistently high urinary Ca\(^{2+}\)/H\(^{1+}\) excretion. In the normal TAL, apical K\(^{+}\)/H\(^{1+}\) secretion, together with basolateral secretion of Cl\(^{-}\)/H\(^{1+}\) results in a transepithelial, lumen positive voltage difference of \(-10\) mV (33, 34). This voltage gradient provides an important driving force for the paracellular flux of Na\(^{+}\), Ca\(^{2+}\), and Mg\(^{2+}\) into the interstitial space. In turn, impaired electrogenic Cl\(^{-}\)/H\(^{1+}\) transport in the TAL inhibits paracellular transport of Ca\(^{2+}\), resulting in hypercalciuria. Remaining high Na\(^{+}\)-Cl\(^{-}\) concentrations in the tubular lumen lead to an adaptive increase in Na\(^{+}\)-Cl\(^{-}\) transport in segments downstream from TAL (1, 80). This will inhibit Ca\(^{2+}\) absorption in these more distal segments and further exacerbate urinary Ca\(^{2+}\) wasting (19). It is puzzling that conservation of Mg\(^{2+}\) is not affected to a similar extent, as NKCC2-deficient patients do not commonly have hypomagnesemia. This is even more surprising because loss-of-function mutations in paracellin-1, which ensures transport of divalent cations through the tight junctions of the TAL epithelia, invariably cause both hypercalciuria and hypomagnesuria. Plasma Mg\(^{2+}\) levels in paracellin-1-deficient patients are often found to be below 0.4 mM (124). The difference between both disorders with respect to Mg\(^{2+}\) handling might be explained by an indissoluble upregulation of both Na\(^{+}\)-Cl\(^{-}\) and Mg\(^{2+}\) reabsorption pathways in DCT cells in case of a NKCC2 defect (55).

Cardinal symptoms of NKCC2 deficiency displayed by humans, such as polyuria, hypercalciuria, PGE\(_{2}\) overproduction, and failure to thrive, similarly were observed in the mouse knockout model. With respect to K\(^{+}\) handling and acid-base balance, however, NKCC2-deficient mice display a clearly different phenotype with metabolic acidosis and hyperkalemia (Table 2). Plasma Na\(^{+}\) and Cl\(^{-}\) concentrations are markedly elevated, suggesting that water diuresis exceeds renal salt loss. This discrepancy is likely attributable to profound congenital hydronephrosis of NKCC2-deficient mice, probably in re-
Table 2. Major discrepancies in the phenotypical presentation of humans and mice deficient for either NKCC2 or ROMK

<table>
<thead>
<tr>
<th>Human NKCC2/ROMK−/−</th>
<th>Mouse NKCC2/ROMK−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyhydramnios</td>
<td>+</td>
</tr>
<tr>
<td>Renal morphology</td>
<td>nephrocalcinosis</td>
</tr>
<tr>
<td>Profound GFR reduction</td>
<td>−</td>
</tr>
<tr>
<td>Polyuria</td>
<td>saliuresis &gt; water diuresis</td>
</tr>
<tr>
<td>Postnatal plasma levels of Na⁺ and Cl⁻</td>
<td>↓</td>
</tr>
<tr>
<td>Acid-base derangement</td>
<td>metabolic alkalosis</td>
</tr>
</tbody>
</table>

GFR, glomerular filtration rate.

