Partial genetic deficiency in tissue kallikrein impairs adaptation to high potassium intake in humans

Joana S. Monteiro[1], Anne Blanchard[2,3,4], Emmanuel Curis[3,5], Régine Chambrey[2,6], Xavier Jeunemaitre[2,6] and Michel Azizi[2,3,4]

[1]Faculty of Medicine, Porto University, Porto, Portugal; [2]Faculté de Médecine, Université Paris Descartes, Sorbonne Paris cité, Paris, France; [3]Assistance Publique Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Centre d'Investigations Cliniques, Paris, France; [4]INSERM, CIC 9201, Paris, France; [5]Université Paris Descartes, Faculté de pharmacie, Sorbonne Paris cité, laboratoire de biomathématiques, Paris, France and [6]INSERM, UMR970, Paris, France

Inactivation of the tissue kallikrein gene in mice impairs renal handling of potassium due to enhanced H, K-ATPase activity, and induces hyperkalemia. We investigated whether the R53H loss-of-function polymorphism of the human tissue kallikrein gene affects renal potassium handling. In a crossover study, 30 R53R homozygous and 10 R53H heterozygous healthy males were randomly assigned to a low-sodium/high-potassium or a high-sodium/low-potassium diet to modulate tissue kallikrein synthesis. On the seventh day of each diet, participants were studied before and during a 2-h infusion of furosemide to stimulate distal potassium secretion. Urinary kallikrein activity was significantly lower in R53H than in R53R subjects on the low-sodium/high-potassium diet and was similarly reduced in both genotypes on high-sodium/low-potassium. Plasma potassium and renal potassium reabsorption were similar in both genotypes on an *ad libitum* sodium/potassium diet or after 7 days of a high-sodium/low-potassium diet. However, the median plasma potassium was significantly higher after 7 days of low-sodium/high-potassium diet in R53H than in R53R individuals. Urine potassium excretion and plasma aldosterone concentrations were similar. On the low-sodium/high-potassium diet, furosemide-induced decrease in plasma potassium was significantly larger in R53H than in R53R subjects. Thus, impaired tissue kallikrein stimulation by a low-sodium/high-potassium diet in R53H subjects with partial tissue kallikrein deficiency highlights an inappropriate renal adaptation to potassium load, consistent with experimental data in mice.

Kidney International (2013) **84,** 1271–1277; doi:10.1038/ki.2013.224; published online 12 June 2013

KEYWORDS: kallikrein; polymorphism; potassium metabolism

Correspondence: *Anne Blanchard, Centre d'Investigations Cliniques, Hôpital Européen Georges Pompidou, 20-40 rue Leblanc 75908, Paris cedex 15, France. E-mail: anne.blanchard@egp.aphp.fr*

Received 13 December 2012; revised 16 April 2013; accepted 18 April 2013; published online 12 June 2013

Extracellular potassium (K) concentration is tightly regulated within physiological limits.[1] In response to a K load, acute changes in plasma K concentration are buffered by an initial shift toward intracellular space,[2] and full restoration of K balance is achieved by the kidneys that adapt urinary K excretion to match the daily K intake.[3,4] Renal K excretion is finely controlled in the distal nephron, where secretion and absorption of K can both occur. Whereas the amiloride-sensitive epithelial Na channel (ENaC) drives K secretion by principal cells and, therefore, promotes kaliuresis, H,K-ATPase (HKA) mediates net K absorption through intercalated cells.[3,5] In physiological conditions, the distal tubule secretes K. High K intake stimulates ENaC activity and renal outer medullary potassium channel (ROMK)–mediated K secretion by both aldosterone-dependent and aldosterone-independent mechanisms.[6,7] On a low-K diet condition, the renal switch from a high rate of K secretion to efficient K conservation is mediated in part by stimulation of H,K-ATPase type 2 (HKA2) in the distal nephron.[8] However, HKA2–deficient mice provided evidences that K adaptation can occur independently of HKA2[9,10] owing to the inhibitory effect of K depletion on both ENaC expression[6,11] and ROMK K activity[12] at the luminal membrane of principal cells. Renal tissue kallikrein (TK), a serine protease, has been shown in a mice knockout model to exert tonic inhibition on HKA2.[13]

Inactivation of the TK gene in mice has demonstrated that TK activates ENaC in principal cells[14,15] and inhibits HKA in intercalated cells.[13] Thus, TK has a kaliuretic effect by stimulating K secretion in principal cells and inhibiting K absorption in intercalated cells. Accordingly, TK-deficient mice develop postfeeding hyperkalemia.[13] These observations support a central role of TK in the maintenance of the stability of plasma K concentration after dietary K loading in mice.[13,16] However, data in humans are lacking.

Renal TK is synthesized in large amounts in the connecting tubule and cortical collecting tubules, and is released in urine and peritubular interstitium.[17] The level of urinary kallikrein (KLK) activity, which reflects the renal synthesis of the enzyme, is influenced in humans by both

genetic factors[18–20] and dietary sodium (Na) and K intakes.[17] A loss-of-function polymorphism in exon 3 of the human TK gene changes an active-site arginine at position 53 to a histidine (R53H), resulting in a substantial loss of kallikrein activity *in vitro*.[18] The *53H* allele is found at a frequency of 3% in white subjects,[18] and ~5–7% of the population are heterozygous for *53H*. On average, urinary KLK activity (UKLKa) is 50% lower in R53H heterozygotes than in R53R-homozygous subjects, reflecting the strong functional effect of the mutation.[18] The R53H polymorphism affects vascular function,[21] as well as intrarenal modulation of renal calcium reabsorption,[22] as initially demonstrated in TK-null mice.[13] This polymorphism thus provides a unique opportunity to study the physiological roles of TK on K balance in humans.

