# The calcium-sensing receptor in the kidney: localisation, function and therapeutic implications

[1]Daniela Riccardi and [2]Giovanna Valenti

[1]*School of Biosciences, Cardiff University, Cardiff, United Kingdom*

[2]*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari 'Aldo Moro'Bari, Italy*

**Address correspondence to:**

Daniela Riccardi, PhD
Division of Pathophysiology and Repair
The Sir Martin Evans Building
Museum Avenue
Cardiff CF10 3AX, United Kingdom
Telephone: +44 (029) 208 79132
Fax: +44 (029) 208 74116
Email: riccardi@cardiff.ac.uk

Giovanna Valenti, PhD
Department of Biosciences, Biotechnologies and Biopharmaceutics
Via Orabona 4,
70125, Bari, Italy
Telephone: +39 (080) 544 3444
Fax: +39 (080) 544 3388
Email: giovanna.valenti@uniba.it

**Abstract**

The ability to monitor changes in the ionic composition of the extracellular environment is crucial to all living organisms. The discovery of the extracellular calcium-sensing receptor, CaSR, from mammalian parathyroid glands is the first example of this kind of mechanism and demonstrates how cells can detect small variations from physiological free ionized calcium in the extracellular fluids and evoke biological responses by altering the secretion of parathormone, PTH, acting on its target tissues, kidney, intestine and bone. Accordingly, aberrant calcium-sensing by the parathyroid glands due to altered CaSR expression or function is associated with impaired divalent cation homeostasis. CaSR activators, termed calcimimetics, have been developed to rectify hyperparathyroidism of various nature while drugs directed against the CaSR, or calcilytics, are in development for hypercalciuric disorders. The kidney also expresses a CaSR and recent evidence suggests that the renal CaSR directly contributes to the regulation of many aspects of kidney function in a PTH-independent manner. This review will focus on the direct roles of the CaSR in the kidney and on the potential impact of pharmacological CaSR modulators on the renal CaSR.

**Introduction**

Parathyroid hormone (PTH) is the major determinant of serum calcium ion levels (Ca). Serum PTH levels are inversely related to those of Ca, with decreases in Ca promoting PTH release and direct Ca reabsorption from the kidney, Ca absorption from the intestine (by promoting the 1-hydroxylation of $25(OH)D_3$ into $1,25(OH)_2D_3$ by the kidney proximal tubule), and Ca resorption from the bone while increases in serum Ca cause opposite effects. Crucial to this process is the extracellular calcium-sensing receptor, CaSR, located in the parathyroid glands[1], which is able to detect and respond to even the smallest changes in serum Ca levels with alterations in PTH secretion. Excessive or defective Ca sensing by the parathyroid glands, either because of genetic or acquired mutations in the CaSR, results in altered divalent cation homeostasis, which can lead to significant, long-lasting morbidity if left untreated[2]. Thus, pharmacologial CaSR modulators have been developed to correct hyperparathyroid or hypoparathyroid disorders of various nature. Calcimimetic drugs have been developed to rectify abnormal CaSR expression or function and have been successfully employed for over a decade to treat hyperparathyroidism secondary to kidney failure[3]. Recently AMG416, a long-acting peptide CaSR agonist suitable for intravenous administration, is being evaluated in Phase 2 clinical trials in patients with secondary hyperparathyroidism receiving haemodialysis[4]. On the other hand, CaSR antagonists, termed calcilytics, are currently being investigated for the treatment of autosomal dominant hypocalcemia with hypercalciuria due to activating CaSR mutations [5] .

The kidney plays a major role in the control of extracellular Ca and urinary Ca excretion is proportional to the filtered Ca load. For instance, increasing serum Ca results in an increase in urinary Ca excretion, an effect which can be observed in the absence of the Ca-regulating hormones, PTH and $1,25(OH)_2D_3$. A number of observations have demonstrated that the kidney is a calcium-sensing organ owing to its ability to sense changes in both urine and serum Ca and a direct role for the CaSR has been proposed in renal calcium handling. For instance, familial hypocalciuric hypercalcemia is an autosomal dominant disease due to inactivating mutations of the *CaSR* gene and manifests with hypercalcemia, parathyroid hyperplasia and inappropriately normal or even low urinary Ca in the face of hypercalcemia.

unusually low Ca:creatinine clearance ratio[6]. In a seminal study which preceded the molecular identification of the CaSR Attie and colleagues investigated the effects of PTH on urinary Ca

excretion in FHH patients with surgical hypoparathyroidism and compared their response to that seen in hypoparathyroid control patients. The authors firstly discovered that Ca clearance in FHH subjects was lower that control subjects upon intravenous calcium infusion. This difference was retained in the presence of acetazolamide, a diuretic which inhibits carbonic anhydrase in the proximal tubule and elsewhere but was completely abolished when the loop diuretic, ethacrinic acid, was administered, with greater calciuric response in FHH than control subjects. The authors therefore concluded that the thick ascending limb of Henle's loop, TAL, is the major site for abnormal Ca transport in FHH patients[7]. Recently Loupy et al have investigated the impact of acute and chronic CaSR inhibition on serum Ca in thyroparathyroidectomised rats in the presence or absence of PTH infusion and shown that the CaSR directly participates to the control of extracellular Ca via the regulation of the paracellular Ca transport the by the TAL[8]. More recently evidence suggests that CaSR directly regulates Ca and Mg permeability via its actions on several tight junction proteins called claudins (Cldn) including Cldn-14[9, 10], -16 and -19[11] (see below).

