Classic diseases revisited

The pathophysiological and molecular basis of Bartter's and Gitelman's syndromes

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Summary

Molecular defects affecting the transport of sodium, potassium and chloride in the nephron through the ROMK K⁺ channel, Na⁺/K⁺/2Cl⁻ cotransporter, the Na⁺/Cl⁻ cotransporter and chloride channel have been identified in patients with Bartter's and Gitelman's syndromes. Defects of the angiotensin II type I receptor and CFTR have also been described. These defects are simple (ie, most are single amino acid substitutions) but affect key elements in tubular transport. The simplicity of the genetic defects may explain why the inheritance of these conditions remains unclear in most kindreds (ie, not just recessive or dominant) and emphasises the crucial importance of the conformational structure of these channels. Application of this molecular information will allow the early genetic identification of patients with these syndromes and enable us to differentiate between the various disorders at a functional level. It may also identify a subgroup in which the heterozygous form may make patients potentially exquisitely sensitive to diuretics.

Keywords: Bartter's syndrome; Gitelman's syndrome; hypokalaemic alkaloses

The inherited hypokalaemic alkaloses are typified by a constellation of metabolic abnormalities, including metabolic alkalosis, hypokalaemia, chloride wasting, hypomagnesaemia, and hyper- or hypocalciuria.¹ Molecular cloning and functional characterisation of renal epithelial channels and transport proteins has expedited our understanding of the molecular basis of the more common syndromes. Genetic analysis of specific tubular disorders has provided definitive links between these syndromes and the cellular defects.

Bartter's and Gitelman's syndromes represent two of the autosomal recessive syndromes producing normotensive hypokalaemic metabolic alkaloses that have been genetically classified.^{2–8} Successful diagnosis is essential to exclude the other causes of hypokalaemic metabolic alkalosis (box 1) and allow effective management. This review summarises the current scientific and clinical aspects of Bartter's and Gitelman's syndromes.

Clinical aspects

Frederic Bartter *et al* first described this syndrome in 1962.⁹ The exciting progress recently made in elucidating the underlying molecular defects has greatly enhanced our understanding of the phenotypic variations seen in the inherited metabolic alkaloses. Bartter's syndrome represents a heterogeneous spectrum of clinical phenotypes.

Prior to this molecular characterisation, several characteristic clinical and biochemical features have differentiated Gitelman's and Bartter's syndromes. Both syndromes present early in life frequently before the twenties.¹⁰ They have an incidence of 1.2 per million with no racial or sexual predilection.⁶ The biochemical effects, resulting from the use of excessive loop and thiazide-sensitive diuretics, which act in the thick ascending limb of the loop of Henle (TAL) and distal convoluted tubule (DCT), respectively, represent good models for the biochemical features found in patients with Bartter's and Gitelman's syndromes, respectively. The presence of hypocalciuria, hypomagnesaemia, presentation later in life,³ milder symptomatology and relative lack of a concentrating defect (in keeping with a defect of the DCT) are all features which may occur in patients with Gitelman's and not Bartter's syndrome.

Children affected by these inherited disorders suffer growth and developmental retardation.^{11–12} Generally, patients experience muscle weakness and cramps, attributable to profound hypokalaemia.^{13–14} Both adults and children describe a range of symptoms (box 2).^{15–16} Classically, patients are normotensive or hypotensive with elevated plasma renin and aldosterone concentrations. Both syndromes have a subnormal responsiveness to infused angiotensin II, and there is often an increase in urinary prostaglandin E₂ excretion in Bartter's patients.¹⁷ Gitelman's patients, however, tend to have normal urinary prostaglandin E₂ concentrations.

In a subgroup of patients, antenatal Bartter's/hyperprostaglandin E syndrome has been described. This disease entity typically presents as a life-threatening condition beginning in utero, with marked foetal polyuria that leads to polyhydramnios and premature delivery. Survivors develop nephrocalcinosis and osteopenia from the marked hypercalciuria. Renal biopsy demonstrates juxtaglomerular hyperplasia, but this finding is non-specific, being associated with general persistent sodium depletion and hyperreninaemia.¹⁸

The aetiology of hypokalaemic metabolic alkalosis can be distinguished by considering both the clinical and biochemical features. The diagnosis of Bartter's/Gitelman's syndrome is, however, one of exclusion, but the constellation of serum and urinary biochemical abnormalities detailed in box 3 are suggestive. Patients can be categorised as either sodium chloride and extracellular fluid volume depleted, and hence present with clinical signs of hypovol-