The mechanism of RAAS activation is virtually identical to that proposed for NKCC2-deficient patients. However, despite the presence of high plasma aldosterone levels, ROMK-deficient patients exhibit transient hyperkalemia in the first days of life (48). Hyperkalemia and coexistent hyponatremia together resemble the clinical picture of pseudohypoaldosteronism type 1 (PHA1). Indeed, several published cases of PHA1 turned out to be misdiagnosed since subsequent mutation analysis identified ROMK mutations as the underlying defect (2, 23, 106). The severity of initial hyperkalemia decreases with gestational age (84). Hyperkalemia is attributed to the additional role of ROMK in the cortical collecting duct (CCD) where it participates in the process of K⁺ secretion (Fig. 1C). Although less pronounced compared with defective NKCC2, most ROMK-deficient patients develop hypokalemia in the course of the disease (48). The only transient nature of hyperkalemia in our patients must be explained by an alternative K⁺ secretion pathway in the CCD. As correction of hyperkalemia does not occur right from the beginning, this alternative pathway might possibly be subject to maturation. One attractive candidate for the alternative route is the large-conductance (>100 pS) K⁺ channel identified in the apical membrane of CCD principal cells (25, 96). Because of its low open probability, the large-conductance K⁺ channel provides no significant apical K⁺ release under physiological conditions. Experimental data, however, suggest that its activity increases with enhanced fluid and solute delivery to the CCD (116, 121).

A tendency toward hyperkalemia is also observed in genetically engineered mice lacking ROMK, which might suggest the predominant role of ROMK for net K⁺ secretion in mice (71, 73). Drawing conclusions from the mouse model, however, again is hampered by concomitant congenital hydronephrosis. Total GFR of ROMK-deficient mice was only 15% of the wild-type, likely due to a reduced number of functioning nephrons (71). Renal insufficiency accounts for metabolic acidosis, which further aggravates hyperkalemia. Untreated, only a small percentage of ROMK-deficient mice survived to weaning. Remarkably, surviving mice preserve their ability to concentrate urine to about twice of the plasma osmolality. This is in contrast to NKCC2-deficient mice and is also not observed in the human phenotype (Fig. 2). Because Na⁺-Cl⁻ reabsorption in the TAL is an essential determinant of urinary concentration capacity, surviving ROMK-deficient mice may harbor compensatory mechanisms to overcome complete inhibition of TAL salt reabsorption. This was confirmed by micropuncture studies, which found Cl⁻ reabsorption between late proximal and early distal tubular sites to be reduced by ~40%, but not completely blunted (71).

Chloride Channel β-Subunit Barttin

The most recently identified molecule in the salt transport machinery of TAL epithelial cells is the renal Cl⁻ channel β-subunit barttin. Discovery of barttin was initiated by chromosomal linkage of a very rare variant of tubular salt wasting associated with sensorineural deafness. Eight affected families have been reported so far, two of whom are treated in our hospital (50). By a positional cloning strategy, a novel gene, BSND, was identified, and inactivating mutations were found in affected individuals (8). Because the gene product, barttin, had no homology to any known protein, its physiological...
function remained unclear until two groups independently described the role of barttin as an essential β-subunit of the ClC-K Cl\(^{-}\) channels (20, 122).

Two Cl\(^{-}\) channels of the CIC family are highly expressed along the distal nephron, with CIC-Ka being exclusively expressed in the thin ascending limb (tAL), and its homolog CIC-Kb predominantly expressed in the distal tubule. Colocalization of both Cl\(^{-}\) channels was observed in rat TAL segments (122). Previous efforts to express functional human CIC-K channels were unsuccessful. Only coexpression of CIC-K with the newly identified protein barttin reconstituted CIC-K-specific Cl\(^{-}\) currents. The function of barttin was defined as to facilitate the transport of CIC-K channels to the cell surface and to modulate biophysical properties of the assembled channel.

In affected individuals, the barttin defect implies a disruption of Cl\(^{-}\) exit across the basolateral membrane in TAL, as well as DCT cells. As a consequence, patients display the most severe phenotype among SLTs. The phenotype is in several features indistinguishable from the clinical picture attributed to a defect of the solute transporters in the apical TAL membrane, which proves the cross-talk between Na\(^{+}\)-Cl\(^{-}\) apical entry and basolateral exit mechanisms.

The first symptom of the disorder is maternal polyhydramnios due to fetal polyuria beginning at ~22 wk of gestation. Again, as in the NKCC2 or ROMK defect, polyhydramnios accounts for preterm labor and extreme prematurity. Postnatally, patients are at high risk of dehydration. Plasma Cl\(^{-}\) levels decrease to ~80 mM; a further decrease can usually be avoided by close laboratory monitoring and rapid intervention in neonatal intensive care units. Polyuria again is resistant to DDAVP and urine osmolalities range between 200 and 400 mosmol/kgH\(_{2}\)O.