In addition to the TAL, Ca sensitivity has also been reported in a variety of cells from the proximal tubule to the collecting duct and juxtaglomerular apparatus[12]. The combined use of detection methods with enhanced sensitivity, of pharmacological CaSR modulators and of tissue-specific gene ablation studies have significantly enhanced our understanding of the role of the CaSR in the kidney. This review article will address the intrarenal distribution of the CaSR, the potential roles of this receptor in the kidney and of the implications of the use of pharmacological modulators on renal function. The hypothesis that the CaSR might mediate the sensitivity of cyclic adenosine monophosphate (cAMP)-linked hormones to type VI adenylyl cyclase (AC6) is also being discussed.

**CaSR expression in the kidney**

Determination of the exact intrarenal distribution of the CaSR is crucial to our understanding of receptor's functional role in this organ, but has been thus far controversial and hindered by the lack of suitably sensitive methods required for detecting low CaSR expression levels. Thus, while CaSR expression in cells of the parathyroid glands or the kidney TAL is unrefuted, CaSR expression in other tubular and glomerular cells of the kidney has been the object of significant debate. Inconsistent findings have been ascribed to species differences, to alternative methodological approaches or molecular tools employed. Recently Graca et al have used immunohistochemistry using commercially available and custom-made antibodies, directed against the amino- and carboxy-termini of the CaSR, as well against the whole fusion protein to investigate the intrarenal distribution of this receptor across human, rat and mouse kidney to enable the full physiological characterisation of the renal CaSR[13]. In addition, the authors employed the most sensitive and specific detection methods currently available that also provide information concerning the exact cellular localization of the CaSR mRNA and protein (namely branched DNA *in situ* hybridisation and proximity ligation assay, respectively). The key findings of this study are summarised in Table 1. These results show that the CaSR is expressed all along the nephron with, as expected, the highest expression within the TAL. CaSR expression was found in the cells of the collecting duct (CD) but with differences in the strenght of the immunoreactivity signal and in the cellular polarity (apical, basolateral or intracellular). This study also shows that intrarenal CaSR protein expression could not be detected in mice with targeted CaSR ablation from the kidney tubules. Proximity ligation assay confirmed CaSR expression within the glomerulus and the proximal tubule (PT), previously two sites of major controversies. This expression pattern was confirmed across species albeit significant variation in the overall expression levels and in the biochemical species expressed (CaSR monomer vs dimer) could be observed. These observations clarify the intrarenal distribution of the CaSR, which will not only allow for further elucidation of the role of the CaSR in the kidney but also inform on the potential therapeutic use, and effects, of pharmacological CaSR modulators in these regions.

**CaSR in the glomerulus and juxtaglomerular apparatus (JGA)**

CaSR expression in the glomerulus has been previously reported with inconsistent findings[14, 15]. Proximity ligation assay demonstrated low but above background CaSR expression in mouse, rat and human glomeruli, in both podocytes and mesangial cells[13]. Intriguingly, the study by Graca et

al points to significant differences in the expression levels which can be observed even within adjacent glomeruli of the same kidney and that could be ascribed to differences in the functional status of the cells[13]. This observation, together with the very low CaSR expression levels, could account for some of the discrepant reports of receptor expression in the glomerulus.

Evidence for a role for the CaSR in the regulation of glomerular function has derived from studies in wich glomerular damage was induced *in vitro* and *in vivo* by the toxin puromycin aminonucleoside (PAN) [16]. These studies show that the CaSR is expressed in murine podocytes where calcimimetics prevent PAN-induced apoptosis and cytoskeletal damage in vitro[16]. In addition, calcimimetic treatment also prevented PAN-induced proteinuria and glomerulosclerosis in rats *in vivo*[16]. Calcimimetics also decreased the progression of renal damage by preventing podocyte loss and interstitial fibrosis in uninephrectomised, apolipoprotein E-deficient mice[17]. The calcimimetic effects were observed in the absence of either serum PTH or systemic blood pressure changes suggesting a direct involvement of the glomerular CaSR.

In human cultured mesangial cells, CaSR activation leads to intracellular Ca mobilisation by both Gq-linked activation of inositol trisphosphate and extracellular Ca influx via TRPC3/6 channels[15]. Aberrant Gq signalling causes glomerular damage via activation of TRPC6 in a murine PAN nephrosis and of diabetic nephropathy[18] but whether direct CaSR activation in mesangial cells negatively impacts on glomerular function via Gq-linked opening of TRPC6 is unknown. Thus, further studies are necessary to investigate the existence of a potential cross-talk between CaSR and TRPC6 in mesangial cells and the impact of long-term clinical use of pharmacological CaSR modulators on glomerular function.

In the juxtaglomerular apparatus (JGA) a variety of stimuli lead to renin secretion largely by increasing the intracellular cAMP pool[19]. Acute CaSR activation by calcimimetic inhibits renin secretion both *in vitro*, in mouse primary juxtaglomerular cells, and *in vivo*[20]. Furthermore, while a rise in intracellular Ca generally promotes exocytosis in secretory cells, in JGA cells an increase in extracellular Ca suppresses renin secretion via CaSR-mediated activation of Gq and attendant intracellular Ca mobilisation, a phenomenon alluded to as the “calcium paradox”[21]. In addition, an increase in extracellular Ca also leads to suppression of the intracellular cAMP pool by activation of calcium-regulated adenylyl cyclases (V and VI) and of phosphodiesterases[22]. In contrast to what observed in acute settings, chronic calcimimetic treatment had no effect on either baseline or stimulated plasma renin activityin rats *in vivo*[20]. If these observations were to translate to humans

they would suggest that long-term treatment with calcimimetics in patients is unlikely to significantly affect renin plasma levels.

