Recent advances in renal tubular calcium reabsorption

Arjen R. Mensenkamp, Joost G.J. Hoenderop and René J.M. Bindels

Purpose of review

Knowledge of renal Ca^{2+} reabsorption has evolved greatly in recent years. This review focuses on two recent discoveries concerning passive and active Ca^{2+} reabsorption.

Recent findings

The thiazide diuretics are known for their hypocalciuric effect. Recently, it has been demonstrated that TRPV5-knockout mice, in which active Ca^{2+} reabsorption in the distal convoluted tubule is completely abolished, show the same sensitivity towards thiazides as wild-type mice. This indicates that thiazide affects Ca^{2+} reabsorption indirectly via contraction of the extracellular volume, independent of active Ca^{2+} reabsorption in the distal convoluted tubule, thereby increasing passive paracellular Ca^{2+} transport in the proximal tubule. Moreover, the antiaging hormone Klotho regulates Ca^{2+} reabsorption in the distal convoluted tubule via a novel molecular mechanism. Klotho stabilizes the TRPV5 Ca^{2+} channel in the plasma membrane by deglycosylation of the protein.

Summary

By showing that thiazide-induced hypercalciuria is due to increased passive Ca^{2+} reabsorption in the proximal tubule, a long-standing issue has been solved, underlining the importance of proximal paracellular Ca^{2+} reabsorption. Moreover, the molecular mechanism by which the antiaging hormone Klotho regulates TRPV5 activity may prove to be generally applicable in Klotho-mediated prevention of aging.

Keywords

Ca^{2+} transport, Klotho, thiazides

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Department of Physiology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, The Netherlands

Correspondence to René J.M. Bindels, PhD, 286 Cell Physiology, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands
Tel: +31 24 3614211; fax: +31 24 3616413; e-mail: r.bindels@ncmls.ru.nl

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Abbreviations

CNT	connecting tubule
DCT	distal convoluted tubule
ECV	extracellular volume
PT	proximal tubule
PTH	parathyroid hormone
TRP	transient receptor protein

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Introduction

The regulation of calcium (Ca^{2+}) reabsorption in the kidney is crucial for the maintenance of Ca^{2+} balance [1,2••]. It is generally known that only 1–3% of Ca^{2+} that is filtered by the kidney is excreted. The majority of Ca^{2+} reabsorption occurs passively along the proximal tubule (PT) and the thick ascending limb of Henle's loop (TAL). Fine tuning of Ca^{2+} reabsorption takes place along the distal convoluted tubule (DCT) and the connecting tubule (CNT), where the remaining 15% of filtered Ca^{2+} is transcellularly reabsorbed [2••,3] (Fig. 1a). This latter process can be divided into three discrete steps. The first step requires Ca^{2+} influx across the apical membrane. Hoenderop *et al.* [4] identified the transient receptor protein-vanilloid TRPV5 as the responsible protein in this process. The second step is the facilitated diffusion of Ca^{2+} through the cytosol. Here, calbindin-D_{28k} binds intracellular Ca^{2+} transported via TRPV5 and shuttles it through the cytosol towards the basolateral membrane where Ca^{2+} is extruded via the Na^+/Ca^{2+} exchanger NCX1 and the Ca^{2+}-ATPase PMCA1b, the final step in this process.