Unlike patients with loss-of-function mutations in ROMK and NKCC2, barttin-deficient patients exhibited only transitory hypercalciuria (50, 105). Medullary nephrocalcinosis was absent; instead, kidney biopsies showed pronounced tissue damage (glomerular sclerosis, tubular atrophy, and mononuclear infiltration). In contrast to classical HPS, progressive renal failure is common, two reported patients underwent renal transplantation (50), and one expired from acute renal failure (unpublished observation). Lack of hypercalciuria can be explained by the additional role of CIC-K-mediated Cl\(^{-}\) transport in the DCT. As chronic pharmacological inhibition of Na\(^{+}\)-Cl\(^{-}\) transport in DCT by thiazides leads to hypocalciuria, the net effect of a combined defect in Na\(^{+}\)-Cl\(^{-}\) transport in TAL and DCT might be neutral with respect to urinary Ca\(^{2+}\) excretion. In contrast, renal Mg\(^{2+}\) conservation is severely impaired, leading to a progressive decline of plasma Mg\(^{2+}\) concentration which is possibly the result of the disruption of both Mg\(^{2+}\) reabsorption pathways, the paracellular one in the TAL and the transcellular one in DCT, respectively. Likewise, the concerted action of furosemide and thiazides is associated with a greater risk to develop clinically relevant Mg\(^{2+}\) deficiency (57).

The barttin defect is invariably associated with sensorineural deafness. Clarification of the pathogenesis of this rare disorder has provided a deeper insight into the mechanisms of endolymph secretion in the inner ear: Marginal cells of the stria vascularis contribute to the endolymph composition by establishment of a high K\(^{+}\) gradient. Transcellular K\(^{+}\) passage is mediated by the furosemide-sensitive Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) symporter type 1 (NKCC1) ensuring K\(^{+}\) entry into the marginal cells. Voltage-dependent K\(^{+}\) channels mediate K\(^{+}\) secretion into the endolymph. Functional operation of NKCC1 requires Cl\(^{-}\) recycling from the cell into the interstitium. The barttin-deficient phenotype demonstrated for the first time that this recycling is provided by the CIC-K/barttin complex. Disruption of ion movement either by mutations in barttin or the apical K\(^{+}\) channels abolishes K\(^{+}\) secretion into the endolymph and causes inner ear deafness (111). Ototoxicity of furosemide which, next to NKCC2, also blocks the NKCC1 symporter, is also related to this pathway.

Renal Chloride Channels CIC-Ka and CIC-Kb
(Digenic Disorder)

The concept of the physiological role of barttin as a common β-subunit of CIC-K channels was substantiated by the very recent description of an individual harboring inactivating mutations in both the CIC-Ka and CIC-Kb Cl\(^{-}\) channel, respectively (97). The patient’s phenotype is indistinguishable from barttin-deficient patients (Fig. 2) (39). This observation not only proves the concept of the functional interaction of barttin with both CIC-K isoforms but also excludes other important functions of barttin, not related to CIC-K channel interaction.

DISORDERS OF THE DISTAL TUBULE, THE THIAZIDE-LIKE SLTs

Renal Chloride Channel CIC-Kb (Monogenic Disorder)

Given a normal CIC-Ka function, an isolated defect of the CIC-Kb gene leads to a more variable phenotype. Several studies have indicated that the clinical variability is not related to a certain kind of mutation (62, 130). Even the most deleterious mutation, which implies the absence of the complete coding region of the CIC-Kb gene and which affects nearly 50% of this patient cohort, can cause varying degrees of disease severity. Features of tubular dysfunction distal from the TAL predominate (Fig. 2), suggesting a major role of CIC-Kb in the thiazide-sensitive tubular segment. Although TAL salt transport can be impaired to a variable extent, its function is never completely blunted. Obviously, other routes of basolateral Cl\(^{-}\) exit can be recruited in the TAL segment, most likely via CIC-Ka.