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Accepted 31 March 1999

Main causes of hypokalaemic metabolic alkalosis

With normotension

Inherited

- Fanconi syndrome
- cystinosis
- chloride-wasting diarrhoea
- cystic fibrosis (excessive sweat loss)
- hyperprostaglandin E/antenatal Bartter's syndrome
- Gitelman's syndrome
- Bartter's syndrome

Acquired

- diuretic use/abuse
- surreptitious vomiting
- laxative abuse
- dietary chloride deficiency
- primary magnesium-losing nephropathy
- aminoglycosides
- chemotherapy (eg, cisplatin, nephrotoxicity, ifosfamide)
- amphotericin
- infusions of large doses of penicillin

With hypertension

- Liddle's syndrome
- apparent mineralocorticoid excess
- glucocorticoid-suppressible hyperaldosteronism

Box 1

Symptoms described in hypokalaemic metabolic alkalosis

- muscle cramps
- weakness
- polyuria
- nocturia
- nephrogenic diabetes insipidus
- failure to thrive
- salt-craving
- enuresis
- constipation
- seizures
- tetany
- joint pains, due to chondrocalcinosis

Box 2

Biochemical changes suggestive of Bartter's/Gitelman's syndromes

Serum

- hypokalaemia
- metabolic alkalosis
- hyponatraemia
- hypochloraemia
- hyperreninaemia
- hyperaldosteronism
- magnesium wasting (Gitelman's)

Urine

- hypocalciuria (Gitelman's)
- hypercalciuria (Bartter's)
- hyperprostaglandinuria (Bartter's)
- hypochloraemia

Box 3

aemia, or normovolaemic/hypervolaemic from the increased mineralocorticoid activity. Measurement of urinary chloride (Cl^-) is useful in the initial diagnostic evaluation to serve as a marker to distinguish between volume-depleted and volume-expanded groups. A low urinary Cl^- (less than 10 mmol/l) is suggestive of disease processes producing Cl^- and volume depletion or after recent, within 12 hours, diuretic administration. In this situation the kidney is avidly reabsorbing sodium chloride at all the tubule sites, especially the TAL. An exception to this is the hereditary alkalosis where there is an inappropriately high urinary Cl^- (greater than 40 mmol/l) resulting from the reduced NaCl reabsorption in the TAL.

Therapeutic options

At present, Bartter's and Gitelman's syndromes remain incurable. Reversal or inhibition of the underlying metabolic/electrolyte disturbance is desirable, but available treatment is primarily aimed at ameliorating symptoms by elevating the profoundly low serum potassium (K^+). Potassium supplementation usually fails to normalise serum K^+ concentrations. Addition of potassium-sparing diuretics, eg, amiloride, triamterene or spironolactone, improves serum K^+ concentrations^{19, 20} but normalisation remains elusive. In a proportion of those patients with associated hypomagnesaemia, magnesium (Mg^{++}) supplementation augments the partial K^+ correction (box 4).²¹ This hypokalaemia, however, remains resistant to accessible recognised therapeutic manoeuvres.

Non-steroidal anti-inflammatory drugs suppress prostaglandin-stimulated renin release and restore the normal vasopressor responsiveness to angiotensin.^{22, 23} They also appear to improve the defective platelet aggregation that is sometimes evident in Bartter's syndrome,²⁴ but serum K^+ concentration remains low. Angiotensin-converting enzyme inhibitors, which reduce kaliuresis by blockade of the renin-angiotensin system, have shown varied success.²⁵⁻³⁰ Their main drawback, however, is their marked hypotensive effect. Angiotensin II receptor blockers, acting specifically on type I receptors in renal and extra-renal tissues, have yet to be studied.

LONG-TERM SEQUELAE

The prognosis with the available therapeutic options remains variable, as prolonged hypokalaemia may lead to tubulointerstitial disease and, potentially, end-stage renal failure. Fatal cardiac arrhythmias, however, remain surprisingly infrequent. A combination of therapeutic strategies provides the most logical and optimal approach to these patients.

RENAL TRANSPLANTATION

Data in this area are scarce as few patients appear to reach end-stage renal disease. However, one would expect transplantation to provide such patients with an organ containing the appropriate functioning wild-type protein rather than the native mutant protein.

Pathophysiology and molecular changes

The similarity of both biochemical and clinical alterations between hypokalaemic alkalosis and diuretic use indicated that the molecular basis of these disorders involved effects on molecular transporters/channels. Several transporters/channels have now been cloned from the kidney, including an inward rectifying K^+ channel (ROMK1), the thiazide-sensitive Na/Cl cotransporter (TSC), the loop diuretic-sensitive $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter (NKCC2), and the chloride channel (CLCKNB).³¹⁻³³ Mutations of these transporters/channels have now been shown to be present in many patients suffering from Gitelman's and Bartter's syndromes.