**CaSR in the proximal tubule**

The proximal tubule (PT) plays a crucial role in phosphate transport and PTH, acting on its type 1 receptor (PTH1R) is the principal regulator of phosphate reabsorption[23]. In the PT CaSR is expressed apically in the mouse, rat and human kidney [24,13, 25]. Furthermore, CaSR expression is retained in conditionally immortalized proximal tubular epithelial cell line (ciPTEC) obtained from urine of a healthy subject[26]. Evidence gathered from studies carried out in isolated S3 segments suggests that activation of the CaSR directly and rapidly blunts the phosphaturic actions of PTH thereby modulating PTH-inhibitable phosphate uptake[25]. In turn, chronic high dietary Pi intake or acute PTH administration suppress proximal tubule CaSR expression[27]. Furthermore, $1,25(OH)_2D_3$ increases serum Ca levels by enhancing Ca absorption form the gut and Ca reabsorption from the distal convoluted tubule. At the systemic level, these effects are countered by the CaSR via suppression of PTH release and increased urinary Ca excretion. In addition, in the human proximal tubular cell line HK-8 cells 1-hydroxylation of $25(OH)D_3$is inhibited by high Ca[28] and studies carried out in mice lacking both the CaSR and PTH genes show that the CaSR mitigates the calcemic response to $1,25(H)_2D_3$ *in vivo*[29]. Because these studies were carried out in the context of PTH gene deficiency, the authors concluded that the exaggerated calcemic response to $1,25(OH)_2D_3$ in the double CaSR/PTH-deficient mice could only be explained by direct, PTH-independent effects of the CaSR on the kidney[29]. In addition, Capasso *et al.* used both pharmacological (calcimimetic) and genetic (CaSR knockout) tools to demonstrate that, in micropuncture experiments in rats *in vivo* and in perfused mouse proximal tubule *in vitro*, activation of the CaSR by Ca or by calcimimetics leads to increased luminal acidification[30]. The proximal tubule accounts for ~65% of the fluid reabsorption via both the transcellular and paracellular routes. NHE3 is crucial to fluid reabsorption and to proton secretion. Activation of NHE3 leads to interstitial $Na^+$ accumulation, with a local increase in its osmolarity, thereby driving paracellular water reabsorption, hence solutes (amongst which, Ca) which are reabsorbed via a process known as “solvent drag”. Because CaSR activation in the proximal tubule drives Na transport, which is likely to occur by activation of NHE3, this effect would result in an increase in fluid and solutes reabsorption. It is known that PTH also induces natriuresis/diuresis by promoting the internalisation of NHE3 and Npt2 via cAMP-protein kinase A stimulation[31]. Thus, activation of the apical CaSR could counteract these actions

therefore resulting in increased Ca and fluid reabsorption. Overall, these observations suggest that, in the proximal tubule, the CaSR directly "fine tunes" PTH actions thereby directly controlling Ca and Pi reabsorption, effect which is independent of systemic changes in calciotropic hormones (see Figure 1A). These CaSR actions are overridden by systemic changes in serum PTH or Pi, which downregulate CaSR expression, selectively by the proximal tubule[27].

**CaSR in the thick ascending limb**

Approximately 25% of the filtered Ca is reabsorbed in this segment, largely by the cortical TAL (CTAL) but also to some extent the medullary TAL (MTAL), a process which is coupled to the reabsorption of NaCl[32]. In this nephron segment PTH stimulates renal tubular calcium reabsorption by increasing both the transcellular and paracellular Ca permeability[8, 33]. In the CTAL an increase in peritubular Ca suppresses PTH-induced increase in intracellular cAMP[34]. CaSR activation in the CTAL also decreases hormone (calcitonin, glucagon, vasopressin)-stimulated intracellular cAMP accumulation by inhibiting AC6[35]. Paracellular Ca permeability is reliant upon the uptake of NaCl across the luminal membrane of this segment which occurs via the bumetanide-sensitive, Na/K/2Cl cotransporter (NKCC2). Potassium ions (K) brought into the cell by NKCC2 are recycled back into the lumen through an apical, 70 pS renal outer medulla K channels. Intracellular Na and Cl are extruded basolaterally via the Na-K-ATPase and voltage-gated Cl channels (CLCNKB)[37]. Together, these processes establish a lumen positive transepithelial potential difference, which provides the driving force for passive (ie., without the need for further energy expenditure) reabsorption of monovalent and divalent cations (Na, Ca and Mg ions) via the paracellular route.

In the TAL, the 70-pS channel is formed by ROMK2 and yet-to-be identified subunit(s); ROMK2 encodes a 30-pS channel[36]. An increase in peritubular Ca (or Mg) activates the CaSR, located basolaterally in this nephron segment, which, in rat microdissected TALs, directly inhibits the 70 pS apical K channels via cytochrome p450 and increase in 20-hydroxyeicosatetraenoic acid (20-HETE) production[38] (Figure 1B). Whether CaSR activation also leads to inhibition of the other apical K entry via the 30 pS ROMK2 is currently unknown. Because K recycling is the rate-limiting step for NKCC2 activity, such an effect would be expected to result in an abrogation of the driving force for paracellular cation reabsorption. In addition, in isolated rat kindey TALs CaSR activation inhibits the basolateral 50-pS K channels, consistent with Kir4.1, an effect which results in an inhibition of basolateral K recycling, hence of transepithelial cation transport[39]. However, evidence that such a mechanism occurs *in vivo* is still lacking. Furthermore, direct CaSR effects on the paracellular

permeability have also been postulated since calcimimetics and calcilytics affect PTH-stimulated Ca reabsorption in the absence of changes in the transepithelial membrane potential[40] (see below). In any event, hypercalcemia-mediated polyuria (diabetes insipidus) mimicks the effects of loop diuretics, which target NKCC2, yielding a "Bartter-like" phenotype with hypokalemia, hyperreninemia with secondary hyperaldosteronism and metabolic alkalosis[41]. Indeed, Bartter syndrome (type 5) can also be present in in a subset of individuals with autosomal dominant hypocalcemia with hypercalciuria due to activating CaSR mutations[42], and that shares many of the phenotypic hallmarks of Bartter type 1 and 2, which are caused by mutations in the genes encoding NKCC2 and ROMK, respectively (see Figure 1B)[43, 44].