The neonatal period in CIC-Kb-deficient patients is usually uneventful. Only one of four patients had a history of mild maternal polyhydramnios. Median duration of pregnancy is 38.4 wk in our patient cohort. More than half of the patients are diagnosed within the first year of life. Symptoms at initial presentation include failure to thrive, dehydration, muscular hypotonia, and lethargy. Laboratory examination reveals plasma Cl\(^{-}\) concentration as low as 60 mM, plasma Na\(^{+}\) concentration of ~120 mM, and severe hypokalemic alkalosis ([K\(^{+}\)]\(_{\text{plasma}}\) frequently below 2 mM). At first presentation, electrolyte derangement is usually more pronounced compared with the other groups, because renal salt wasting progresses slowly and is virtually not accompanied by polyuria, which delays medical consultation. Plasma renin activity is markedly increased, whereas plasma aldosterone concentration is only slightly elevated. This discrepancy might be attributed to inhibition of aldosterone secretion by hypokalemia and alkalosis. Therefore, normal or slightly elevated aldosterone levels
under conditions of profound hypokalemic alkalosis are, in fact, inappropriately high.

Urinary concentration ability is preserved at least to a certain extent. Several patients achieved urinary osmolalities above 700 mosmol/kgH₂O in morning urine samples (Fig. 2). Because medullary hypertonicity critically depends on Na⁺-Cl⁻ reabsorption in the TAL, the ability to concentrate urine above 700 mosmol/kgH₂O indicates intact TAL function despite ClC-Kb deficiency. The integrity of TAL function is also reflected by the finding that hypercalciuria is present in only half of the patients and exists only temporarily. Indeed, the majority of patients exhibit normal or even low urinary Ca²⁺/Mg²⁺ concentrations, which can be attributed to gender, age, and high urinary Mg²⁺ elimination together with Mg²⁺ reabsorption of mineral calcium, as demonstrated by increased bone density, as well as chondrocalcinosis (29). Although the coexistence of hypomagnesemia and hypocalciuria is typical for NCCT deficiency, it is neither a specific nor universal finding. Clinical observations in NCCT-deficient patients disclosed intraindividual and interindividual variations in urinary Ca²⁺/Mg²⁺ concentrations, which can be attributed to gender, age-related conditions of bone metabolism, intake of Mg²⁺ supplements, changes in water diuresis and urinary osmolality, respectively (68, 91). Likewise, hypomagnesemia might not be present from the beginning. Because <1% of total body Mg²⁺ is circulating in the blood, renal Mg²⁺ loss can be corrected temporarily by release from bone and muscle deposits, as well as by increased intestinal Mg²⁺ reabsorption. Therefore, the strict definition of hypomagnesemia with coincident hypocalciuria to separate Gitelman (NCCT) syndrome from Bartter (ClC-Kb) syndrome appears arbitrary (129, 130).