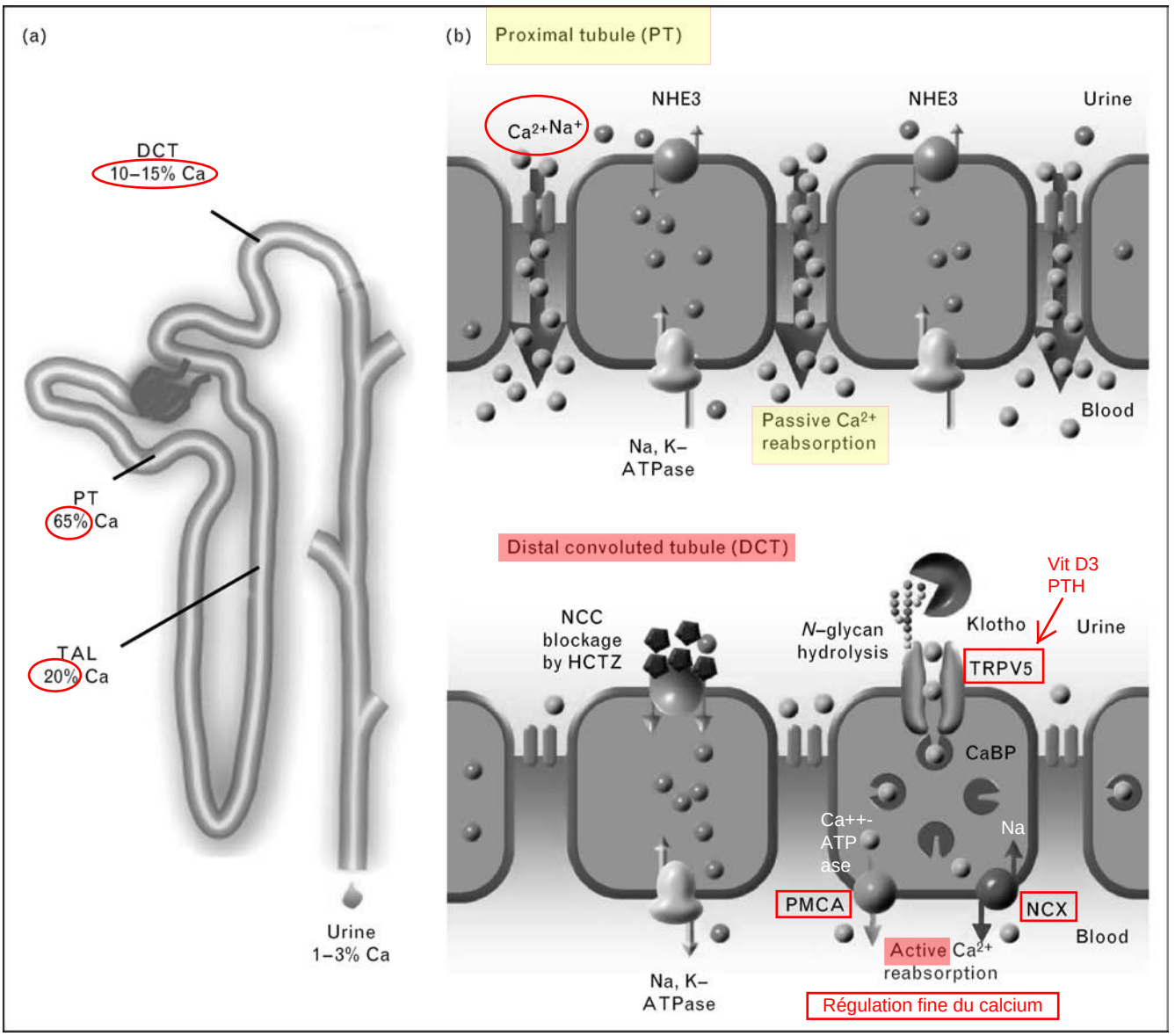
85 % réabs.Ca++ PASSIVE dansTP et Henle asc.