*CaSR regulates paracellular permeability by the thick ascending limb*

A combination of tissue-specific gene ablation and pharmacological studies, together with observations in subjects carrying activating or inactivating mutations in the *CaSR* gene unanimously reveal the primary role for the CaSR renal in the control of urinary Ca excretion (reviewed by [40]).

In the TAL, the paracellular permeability is controlled by tight junctional proteins called claudins, namely claudin-14, -16, and -19, all of which are linked to hypercalciuria, nephrolithiasis and reduced bone mineral density in humans[9-11]. Claudin-16 and -19 heterodimerise to form the pore through which divalent cations are reabsorbed, and whose gating is controlled by claudin-14. Claudin-14 expression is increased by high dietary Ca intake and by calcimimetic treatment *in vivo* resulting in decreased paracellular divalent cation permeability[45]. Recent studies by Hou *et al* have demonstrated that claudin 14 in the TAL is downstream of CaSR activation via the calcineurin-nuclear factor of activated T cell-microRNA (NFATc1-miR) and chromatin remodelling. Specifically, CaSR controls the transcription of miR-9 and miR-374 that target the 3'-untranslated region of the claudin-14 gene[11]. The calcineurin inhibitor, cyclosporine, prevents CaSR-dependent regulation of claudin-14 and urinary Ca excretion[11] (Figure 1B). While physiologically the CaSR-claudin 14 axis in the TAL prevents Ca overload in the presence of elevated serum Ca levels, disturbances in this process could contribute to pathological hypercalciuria and nephrocalcinosis (see below). Furthermore, studies carried out by Toka *et al*. using CaSR-specific deletion from the kidney tubule demonstate that loss of the renal CaSR results in hypocalciuria which was not accompanied by significant changes in serum and urinary Mg excretion [46]. Because these mice also show normal serum Ca and PTH levels, these studies clearly indicate that the main role of the renal CaSR is to

inhibit Ca reabsorption in a PTH-independent fashion. This effect is accounted for, at least in part, by an increase in the paracellular pathway, as evidenced by the concomitant downregulation claudin-14 and of NKCC2 activation in these mice.

Thus, calcimimetic drugs would be expected to increase urinary Ca output via PTH-dependent and PTH-independent (renal CaSR-mediated) actions, hence potentially increasing the risk of Ca stone occurrence. While it is known that single dose of calcimimetic increases urinary Ca exretion in renal transplant patients with secondary hyperparathyroidism[47] and that clinical use of calcimimetics does not appear to elicit polyuria or renal Na wasting, the impact of calcimimetics or calcilytics on nephrolethiasis remains to be investigated.

**CaSR in the distal convoluted tubule and collecting duct**

The DCT is a short segment which plays a key role for fine-tuning calcium and magnesium reabsorption. Apical Ca reabsorption occurs through the calcium channel transient receptor potential vanilloid member 5 (TRPV5), and is transported across the basolateral membrane by the calcium ATPase (PMCA) and a Na/Ca exchanger (NCX)[48, 49]. In this nephron segment PTH stimulates transcellular calcium reabsorption by cAMP-mediated activation of TRPV5[48] (Figure 1C). Previous studies reported that the CaSR is expressed on the basolateral membrane and in the apical surface and in vesicles associated with TRPV5[24, 50-52], findings confirmed by Graca et al[13]. Interestingly, Topala and co-workers demonstrated that, in HEK293 cells stably transfected with the human CaSR, activation of this receptor increases calcium reabsorption via TRPV5[50]. Because in the TAL Na and Ca reabsorption are largely associated, a natriuretic stimulus would be expected to increase Ca delivery to the DCT. The functional interaction between TRPV5 and CaSR would allow for removal of Ca from the pro-urine without affecting Na reabsorption, thus minimising renal calcium wasting. However this concerted interplay is unlikely to prevent the hypercalciuric effect of loop diuretics, or of activating mutations of the *CaSR* gene. Moreover, the CaSR has been shown to interact with the K channel Kir4.1 expressed on the basolateral membrane of DCT. This interaction with Kir4.1 causes its inactivation which in turn could reduce K recycling through the basolateral Na-K-ATPase thereby contributing to the inhibition of Na reabsorption in this segment[51] (Figure 1C).