Thiazide-Sensitive Na⁺-Cl⁻ Cotransporter

Because the NCCT cotransporter is specifically expressed in DCT, the human phenotype deficient for NCCT serves as a valuable tool to assess the specific contribution of this segment to renal salt and fluid conservation. DCT epithelia contain two cell types (82): DCT1 cells that express the NCCT as its predominant apical Na⁺ entry pathway, and further distal residing DCT2 cells that express the epithelial Na⁺ channel as an additional pathway for apical Na⁺ reabsorption (Fig. 3) (70). Both Na⁺ entry pathways are inducible by aldosterone. DCT1 and DCT2 cells probably also differ in divalent cation transport closely depends on the function of ClC-Ka and ClC-Kb orthologs were found to be coexpressed in the TAL segment in rats (122). An isolated defect of ClC-Ka protein, as demonstrated by a ClC-Ka-deficient mouse model, induces a mild diabetes insipidus-like phenotype without major disturbances of renal salt homeostasis (76). It was also suggested that Cl⁻ may pass across the basolateral membrane via K⁺/Cl⁻ cotransporters, which also reside in the TAL segment (17, 35), although knockout mice deficient for K⁺/Cl⁻ cotransporters do not suffer from renal salt wasting (11, 12). Again, physiological variations between different mammalian species have to be considered. In humans, the barttin- and combined CIC-Ka/b-deficient phenotypes suggest that the CIC-K channels predominantly mediate basolateral Cl⁻ efflux in the TAL segment. The relative contribution to this process can change between both channels, for example, by confounding genetic factors. Very recently, we described a frequent genetic variant of ClC-Kb, T481S, which causes pronounced channel activation (51). This polymorphism turned out to be more frequent in Western Africa and is associated with essential hypertension (52). Therefore, we hypothesize that genetic confounders, which are unequally distributed among different ethnicities, might also influence the ability to recruit ClC-Ka for compensation of ClC-Kb deficiency in the TAL, accounting for the more variable manifestation of the ClC-Kb disorder.
Yet, the pathophysiology that compromises distal Mg\(^{2+}\) reabsorption and favors reabsorption of Ca\(^{2+}\) has not been identified conclusively. However, two observations in our and other patient studies provide some insights: 1) The occasional coexistence of hypomagnesemia and hypocalciuria in ClC-Kb-deficient patients indicates that the dissociation of renal Ca\(^{2+}\) and Mg\(^{2+}\) handling is not restricted to NCCT defects but rather a consequence of impaired transcellular Na\(^{+}\)-Cl\(^{-}\) reabsorption in the DCT segment; and 2) 70% of the filtered load of Mg\(^{2+}\) and 30% of filtered Ca\(^{2+}\) are reabsorbed in the TAL via the paracellular route. Disruption of the paracellular route due to mutations of the tight-junction protein paracellin-1 causes severe hypomagnesemia and hypercalciuria. NKCC2 and ROMK patients with defective TAL Na\(^{+}\)-Cl\(^{-}\) reabsorption also display hypercalciuria but do not develop severe hypomagnesemia. Different from the paracellin-1 disorder, defective salt reabsorption in the TAL increases the Na\(^{+}\) load in the distal tubule and induces an upregulation of NCCT/ClC-Kb and ENaC (1, 80). Because loss-of-function of NCCT and ClC-Kb is associated with hypermagnesuria, one might safely predict that upregulation of NCCT/ClC-Kb will increase distal Mg\(^{2+}\) reabsorption. A significant influence of ENaC on distal Mg\(^{2+}\) uptake appears less likely as neither activating nor inactivating mutations cause major disturbances in renal Mg\(^{2+}\) handling.

Recently, the genetic analysis of a rare Mg\(^{2+}\)-wasting disorder, hypomagnesemia with secondary hypocalcemia, identified TRPM6, a new member of the transient receptor potential (TRP) family of cation channels, as the first component of active transcellular Mg\(^{2+}\) reabsorption in the DCT (98). TRPM6 colocalizes with the NCCT protein, and is thought to participate in the formation of the apical Mg\(^{2+}\)-permeable ion channel in DCT1 cells (Fig. 3) (120). Yet, a direct link between active Na\(^{+}\)-Cl\(^{-}\) absorption and uptake of Mg\(^{2+}\) in DCT1 cells is still missing. However, indirect evidence is provided by the laboratory condition found in NCCT (and ClC-Kb)-deficient patients, as well as by morphological studies upon pharmacological inhibition of NCCT in rats. Long-term administration of thiazides reduced DCT cell mass in rat kidney, probably by inducing apoptosis (69). This observation was also confirmed by morphometric analyses of distal convolutions in NCCT-deficient mice (100). Laboratory examination further confirmed the dissociation of renal Ca\(^{2+}\) and Mg\(^{2+}\) handling as previously described for GS patients, although these mice did not exhibit metabolic alkalosis and hypokalemia. Whereas NCCT (and TRPM6)-containing DCT1 cells undergo apoptosis, the DCT2 cells that predominantly express ENaC can proliferate. Because DCT2 cells also express the epithelial Ca\(^{2+}\)-permeable transcellular ECaC (Fig. 3) (70), subsequent increase of the absorption capacity for Ca\(^{2+}\) could explain low urinary Ca\(^{2+}\) in Bartter/Gitelman syndrome, as well as the hypocalciuric effect of long-term administration of thiazides.