The purpose of this study was to investigate the effect of a partial human TK deficiency on renal handling of K and the regulation of plasma K concentration.

RESULTS

Baseline values

The clinical, biological, and hormonal characteristics of the subjects have been previously reported.[21,22] We report here the plasma Na and K data (Table 1). There was no significant difference between genotypes in terms of plasma Na and K and creatinine concentrations and glomerular filtration rate (GFR) on the *ad libitum* diet. As previously reported, the baseline 24-h UKLKa of R53H subjects was 64% lower than those of R53R subjects.[18,21] Plasma active renin and aldosterone concentrations did not significantly differ between the two genotypes (Table 1).[21]

Effect of R53H polymorphism on diet-induced change in K metabolism

After 7 days of controlled diet, the 24-h urinary Na and K excretion showed that a new electrolyte balance was achieved with no significant difference between the two genotypes. Plasma renin and aldosterone concentrations were similarly affected by the two diets in both groups (Table 2).[21]

The plasma K concentration on day 7 of the low-Na/high-K diet was significantly higher than on the high-Na/low-K diet and was influenced by the R53H polymorphism (Table 2 and Figure 1). Plasma K increased by +0.78 mmol/l (+0.44 to +1.12) with low-Na/high-K in R53H subjects, whereas the diet-induced changes in plasma K were much smaller in R53R subjects (+0.23 mmol/l (+0.03 to +0.42); *P* for diet/genotype interaction = 0.0017; Table 2 and Figure 1). The plasma K concentration thus achieved on the low-Na/high-K diet was significantly higher in R53H subjects than in R53R subjects (Table 2). In contrast, within high-K- or low-K-fed groups, the 24-h urinary K excretion did not differ significantly between the two genotypes (Table 3). The higher plasma K in R53H was not associated with any significant increase in plasma aldosterone as compared with R53R subjects on the same diet (Table 2). Interestingly, the fasting K reabsorption on day 7 of the low-Na/high-K diet was significantly higher in R53H subjects than in R53R subjects, suggesting a renal defect in adaptation to K load. This effect was not observed on the high-Na/low-K diet (Table 3).

R53H subjects had higher plasma potassium concentration despite similar urinary potassium excretion and plasma aldosterone concentration. This suggests a primary defect of kidney adaptation to chronic K load in R53H subjects (Tables 2 and 3).

Plasma Na concentration was significantly lower in the low-Na/high-K than the high-Na/low-K diet, with no significant difference between genotypes (Table 2 and Figure 1). The magnitude of dietary-induced plasma Na changes was similar between the two genotypes ($\Delta(Na)_P$ = −3.37 mmol/l (−5.23 to −1.51) vs. −2.66 mmol/l (−3.74 to −1.58), R53H vs. R53R, *P* for diet/genotype interaction = 0.46). This tendency toward hyponatremia was likely related to hypovolemic stimulation of vasopressine secretion. For a given diet, the 24-h urine Na excretion as well as Na reabsorption did not differ between genotypes (Table 3).

Finally, we found no significant difference in plasma and urinary levels of electrolytes and hormones between genotypes on the high-Na/low-K diet (Tables 2 and 3).[21]

Effect of furosemide infusion on Na and K excretion

As expected, furosemide infusion induced a massive increase in urinary Na and K excretion (Table 4). The magnitude of the furosemide-induced natriuretic and kaliuretic response was influenced by the Na/K content of the diet, as was the furosemide-induced increase in GFR. The furosemide-induced increase in FE_{Na} was higher on the high-Na/low-K diet than on the low-Na/high-K diet (ΔFE_{Na}: 7.62% (6.90–8.41) vs. 4.15% (3.76–4.58), respectively; $P < 0.001$).

The R53H genotype did not influence the natriuretic effect of furosemide on the low-Na/high-K diet, but influenced its kaliuretic effect (Table 4). Indeed, the furosemide-induced decrease in K reabsorption was significantly greater in R53H than in R53R subjects (ΔK_{reabs}: −2.28 mmol/l GF (mmol per liter of glomerular filtrate; −2.62 to −1.92) vs. −1.74 mmol/l GF (−1.94 to −1.54), respectively; $P = 0.005$, Table 4 and Figure 2). Accordingly, the decrease

Table 1 | Baseline biochemical and hormonal parameters measured on *ad libitum* Na/K diet in R53H and R53R subjects

	R53H (*N* = 10)	R53R (*N* = 30)	*P*-value
Plasma			
Na (mmol/l)	139 (139–141)	140 (139–141)	0.3923
K (mmol/l)	3.9 (3.7–4.0)	4.0 (3.7–4.2)	0.7680
Cl (mmol/l)	97.5 (96.0–99.0)	98.0 (97.0–100.0)	0.3471
Creatinine (μmol/l)	81 (78–93)	79 (73–83)	0.1987
Active renin (pg/ml)	12 (9–7)	15 (11–22)	0.1285
Aldosterone (pg/ml)	68 (62–87)	70 (58–89)	0.8298

Abbreviations: Cl, chloride; K, potassium; Na, sodium.
Data are expressed as median (interquartile range). Plasma renin and aldosterone concentrations have been previously reported.[21,22]