In the collecting duct the CaSR shows decreasing expression for the cortex to the medulla[12, 13]. In the cortical CD is expressed in intecalated cells as well as in principal cells[12, 13] (Figure 1D). In

intercalated cells CaSR display a predominant apical distribution and it has been suggested that, in dissected mouse outer medullary collecting ducts, its activation by high extracellular Ca promotes urine acidification by the stimulation of the luminal $H^+$-ATPase[53]. Evidence suggests that formation of less acidic urine predisposes to urolithiasis[54, 55]. Of note, TRPV5 –/– mice are hypercalciuric and polyuric with increased urinary acid excretion, but they do not develop renal calcification because they exhibit significant urine acidification[53]. However, when acidification is prevented by targeted gene ablation of the collecting duct-specific B1 subunit of the renal $H^+$-ATPase in TRPV5 -/- mice significant tubular precipitation of calcium phosphase crystals was observed and animals died prematurely of hydronephrosis likely due to the presence of renal stones. In the collecting duct principal cells CaSR is localized on the apical membrane and co-localized with AQP2 in intracellular vesicles[12, 56, 57] where several lines of evidence from *in vitro* studies, from animal models *in vivo* and from observations in humans suggest that CaSR signaling inhibits vasopressin-induced trafficking and expression of the aquaporin-2 (AQP2) water channel[58-66]. The postulated mechanism implies that, under vasopressin action promoting water reabsorption from the lumen, an increase in urinary Ca concentration, due to urine concentration, activates the CaSR located on the apical membrane of collecting duct principal cells. This activation reduces the vasopressin-stimulated insertion of AQP2, and in turn the rate of water reabsorption, leading to the formation of more dilute urine thereby reducing the risk of calcium supersaturation[61, 64, 67]. More recently, observations carried out in renal cells and microdissected collecting duct demonstrate that the inhibitory effect of CaSR signaling on AQP2 trafficking to the plasma membrane is mainly due to a strong reduction in cAMP-induced AQP2-pS256 phosphorylation and trafficking with the physiological consequence of blunting the osmotic water permeability response[65]. Further studies are necessary to determine the relevance of the CaR-AQP2 interplay in humans and the possibility of using CaSR modulators to rectify severe hypercalciuria and nephrocalcinosis[68].

Table 2 summarizes the principal evidence of reported effect of extracellular Ca or calcimimetics (either luminal o basolateral) on the renal physiology, thought to be mediated by CaSR.

**Inhibition of hormone-dependent cAMP accumulation by Ca: role of CaSR and AC6 co-expression**

Evidence that the CaSR and the calcium-inhibitable AC6 are co-expressed in the same cells in defined nephron segments has led to the hypothesis of a functional interplay of CaSR signaling on the inhibition of intracellular cAMP content in response to an increase in extracellular Ca. Specifically, functional expression of the AC6 has been demonstrated in the juxtaglomerular cells

[18], in the proximal tubule[69], in the TAL and in the collecting duct[70].

As discussed previously, by antagonizing the effects of PTH in the kidney, CaSR signaling affects Ca and Pi metabolism and regulates tubular transport of Mg, Na, K, Cl and water excretion[40].

~~As discussed, in the kidney CaSR signaling affects Ca and Pi metabolism by antagonizing the effects of PTH on their tubular transport and tubular regulation of Ca, Mg, Na, K, Cl and water[40].~~

These effects are modulated by the action of distinct hormones acting on the selected tubular segments where the CaSR is expressed. Previous observations demonstrated that, in renal epithelial cells, CaSR activation by an increase in extracellular Ca (as well modulation of its function by pH and nutrients) results in modulation of intracellular cAMP levels [35,65,71] suggesting the possibility that the CaSR converts an extracellular Ca stimulus into a negative feedback on Gs-linked hormones acting via cAMP. Based on the observation that CaSR and AC6 are co-expressed in the same cells in defined nephron segments, it can be speculated that the negative feedback of CaSR on hormones acting through the Gs/AC6/cAMP pathway confers high sensitivity of hormone effects to changes in Ca.

CaSR is expressed in renal juxtaglomerular cells and its activation stimulates inctracellular Ca-mediated decreases in cAMP content and inhibition of renin release through activation of AC5 and AC6[22, 72].

In the proximal tubule Fenton and coworkers have shown that PTH increases Pi excretion by stimulating internalization of the sodium-dependent phosphate transporters (Npt2a and Npt2c) from the apical plasma membrane via activation of the AC6 through PTH type 1 receptors associated to the cAMP/protein kinaseA signaling pathway[69]. Evidence that AC6 is the isoform mediating this effect was based on data derived from AC6 -/- mice which displayed Pi wasting and no response to PTH in terms of phosphate excretion and urinary cAMP excretion[69]. The authors concluded that AC6 in the proximal tubule modulates acute PTH-stimulated cAMP formation and urinary Pi excretion. In this scenario CaSR in the proximal tubule can represent a major regulator of the PTH action by antagonizing its effects on Pi excretion through downregulation of the Gs/AC6/cAMP pathway (see Figures 1A and 2).

As stated above, in the TAL the main function of CaSR is the regulation of urinary Ca excretion having a crucial impact on calcium balance. In this nephron segment NKCC2 and ROMK working in concert establish a lumen-positive transepithelial potential differences allowing Ca reabsorption

via the paracellular pathway. NKCC2 trafficking and activity is positively regulated by phosphorylation at ser126 under vasopressin action[73, 74]. Interestingly, observations carried out in AC6 knockout mice identified AC6 as the isoform mediating the vasopressin-induced phosphorylation of NKCC2[70]. The authors found that, in AC6 knockout mice, phosphorylation of NKCC2 at ser126 in the medullary portion of the TAL was lacking[70].

Moreover previous evidence in microdissected cortical TAL showed that CaSR and AC6 are coexpressed within the same cells and that activation of CaSR by extracellular Ca induced a dose-dependent inhibition of intracellular cAMP content resulting from both stimulation of cAMP hydrolysis and inhibition of cAMP synthesis[35].