TSC MUTATION (GITELMAN'S SYNDROME)

Gitelman's syndrome has mainly autosomal recessive inheritance, although autosomal dominant cases have been described.³⁴ Simon *et al*³⁵ cloned and characterised the renal TSC gene and localised it to chromosome 16. They and others³⁵⁻³⁸ demonstrated many mutations of the TSC in a population of patients with hypokalaemic alkalosis that were not present in controls. Pollak *et al* confirmed these findings in a larger kindred of patients with Gitelman's syndrome.^{35, 39} They cloned the human analogue of the rat TSC, mapped it to chromosome 16q13 and discovered that recombinants localised the gene to a 15-centimorgan region on the long arm of chromosome 16.³⁹

Defects of the TSC in the DCT lead to reabsorptive failure of sodium (Na^+) and Cl^- and subsequent water loss with ensuing hypovolaemia (figure 1). The reduced vascular volume stimulates the renin-angiotensin system, resulting in

Treatment options for hypokalaemic metabolic alkalosis

- potassium supplements
- magnesium supplementation
- potassium-sparing diuretics
- non-steroidal anti-inflammatory drugs
- angiotensin-converting enzyme inhibitors
- angiotensin II receptor antagonists

Box 4

Figure 1 Cell model of distal convoluted tube

elevation of renin and aldosterone concentrations. These pathophysiological changes cause an increase in apical Na^+ reabsorption via epithelial cortical collecting duct Na^+ channels and stimulation of the basolateral Na^+/K^+ -ATPase to allow flow of Na^+ down its electrochemical gradient (figure 2). The increased aldosterone concentrations stimulate cortical and medullary collecting ducts H^+ -ATPase pumps leading to an increased apical H^+ ion secretion. K^+ and H^+ ion excretion increases as K^+ enters from the basolateral membrane via the Na^+/K^+ -ATPase pumps, resulting in hypokalaemic metabolic alkalosis. The resultant low intracellular Na^+ increases DCT Ca^{++} reabsorption via basolateral $\text{Na}^+/\text{Ca}^{++}$ exchangers, causing hypocalciuria. Magnesium loss, via apical $\text{Mg}^{++}/\text{Na}^+$ exchangers, increases due to the net negative transepithelial potential. This reduced Mg^{++} may also stimulate parathyroid hormone release, which further increases Ca^{++} reabsorption.

Figure 2 Cell model of the cortical collecting duct

Molecular classification of hypokalaemic metabolic alkalosis

Transporter defects

- TSC mutation
- NKCC2 mutation

Channel defects

- ROMK mutation
- CLCKNKB chloride channel defect

Other undefined mutations

- CFTR
- angiotensin II receptor
- renin gene
- atrial natriuretic peptide

Box 5

NA-K-2CL MUTATION (BARTTER'S SYNDROME)

Bartter's syndrome occurs with a familial tendency and is an autosomal recessive disorder.⁴⁰⁻⁴² Simon *et al* have linked Bartter's syndrome to a defect in the renal NKCC2 gene and identified mutations in a kindred with Bartter's syndrome that were not present in controls.⁴³ More recently, mutations of NKCC2 have also been identified which were found to be associated with antenatal Bartter's syndrome.⁴⁴

Mutation of the NKCC2, located on chromosome 15, leads to reduced Na^+ and Cl^- reabsorption in the TAL, with subsequent salt wasting and hypovolaemia and a similar progression as above (figure 3). As approximately 30% of the filtered Ca^{++} load is coupled to Na^+ transporter activity, this would also account for the observed hypercalciuria through the lack of a lumen positive potential to generate a net calcium reabsorption and thus lead to an increased urinary Ca^{++} concentrations. Magnesium, which is mainly reabsorbed in the TAL, would presumably be maintained through increased distal tubule reabsorption via $\text{Na}^+/\text{Mg}^{++}$ exchangers (figure 2).

ROMK MUTATION (BARTTER'S SYNDROME)

Potassium channels have been implicated in the regulation of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransport in the TAL, as K^+ channel antagonists abolish NKCC2 activity.⁴⁵ Two candidate mammalian K^+ channels have been isolated, ROMK and RACKT.^{33, 46} These belong to the K^+ channel family of inward rectifiers (ie, current is mainly in the inward direction) in the apical membrane of the distal nephron. In nine kindreds with Bartter's syndrome without mutations of NKCC2, mutations in ROMK have been demonstrated.⁴⁷ Others have recently identified mutations that have been linked with antenatal Bartter's syndrome.⁴⁸⁻⁵¹ Early work has demonstrated that many of the mutations produce non-functional channels or reduced K^+ fluxes, perhaps related to abnormalities in phosphorylation and protein trafficking.^{52, 53}