**THE ENIGMATIC ROLE OF PROSTAGLANDIN E\(_2\) IN SLT**

The involvement of prostaglandins in the pathogenesis of SLTs has been recognized early by several investigators (22, 31, 119). Bartter hypothesized PGE\(_2\) hyperactivity being a
phenomenon secondary to hypokalemia. However, this hypothesis is not supported by several more recent experimental and clinical studies. In SLT patients, PGE2 excretion did not correlate with the degree of hypokalemia. For example, ROMK-deficient patients who exert only modest hypokalemia, if at all, displayed much higher urinary PGE2 concentrations compared with BS/GS patients with more severe hypokalemia (Table 4).

It is now well established that salt depletion induces renal PGE2 overproduction (63). Rats on a low-salt diet (44), as well as SLT patients (59) and humans treated with furosemide (59), exhibit enhanced expression of COX-2 and microsomal prostaglandin E2 synthase (mPGES) (26, 60) in the MD. Both enzymes are key components of the prostanoid pathway and are not expressed in this nephron segment in healthy controls. In cultured rabbit cortical TAL cells, intracellular depletion of chloride due to either administration of furosemide or lack of Cl− in the incubation medium has been shown to induce COX-2 via activation of the MAP kinase p38 (14). A similar mechanism may also underlie the induction of COX-2 (and possibly mPGES) in SLT patients, because entry of Cl− is impaired in patients with loss-of-function mutations in either ROMK, Barttin, or NKCC2 genes. Thus the sequence of events from impaired chloride entry into MD cells to the generation of PGE2 is now characterized to some extent. In contrast, the effects downstream of enhanced PGE2 generation are poorly understood: several groups have consistently shown in salt- and/or volume-depleted humans (15, 90) and experimental animals (43) that COX-2 metabolites stimulate secretion of renin. Thus one may speculate that in normal salt-depleted individuals, COX-2 expression promotes salt retention via activation of the RAAS. Surprisingly, in SLT patients, salt losses decrease when hyperreninemia is blunted by COX-2 inhibition. Several hypotheses have been presented to explain the effects of PGE2 on diuresis and saluresis and the antidiuretic effects of prostaglandin-inhibitors such as indomethacin.

First, indomethacin reduces polyuria by reducing GFR. Although COX inhibition is associated with a moderate decrease of GFR by an average of 22% in these studies, this does not completely explain the correction of saluresis by about 50% (range 34 to 65%) and a rise in urinary osmolality from a median of 155 to 187 mosmol/kgH2O (99) (236 to 270 mosmol/kgH2O; more recent unpublished data) in furosemide-like SLTs (31, 67, 81, 90, 99, 103).

Second, indomethacin reduces polyuria by inhibiting the diuretic effects of PGE2. Single nephron perfusion studies from the late 1980s have shown that coadministration of furosemide and indomethacin reduces the diuretic effect observed with furosemide only in the loop of Henle by ~50% (37). Add-back experiments with various prostaglandins have shown that only PGE2 restores the diuretic effect of furosemide when coadministered with indomethacin (56). This finding indicates that the diuretic action of furosemide requires induction and action of PGE2. PGE2 exerts its biological effects through interaction with various tubular G protein coupling receptors, named EP-receptors. In rabbit isolated perfused collecting ducts, activation of EP1 inhibits Na+ and water reabsorption via a Ca2+-coupled mechanism (38). EP3 inhibits cAMP formation by coupling to pertussis-sensitive inhibitory G-protein. Activation of EP3 has been shown to inhibit AVP stimulated salt and water absorption in the thick ascending limb and the collecting duct, respectively (45). The notion that PGE2 has direct tubular effects is in keeping with the observation that indomethacin reduces polyuria to a greater extent than GFR in SLT patients (90).