15% réabs. avec régulation fine dans TCD et TC

TRPV5

TRPV5 belongs to the transient receptor protein (TRP) super family, representing channels with a wide range of functions. Its diversity is reflected in tissue distribution, ion permeability, modes of activation and involvement in biological processes [5,6•]. Although all TRP channels mediate the influx of cations, TRPV5 has a strong preference for Ca^{2+}, allowing it to transport Ca^{2+} effectively in the presence of relatively high Na^+ concentrations [7]. TRPV5 is expressed in DCT2 and CNT where it colocalizes with calbindin-D_{28k}, NCX1 and PMCA1b at the segment involved in active Ca^{2+} reabsorption. TRPV5 is both transcriptionally and posttranscriptionally regulated. Parathyroid hormone (PTH) is an essential component of Ca^{2+} homeostasis. High plasma Ca^{2+} concentrations are sensed by the parathyroid Ca^{2+}-sensing receptor, resulting in a decrease in PTH secretion from the parathyroid glands [8]. Van Abel *et al.* [9] have shown that PTH increases the expression of genes involved in transcellular Ca^{2+} transport, resulting in increased Ca^{2+} reabsorption. TRPV5 gene expression is also under control of 1,25-dihydroxy vitamin D_3 ($1,25\text{-}(OH)_2D_3$) [10–12]. Posttranscriptionally, TRPV5 activity is modulated by other proteins, most likely by affecting its protein conformation or by interfering with the intracellular trafficking of TRPV5 [13–16].

Figure 1 Renal tubular Ca^{2+} transport



(a) Overview of Ca^{2+} reabsorption along the nephron; thick ascending limb of Henle's loop (TAL). (b) Detail of proximal tubule (PT) (upper panel) and distal convoluted tubule (DCT) (lower panel). NCC blockage by hydrochlorothiazide leads to reduced Na^+ reabsorption in DCT. This will induce extracellular volume (ECV) contraction due to renal salt and water loss [47]. To accommodate this, ECV contraction will result in a compensatory increase in renal proximal Na^+ reabsorption, thereby enhancing the electrochemical gradient ultimately leading to increased passive paracellular Ca^{2+} reabsorption. Klotho exerts its effect by (partial) hydrolysis of the N-glycosylated TRPV5 channel, thereby stabilizing it in the apical plasma membrane. This will enable prolonged transcellular Ca^{2+} transport across the epithelium.

Recently, two new findings were presented that greatly enhance our knowledge about proximal [17••] and distal [18••] Ca^{2+} reabsorption and the role of TRPV5 herein. In this review, both milestones are discussed in detail.

Thiazide-induced hypocalciuria due to increased passive Ca^{2+} reabsorption

Thiazide is a well known diuretic frequently used to treat arterial hypertension [19,20]. The development of hypocalciuria, as one of its side-effects, is used to its advantage in the treatment of idiopathic hypercalciuria [21]. The responsible molecular mechanism remains to be conclusively identified, however. It has been known for a long time that the point of action of thiazides is located in DCTs, where Na^+ reabsorption is inhibited by blockage of the apical Na^+-Cl^- transporter, known as NCC [22]. This results in renal Na^+ and water wasting, but is often also associated with hypomagnesemia and hypocalciuria [19,20]. Linkage analysis for Gitelman's

syndrome resulted in the identification of NCC in humans [23–25]. The patients presented with hypomagnesemia and hypocalciuria, in addition to hypokalemia and metabolic alkalosis [26]. Schultheis *et al.* [27] created an NCC-knockout ($NCC^{-/-}$) mouse exhibiting identical phenotypes as described for Gitelman's syndrome and chronic thiazide treatment, supporting the central role of NCC in Na^+ reabsorption in DCT.

The question remained of what the origin was of the hypocalciuria. Two options have been proposed [28,29]: increased passive proximal Ca^{2+} reabsorption due to extracellular volume (ECV) contraction [30] or increased active Ca^{2+} reabsorption in DCT possibly due to hyperpolarization of the apical membrane [31].