Pathophysiologically, loss of ROMK function leads to the inability of K^+ to recycle out of the cells of the TAL into the lumen, resulting in a reduced luminal K^+ concentration (figure 3). This in turn leads to an inhibition of $\text{Na}^+/\text{K}^+/\text{Cl}^-$ activity with resultant salt wasting from the TAL. The consequent reduced volume causes an increased renin release, secondary hyperaldosteronism, and

Figure 3 Cell model of the thick ascending limb of Henle

increased Na^+ reabsorption from the distal nephron in exchange for K^+ and H^+ ions, which are secreted.

CLCKNKB MUTATION (BARTTER'S SYNDROME)

Hypokalaemic alkalosis has been described in terms of a defect of Cl^- reabsorption in the TAL.⁵⁴⁻⁵⁵ Defects in Cl^- transport have been previously reported.⁵⁶⁻⁵⁷ Two closely related cloned Cl^- channels located on chromosome 1 have been postulated to mediate chloride reabsorption across the basolateral membrane of the TAL.⁵⁸ Linkage analysis in patients with hypokalaemic alkalosis without ROMK/NKCC2 defects has revealed mutations of this Cl^- channel.⁵⁹ Loss or impaired Cl^- channel activity from the basolateral membrane of the TAL leads to a rise in intracellular Cl^- resulting in a reduced Na^+ reabsorption and thus salt wasting and hypovolaemia (figure 3). These ionic changes stimulate the renin-angiotensin system, resulting in Na^+ reabsorption and K^+ secretion distally. The reduced lumen positive potential from the excess intracellular Cl^- leads to a reduction in Ca^{++} reabsorption and hypercalciuria.

ANGIOTENSIN II RECEPTOR TYPE 1 DEFECT

Yoshida⁶⁰ found a point mutation at position 931 of the angiotensin II type 1 receptor. This mutation produced an amino acid substitution present in one of five Bartter's syndrome patients but no healthy patients. This has been postulated to result in different tissue-specific effects of G-proteins involved in the signal transduction of the angiotensin receptor, thus explaining the impaired vascular responsiveness. However, this finding in a single patient may be incidental to the presence of one of the transport/channel defects which was not screened for in their study.

OTHER POTENTIAL MUTATIONS

Several other potential candidate genes have been identified, including those for atrial natriuretic peptide and the renin gene. At present, no molecular abnormalities of these genes have been detected in Bartter's syndrome patients.⁶¹ Several families lack mutations of ROMK, NKCC2, TSC or CLCKNKB. This subgroup may represent those with potential mutations of other K^+ channel or other unidentified membrane proteins. The heterogeneity of Bartter's syndrome suggests that further mutations exist. There are multiple reports of 'pseudo-Bartter's syndrome' in cystic fibrosis but it is unclear whether extra-renal sweat loss is the dominant cause. Moreover, isolated hypokalaemic alkalosis has been described in patients with milder forms of cystic fibrosis.⁶²⁻⁶³ Co-expression of CFTR with ROMK restores glibenclamide sensitivity⁶⁴⁻⁶⁵; hence, it is reasonable to suggest that some CFTR mutations result in dysfunction of the TAL.

Confusion and contention regarding the classification of hypokalaemic alkalosis still exists. The previously proposed pathophysiological classification is now inadequate and molecular labels could perhaps replace it (box 5).¹ This classification, however, is insufficient, as best seen with the infantile form of Bartter's syndrome which is a distinct clinical entity, but for which the molecular basis is at present incomplete.

Future developments

More detailed electrophysiological examination of the functional characteristics of mutations will demonstrate whether functional channels are produced and, if so, what their biophysical characteristics are relative to wild-type channels. Creation of transgenic models of these mutations will allow *in situ* examination of the clinical effects of specific therapies on biochemical parameters in patients with Bartter's and Gitelman's syndromes.

Summary points

- Bartter's and Gitelman's syndromes are tubular disorders in which the main manifestations are symptoms of hypokalaemia and salt wasting
- without treatment children may suffer from growth retardation
- the treatment of hypokalaemic metabolic alkalosis consists of ameliorating symptoms, specifically by raising plasma potassium and magnesium
- molecular studies have identified defects of ROMK, NKCC2, TSC, and CLCKNKB
- genetic identification will make the future diagnosis of the inherited hypokalaemic alkalosis more accurate and simple
- urinary chloride is a useful measure in the initial diagnostic evaluation of hypokalaemic alkalosis

Box 6

The Kaberry Research Fund is acknowledged for their generous support.

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