This effect on intracellular cAMP associated to CaSR signaling in the TAL indicates that CaSR activation impairs vasopressin action in this nephron segment causing changes in transepithelial potential due to changes in ion transport as well as to a direct control of components of the tight junction proteins including claudins. Using a similar signal transduction pathway, the CaSR is expected to counteract PTH-induced Ca reabsortion in TAL. Overall, antagonizing vasopressin and PTH action through impairment of the Gs/AC6/cAMP pathway results in reduced Ca reabsorption and increase in Ca excretion (see Figures 1B and 2).

The collecting duct represents the nephron segment defining the final composition of urine fine-tuning electrolytes and water reabsorption. Although little Ca is transported in this segment, the extent of water reabsorption and the urine acidification can deeply modify Ca solubility and therefore the risk of Ca stone formation. Both AC3 and AC6 are expressed in collecting duct principal cells and have both been suggested to contribute to the overall rise in cAMP during vasopressin stimulation[75]. However, several line of evidence suggest that the calcium–inhibitable AC6 type is the major enzyme in modulating vasopressin-regulated water reabsorption[76-78]. Collecting duct-specific knockout of AC6 results in a urinary concentration defect associated with reduced vasopressin-stimulated cAMP accumulation[79]. Therefore in the collecting duct Ca "sensing" by the CaSR may sensitize vasopressin to luminal Ca resulting in attenuation of the antidiuretic response to limit water reabsorption (see Figures 1D and 2). In line with this view, data from renal cells and microdissected collecting duct demonstrated that the negative feedback of CaSR signaling on vasopressin action are primarily due to reduction of hormone dependent cAMP generation and possibly hydrolysis[65].

Overall, the emerging concept is that within the nephron, co-expression of CaSR and the Ca inhibitable AC6 isoform elicits a negative feedback on those hormones acting through the Gs/AC6/cAMP pathway, conferring high sensitivity of hormone effects to extracellular Ca concentration. This negative feedback mainly relies on inhibition of intracellular cAMP levels as a result of CaSR activation by extracellular Ca and consequent inhibition of cAMP synthesis by the Ca inhibitable AC6 isoform (Figure 2).

**Genetic polymorphisms associated with calcium nephrolithiasis**

*CaSR and claudin-14 as two important loci for kidney stone diseases*

As discussed above, CaSR expressed in the TAL inhibits paracellular Ca reabsorption through the paracellular pathway by blocking claudin-16 via activation of claudin-14 transcription[9]. In the TAL, CaSR also decreases the NKCC2 activity that sustains the electrochemical gradient driving the paracellular Ca reabsorption[45]. Therefore CaSR activation by serum Ca in the TAL promotes calciuria. On the other hand, in the proximal tubule, CaSR activation antagonises the phosphaturic action of PTH and promotes proton secretion through the NHE3[25, 30] whereas in the collecting duct CaSR activation decreases water reabsorption by inhibiting the tubular response to vasopressin and increases proton excretion by stimulating proton pump activity. Therefore, CaSR activity in the proximal tubule and collecting duct may counterbalance the risk of calcium phosphate precipitation associated to the effect of CaSR to enhance Ca excretion in TAL.

Based on these findings both CaSR and claudin-14 have been investigated as two important loci for kidney stone diseases. Indeed, allelic CaSR variants as well as the tight junctional protein, claudin 14, show association with nephrolithiasis[80-85].

The possible involvement of the CaSR in the pathophysiology of calcium nephrolithiasis has been investigated by several groups with contrasting results. In a cohort of French patients with familial calcium nephrolithiasis[86] as well as in a large group of Canadian brothers[87] no evidence for mutations in the *CaSR* gene predisposing to the risk of renal stones has been observed. However, other groups showed that single-nucleotide polymorphisms (SNPs) of the CaSR are associated with nephrolithiasis and kidney stones in patients. An association between the R990G polymorphism of the *CaSR* gene and primary hypercalciuria was found to be more frequent in hypercalciuric calcium stone-forming patients, compared to normocalciuric stone formers or healthy individuals. In line with these findings, *in vitro* data indicated that R990G polymorphism results in a gain-of-function of the CaSR[88]. Other investigators however found no association between this CaSR

Arg990Gly polymorphism and indices of serum Ca homeostasis[89].

Calcium nephrolithiasis was also found to be associated with the rs6776158 SNP, located within *CaSR* gene promoter 1[90]. In vitro studies showed that the minor G allele at rs67780158 caused a decrease in the transcriptional efficiency of the promoter 1 in two renal cell models[90]. Moreover recent data showed that the simultaneous presence of both rs1501899 and Arg990Gly may potentiate the kidney stone risk in patients with primary hyperparathyroidism despite their apparently opposite effects on CASR function in the kidney[91].

Regarding the molecular basis of the pathophysiology of calcium nephrolithiasis of the gain-of-function Arg990Gly polymorphism, the increase in calcium excretion is expected to predispose to intratubular precipitation of calcium-phosphate crystals.

Conversely, the CaSR polymorphisms rs6776158 SNP at the regulatory region associated with decreased CaSR expression may improve the tubular efficency to reabsorb Ca. This may lead to increased Ca concentration in the interstitium predisposing to hydroxyapatite precipitation in the Randall's plaque followed by precipitation of urinary calcium oxalate and formation of calcium-oxalate stones. Deficient expression may also impair CaSR-associated urine acidification and dilution in collecting ducts and phosphate retention in proximal tubules exposing patients to the risk of calcium phosphate precipitation in the tubular fluid.

Based on this evidence, some CaSR polymorphisms may be included amongst factors predisposing to stones and can potentially represent a marker to identify patients who may develop calcium nephrolithiasis.