Finally, excessive PGE2 formation may result in an enhanced renal medullary perfusion (94), resulting in a washout of the osmotic gradient and may thus further compromise renal concentrating ability in patients affected by hypokalemic SLT.

Presently unresolved is the issue about the precise molecular mechanisms that activate COX-2 and microsomal PGE2 synthase in the salt-wasting state, as well as the precise molecular targets of PGE2. Nevertheless, unspecific COX inhibitors, as well as COX-2-specific inhibitors, are presently the major pharmacotherapeutic option we can offer to our patients with SLTs. They are of unsurpassed benefit, particularly during life-threatening situations in the perinatal period and early infancy due to renal salt and water wasting and the deleterious effects of extrarenal PGE2 hyperactivity like vomiting, diarrhea, and fever. Treatment of SLT patients with COX-2 inhibitors such as rofecoxib compared with classical nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin may be superior in terms of gastrointestinal side effects and specificity. However, recent studies suggest that long-term exposure to rofecoxib may carry a higher cardiovascular risk compared with nonselective inhibitors such as naproxen (24). Therefore, treatment of SLT patients should be initiated with classical NSAIDs such as indomethacin.

**SOLUTION OF THE CASE REPORT**

When treatment with indomethacin was initiated, initially, hyperprostaglandin E syndrome was suspected. However, the demonstration of sensorineural deafness, as well as the absence of medullary nephrocalcinosis, which was established shortly thereafter, rather suggested a barttin defect. Surprisingly, neither the haplotype data nor the direct sequencing of the barttin gene could confirm this assumption. A novel explanation was a digenic disorder that affects both ClC-Ka and ClC-Kb previously defined in barttin-handling salt-losing tubulopathies. Indeed, this hypothesis turned out to be true when we identified for the first time a homozygous point mutation in the ClC-Ka channel, together with a complete deletion of the ClC-Kb channel genes. By contrast, the Barttin gene could confirm this assumption. A novel explanation was a digenic disorder that affects both ClC-Ka and ClC-Kb previously defined in barttin-handling salt-losing tubulopathies. Indeed, this hypothesis turned out to be true when we identified for the first time a homozygous point mutation in the ClC-Ka channel, together with a complete deletion of the ClC-Kb channel genes (97). Thus the patient’s unique clinical picture with severe salt-wasting, sensorineural deafness, and absence of nephrocalcinosis together with the knowledge about the function of ClC-Ka and ClC-Kb previously defined in barttin-deficient patients, led to the identification of the underlying genetic defect. Without this premise, the detection of an extremely rare digenic defect affecting two Cl− channel genes probably would not have been possible.

<table>
<thead>
<tr>
<th>NKCC2−/−</th>
<th>ROMK−/−</th>
<th>Barttin−/−</th>
<th>CIC-Ka−/−</th>
<th>NCCT−/−</th>
</tr>
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<tbody>
<tr>
<td>n = 17</td>
<td>n = 20</td>
<td>n = 10</td>
<td>n = 20</td>
<td>n = 17</td>
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<td>79 (17–624)</td>
<td>114 (26–371)</td>
<td>174 (27–238)</td>
<td>41 (6–265)</td>
<td>32 (7–183)</td>
</tr>
</tbody>
</table>

Values are in ng·h−1·(1.73 m2)−1. Normal range is 4–27 ng·h−1·(1.73 m2)−1 (Ref. 66).
Perspectives

Although careful clinical examination of patients suffering from inherited salt-losing kidney disorders taught us a lot about the role of the affected transport proteins in the process of renal tubular salt reabsorption, there still remain several open questions: How do we identify regulators of compensatory activation of Na\(^+\)-Cl\(^-\) conserving mechanisms in response to pharmacological or inherited blockade of a single solute transporter? How can we explain the dramatic effect of cyclooxygenase inhibitors on renal tubular salt handling? And what are the reasons for the phenotypic differences between the human diseases and the animal models? We hope that with this review, we have clearly pointed out that to answer these questions, we depend on the close interaction between basic science and clinical medicine.

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REFERENCES


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SALT HANDLING IN THE DISTAL NEPHRON

Invited Review