First models of thiazide action

The effect of thiazide on Ca^{2+} reabsorption was observed by Lamberg and Kuhlback [32] in 1959 and Seitz and Jaworski [33] shortly thereafter. Around the same time, it was shown that chlorothiazide treatment results in ECV contraction, as measured by an increase in hematocrit [34], although this effect was temporal. Micropuncture studies in rats initially pointed towards a prominent role for DCTs in the change in Na^+/Ca^{2+} reabsorption ratio after acute thiazide treatment. Edwards *et al.* [35] showed that chlorothiazide treatment in dogs results in reduced glomerular filtration rate (GFR), increased Na^+ excretion, and a decreased fractional Ca^{2+} excretion. Micropuncture data showed that neither Na^+ nor Ca^{2+} reabsorption is affected in PTs, and distally only Na^+ reabsorption was decreased. Costanzo and Weiner [36] concluded that volume depletion is not a necessary condition for the hypocalciuric response as injection of thiazide in the left renal artery of dogs only affected Ca^{2+} clearance in the left kidney, while volume depletion would be expected to affect both kidneys. Subsequently, Costanzo and Windhager [37] performed micropuncture and microperfusion studies in rats to characterize thiazide-sensitive Ca^{2+} transport. While Na^+ load was increased in DCTs, Na^+ reabsorption was reduced. Luminal Ca^{2+} levels were also higher at the beginning of DCTs, suggesting a decreased Ca^{2+} and Na^+ absorption in PTs. The luminal Ca^{2+} concentration in DCTs was significantly increased, supporting a role for this latter segment in thiazide-induced hypocalciuria.

Role of PTH

Brickman *et al.* [38] showed that patients with hypoparathyroidism do not develop hypocalciuria after thiazide treatment, in contrast to controls or hyperparathyroidic patients, suggesting an important role for PTH. Studies in thyro-parathyroidectomized dogs, however, showed that Ca^{2+} excretion is decreased to a similar extent as control dogs after the acute application of chlorothiazide [36,39]. In contrast, studies by Shimuzu *et al.* [40] showed that the addition of PTH is essential for acute thiazide-induced Ca^{2+} transport in isolated rabbit CNTs . This effect could be inhibited by blocking the basolateral Na^+-K^+-ATPase or NCX1, suggesting that thiazides inhibit transcellular Ca^{2+} transport. As the presence of Na^+ in the luminal compartment was essential, an inhibitory effect of intracellular Na^+ on Ca^{2+} transport by interfering with NCX1 was suggested. Gesek and Friedman [31] showed that chlorothiazide, like PTH [41], can hyperpolarize cultured DCT cells by decreasing the intracellular Cl^- concentration, resulting in increased Ca^{2+} transport. This effect could be inhibited by the Ca^{2+} channel blocker nifedipine, indicative of the presence of L-type voltage-gated Ca^{2+} channels. Other groups suggested the existence of multiple Ca^{2+} channels in DCTs, one of them sensitive to chlorothiazide, another to PTH [42,43].

Thiazides and TRPV5

In 1999, TRPV5 was identified as the major epithelial Ca^{2+} channel in DCTs and CNTs [4]. This discovery prompted the question of whether these channels constituted the missing link in thiazide-induced hypocalciuria. Nijenhuis *et al.* [29] showed that a chronic treatment of rats with a high dose of hydrochlorothiazide results in decreased mRNA levels of genes encoding for TRPV5, calbindin-D_{28k}, and NCX1. These results were at odds with a role for DCTs at the origin of thiazide-mediated hypocalciuria. Furthermore, chronic treatment of rats with hydrochlorothiazide induced ECV contraction and reduced urinary Ca^{2+} excretion. Na^+ repletion abolished ECV contraction and prevented the hypocalciuria. Lee *et al.* [44] studied the effects of acute and chronic treatment with lower doses of thiazide. Only acute treatment with low doses of chlorothiazide resulted in an upregulation of TRPV5 mRNA. Chronic chlorothiazide treatment caused volume depletion, which could be compensated by salt supplementation, confirming the data of Nijenhuis *et al.* [29], but did not affect TRPV5 expression. The authors concluded that volume contraction after chronic thiazide treatment increases Ca^{2+} reabsorption in the PTs.