Claudins are key components of the paracellular pathway. Studies on the physiological function of claudins highlighted a functional interaction between CaSR signaling and claudin gene expression, suggesting a role for claudins in nephrolithiasis. Indeed, defects in the function of claudins, have been implicated in hypercalciuria and nephrolithiasis (see Hou, 2013 for review[84]).

Physiologically, in the thick ascending limb, the claudin 16 channel provides cation permeability to the tight junction essential for Ca and Mg reabsorption driven by the lumen-positive transepithelial potential gradient. On the other hand, claudin 14 blocks the paracellular cation channel made of claudin 16 and claudin 19. The recent demonstration that stimulation of CaSR signaling in the TAL downregulates miR-9 and miR-374 causing increasing in claudin 14 levels, and in turn suppressing claudin 16 and 19 permeabilities, thus promoting Ca excretion, strongly support a role for claudins in nephrolithiasis[84].

**Conclusions and Implications of the latest work and future directions**

Recent studies confirm that the CaSR is expressed all along the nephron, from glomerulus to the inner medullary collecting ducts. However, significant differences in its expression levels can be found, with the highest expression being in the TAL and the lowest in the glomerulus and proximal tubule. Accordingly, direct roles for the CaSR in the kidney have been identified and appear to correlate with the expression levels of this receptor. In the TAL the CaSR is a major determinant of urinary Ca excretion. In other regions the CaSR appears to "fine-tune" and integrate multiple stimuli deriving from Na, pH and mineral ion metabolism, particularly setting the sensitivity threshold for several cAMP-coupled hormones to extracellular Ca.

CaSR can be the target for therapeutic intervention for a number of inherited and acquired conditions in which the level of expression and/or function of theCaSR is altered (see Box 1). Calcimimetics have been proven to be effective in primary and secondary hyperparathyroidism. Conversely calcilytics might be used in the future to treat hypoparathyroidism and, in hypercalciuric stone-former patients, to prevent nephrolithiasis and nephrocalcinosis. The long-term use of these modulators on these, and other aspects of renal function modulated by the CaSR remains to be evaluated.

**Key points**

- Tissue-specific CaSR ablation and pharmacological studies reveal a pivotal role for the renal CaSR in the control of divalent cation excretion, in a PTH-independent fashion
- CaSR, acting on claudin-14 in the TAL, regulates paracellular Ca reabsorption
- In other nephron segments the CaSR "fine tunes" Ca, Mg and Pi transport by integrating multiple inputs from divalent cation concentration, osmolarity and urine acidification.
- Calcimimetics would be expected to increase urinary Ca excretion by acting on the CaSR in the parathyroid glands and the kidney
- Calcilytics represent a novel, promising avenue for the treatment of hypercalciuria, nephrolithiasis and nephrocalcinosis

- Co-expression of CaSR and the calcium-inhibitable AC6 in the nephron sensitises hormones to extracellular calcium and counteracts hormone-induced increase in cAMP

**Display items**

**Box 1. Pros and cons of allosteric modulators of the CaSR**

Calcimimetics and calcilytics are drugs which act on the CaSR. Calcimimetics enhance CaSR sensitivity to extracellular Ca and reduce serum PTH by activating the parathyroid and kidney CaSR. Two kinds of calcimimetics have been developed: “type I” calcimimetics are inorganic or organic polycationic agonists, and “type II” calcimimetics are positive allosteric enhancers of CaSR activity. Cinacalcet is a type II calcimimetic which has been approved to treat advanced secondary hyperparathyroidism, an important complication of end-stage chronic kidney disease. Conversely calcilytics are allosteric antagonists of the CaSR. They evoke a rightward shift in the Ca concentration-response curve at the CaSR, hence higher then normal calcium levels are required to suppress PTH release. Owing to their abillity to evoke oscillating fluctuations in serum PTH, a known bone anabolic stimulus, calcilytics such as ronacaleret[92] and JTT-305/encaleret[93] were initially developed to treat age-related osteoporosis. While their development has been halted due to lack of efficacy, they could be repurposed to treat hypercalciuric disorders due to an overactive CaSR.

Since the CaSR is expressed in a multiplicity of tissues, the potential adverse effects of systemic CaSR allosteric modulators cannot be ignored. As an example, cinacalcet normalizes serum PTH and calcium concentrations in kidney transplant recipients, however long-term treatment with cinacalcet may increase urinary calcium excretion and the risk of renal calcium deposits and may alter renal graft function.

Conversely, calcilytics, expected to reduce urinary calcium excretion, might be used to treat gain-of-function variants of the CaSR associated with hypercalciuria and stone diseases. However, an undesired effect would be to stimulate PTH release, which may worsen the hypercalciuria.

Development of drugs with tissue-specific action able to modulates differentially specific CaSR-regulated processes, particularly in the setting of an under-active or over-active CaSR, is the future challenge in CaSR drug discovery.