A role for TRPV5 in thiazide-induced Ca^{2+} reabsorption was challenged by Loffing *et al.* [45]. The authors showed that the thiazide-sensitive NCC is mainly located in DCT1, in contrast to TRPV5 which is present in DCT2 and CNTs [45]. In $NCC^{-/-}$ mice, DCT1 was virtually absent, while DCT2 was apparently intact and retained expression of TRPV5 and NCX1. Fractional delivery of Ca^{2+} was decreased in PTs, but unaffected in DCTs. These data already suggest that TRPV5 – and DCT2 in general – play a marginal role, if any at all, in thiazide-induced hypocalciuria. The creation of a TRPV5-knockout ($TRPV5^{-/-}$) mouse by Hoenderop *et al.* [46] could finally resolve the issue of the

putative involvement of TRPV5. TRPV5$^{-/-}$ mice are characterized by renal Ca^{2+} wasting as a result of reduced Ca^{2+} reabsorption in DCTs, accompanied by polyuria and reduced urinary pH [46]. Nijenhuis *et al.* [17••] showed that chronic treatment with a relatively low dose of hydrochlorothiazide results in a strong reduction of urinary Ca^{2+} excretion in both wild-type and TRPV5$^{-/-}$ mice. Importantly, micropuncture data showed that Na^+, fluid, and Ca^{2+} reabsorption in PTs is increased after hydrochlorothiazide treatment, while fractional Ca^{2+} delivery in DCTs was reduced. Chronic low-dose treatment did not result in changes in mRNA and protein levels of TRPV5, calbindin-D_{28k} or NCX1, in contrast to high-dose hydrochlorothiazide treatment [29], thereby confirming the data of Lee *et al.* [44]. The hematocrit was significantly increased in wild-type and TRPV5$^{-/-}$ mice, indicative of volume depletion. This was associated with a decreased lithium clearance, an inverse measure for proximal tubular Na^+ reabsorption and indirectly passive paracellular Ca^{2+} transport. Also, protein levels of the proximal tubular Na^+/H^+ exchanger NHE3 were significantly increased. Together, these data demonstrated that the passive paracellular Ca^{2+} transport in PTs is enhanced after chronic thiazide treatment. In addition, a single dose of hydrochlorothiazide resulted in an acute temporary increase in Na^+ excretion without affecting Ca^{2+}. After this, however, hypocalciuria developed both in wild-type and in TRPV5$^{-/-}$ mice, coinciding with a decrease in Na^+ excretion.

In conclusion, recent data show that thiazide-induced Ca^{2+} reabsorption occurs mainly in the proximal part of the nephron. Inhibition of Na^+ reabsorption in DCTs will decrease ECV due to renal salt and water loss [47]. Consequently, ECV contraction results in a compensatory increase in renal proximal Na^+ reabsorption, thereby enhancing the electrochemical driving force for paracellular Ca^{2+} reabsorption (Fig. 1b).

Klotho: a new player in Ca^{2+} reabsorption along distal convoluted tubules

People have been striving for longevity for centuries. Nowadays, molecular biology can be applied to understand the molecular mechanism of aging. In 1997, Kuro-o *et al.* [48] discovered by random mutagenesis a mouse strain with a phenotype resembling premature aging in humans. These infertile mice developed arteriosclerosis, osteoporosis, skin changes, and ectopic calcification in several organs and died at an early age [48]. The allele associated with this phenotype carried a gene now called Klotho, named after the Greek goddess who spins the thread of life. Overexpression of Klotho in mice resulted in a significant extension of life span and a suppression of aging [49••]. In humans, allelic variations are related to life expectancy and occult coronary artery disease [50,51•,52]. The Klotho gene encodes a single-pass transmembrane protein with an N-terminal signal sequence, a putative extracellular domain with two internal repeats and a short intracellular domain on the carboxy terminus. The gene is mainly expressed in DCTs of the kidney, the choroid plexus in the brain [18••,48], and the parathyroid gland [53]. Klotho exhibits β-glucuronidase activity [54] and is activated by cleavage of the N-terminal extracellular tail. This part is then released into the urine, serum and cerebrospinal fluid [18••,55].

Klotho and Ca^{2+} homeostasis

Klotho-deficient mice (*kl/kl*) have slightly increased plasma levels of Ca^{2+} [48]. This was associated with high levels of 1,25-$(OH)_2D_3$ caused by increased expression of renal 1α-hydroxylase, the rate-limiting step in 1,25-$(OH)_2D_3$ synthesis [56,57]. Dietary suppression of 1,25-$(OH)_2D_3$ levels normalized most of the *kl/kl* phenotype [58]. Conversely, Tsujikawa *et al.* [58] showed that administration of 1,25-$(OH)_2D_3$ induces Klotho expression in the kidney. PTH levels were decreased and calcitonin levels were increased in adult *kl/kl* mice, correlating with the increased plasma Ca^{2+} levels. These data suggested that this correction mechanism is not affected in *kl/kl* mice and that Klotho may act in a negative regulatory circuit of vitamin D.

One of the first signs that Klotho plays a role in Ca^{2+} metabolism was the observation that *kl/kl* mice develop bone abnormalities, including osteoporosis [48]. *Kl/kl* mice had an ~20% lower bone mineral density than control mice. This has been confirmed in humans, where some allelic variants are associated with osteoporosis and spondylosis [59,60]. In *kl/kl* mice, both osteoblast and osteoclast differentiation were impaired [61,62]. The decrease in bone formation exceeded that of bone resorption, however, resulting in a net bone loss [61], the phenotype resembling that of humans suffering from senile osteoporosis. Osteoporosis in *kl/kl* mice was associated with abnormal elongation of the trabecular bones in the epiphyses of long bones. This is likely the result of the reduced bone resorption, in part due to a reduced number of osteoclasts [63].

Recently, Chang *et al.* studied the relationship between TRPV5 and Klotho [18••]. Microarray data revealed that Klotho expression is decreased in TRPV5$^{-/-}$ mice. Coexpression of Klotho with TRPV5 in human embryonic kidney 293 (HEK293) cells, or application of purified Klotho to TRPV5-expressing HEK293 cells, significantly stimulated $^{45}Ca^{2+}$ uptake in these cells. Cell surface biotinylation experiments revealed a significant increase in plasma membrane localization of TRPV5 whereas total expression was not affected after Klotho treatment. These effects could be mimicked by a purified β-glucuronidase indicating that the enzymatic activity of

Klotho is responsible for the increased TRPV5 activity. Klotho had no effect on a nonglycosylated mutant of TRPV5 (N358Q), indicating that Klotho may work by affecting the extracellular glycosylation status of the channel, entrapping the channels in the plasma membrane, thereby increasing TRPV5-mediated Ca^{2+} influx activity (Fig. 1b).

During aging, Ca^{2+} loss predominates Ca^{2+} intake [64]. As Klotho deficiency is associated with a phenotype resembling aging, it may well be that impaired Klotho activity in the elderly is responsible for reduced Ca^{2+} reabsorption via TRPV5. Future studies will have to further substantiate this notion. The discovery of the extracellular enzymatic effect of Klotho on TRPV5 activity has greatly enhanced our understanding of Klotho functioning and may prove to be the role model in unravelling the antiaging effect of this novel hormone with enzymatic activity.

Conclusion

New exciting developments in renal Ca^{2+} reabsorption are presented in this review. Thiazides are known to affect the Ca^{2+} balance inducing hypocalciuria, and have been suggested as exerting favorable long-term effects in counteracting osteoporosis. It took more than 40 years to unravel the mechanism responsible for thiazide-induced hypocalciuria, which could only be addressed conclusively after the creation of the $TRPV5^{-/-}$ mouse. Regulation of TRPV5 can be considered as the fine-tuning step of renal Ca^{2+} reabsorption. The finding that Klotho regulates TRPV5, and thereby total Ca^{2+} reabsorption, is a major step forward in our understanding of the relationship between Ca^{2+} reabsorption and aging.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 556–557).

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