**Figure Legends**

**Figure 1. Schematic representation of the CaSR in the kidney tubule**

A) CaSR in the proximal tubule. The CaSR blunts the phosphaturric action of PTH, inhibits the activity of CYP27B1 (1-hydroxylase), promotes acidification via NHE3 which in turns drives Na accumultion in the interstitium and solute reabsorption. B) CaSR in the TAL. Hypercalcemia activates the CaSR in the TAL leading to an inhibition of ROMK channel, thereby prevening apical K recycling, the rate-limiting step for NKCC2 activity, leading to loss of the driving force for paracellular cation reabsorption. Thus, hypercalcemia mimicks the effects of loop diuretics and recapitulates a Bartter type 1 and 2 phenotypes, ascribed to mutations in the genes encoding NKCC2 or ROMK, respectively, an effect which is also mimicked by activating mutatins in the *CaSR* gene (Bartter type 5) with hypercalciuria, hypokalemic alkalosis and hyperreninemic hyperaldosteronism. Bartter type 3 and 4 are due to mutations in the genes encoding the basolateral Cl channel, ClC-Kb or its auxiliary subunit, Barttin, respectively. CaSR activation also directly regulates paracellular permeability via NFATc1-microRNA-Cldn-14. C) CaSR in the DCT. At the apical membrane CaSR is biochemically associated with the $1,25(OH)_2D_3$-regulated TRPV5. Increased Ca delivery (filled circles) to the DCT results in CaSR activation, hence incrased apical Ca entry via TRPV5. Intracellularly, calbindin D28K (indicated by crescents) delivers Ca ions are to the basolateral membrane where Ca exits the cell via active mechanisms (PMCA and NCX). CaSR also decreases surface expression of Kir 4.1 resulting in an inhibition of NaCl reabsorption. D) CaSR in the CD. In the presence of an antidiuretic stimulus, Ca concentrations in the pro-urine can become supersaturating, potentially leading to Ca stone formation (indicated by stars). When luminal Ca concentration becomes critical it activate the apical CaSR which, in principal cells, blunts vasopressin-mediated apical insertion of AQP2 water channels and in turn the rate of water reabsorption. In intercalated cells CaSR activation leads to luminal acidification. Overall, these two effects result in the production of a more dilute, acidified urine, thereby reducing the risk of nephrolithiasis.

**Figure 2. CaSR confers high sensitivity to extracellular calcium concentration by reducing the effects of distinct hormones selectively acting in selected nephron segments via Gs/AC6/cAMP pathway**

CaSR is expressed in renal juxtaglomerular cells and its activation stimulates calcium-mediated decreases in cAMP content and inhibits renin release through activation of AC5 and AC6.

In the proximal tubule CaSR is expressed on the luminal membrane and its activation impairs PTH action by antagonizing its effects on Pi excretion through downregulation of the Gs/AC6/cAMP pathway. This reduces Pi excretion limiting the risk of formation of Ca-Pi precipitates. In the thick ascending limb CaSR signaling counteracts vasopressin action on activation of NKCC2 phosphorylation causing changes in transepithelial potential resulting in overall reduced Ca reabsorption. In this segment AC6 is the adenylate cyclase isoform activated by vasopressin. Using a similar signal transduction pathway, CaSR is expected to counteract PTH-induced Ca reabsortion in TAL. In the collecting duct, activation of CaSR by luminal calcium reduces the vasopressin-stimulated insertion of AQP2, leading to the formation of more dilute urine favoring the solubility of calcium phosphate salts.

The negative feedback of CaSR on hormones acting through the Gs/AC6/cAMP pathway is principally due to the inhibition of cAMP synthesis by the Ca-inhibitable AC6 as a consequence of CaSR-activated increase in intracellular Ca concentration.

**Table 1. Summary of CaSR mRNA (in situ hybridisation) and protein (immunohistochemistry and proximity ligation assay) expression levels along the nephron.** The highest CaSR mRNA and protein expression levelswere observed in the TAL while only faint protein expression could be osberved in the glomerulus (from: Graca et al, AJP RP, 2015)[13].

**Table 2.** Reported effects of extracellular Ca (either luminal o basolateral) on the renal physiology, believed to be mediated by CaSR.

**Competing interests**

The authors declare no competing interests

**Brief biography (100 words):** current and past roles, training and research, society memberships and other interests.

**Daniela Riccardi, PhD**

I obtained my PhD in Physiopathology from the University of Milan, Italy. From 1993-1997 I was a Research Fellow in Dr Steven Hebert lab in the Renal Division of the Brigham and Women's Hospital. There, I identified the CaSR from mammalian kidney for which I was awarded the first prize, Excellence in Research from the American Society of Nephrology and National Kidney Foundation. In 1997 I moved to the UK, working on the CaSR in the kidney-bone-vascular axis and, more recently, in asthma. Currently I am Full Professor of Physiology and Deputy Head at Cardiff School of Biosciences.

**Giovanna Valenti, PhD**

I worked as research fellow at the CEA Saclay, France (1985-1987) and at the Harvard Medical School of Boston, MA,General Hospital, USA (1992-1993) in Dr Dennis Brown laboratory where for the first time I localized the water channel Aquaporin 1 in the rat kidney. From 1994 to date I have spent several months at the MDC Berlin (Germany) and at the University of Salzburg (Austria) as visiting professor. One main topic of my research is pathophysiology of CaSR and its functional interaction with renal aquaporins in nephrolitiasis. I am currently Full Professor of Physiology at the University of Bari, Italy.

**References**


1. Brown, E.M. et al. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* **366**, 575-80 (1993).
2. Brown, E.M. & MacLeod, R.J. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* **81**, 239-297 (2001).
3. Block, G.A. et al. Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. *N Engl J Med* **350**, 1516-25 (2004).
4. Bell, G., Huang, S., Martin, K.J. & Block, G.A. A randomized, double-blind, phase 2 study evaluating the safety and efficacy of AMG 416 for the treatment of secondary hyperparathyroidism in hemodialysis patients. *Curr Med Res Opin* **31**, 943-52 (2015).
5. Letz, S. et al. Amino alcohol- (NPS-2143) and quinazolinone-derived calcilytics (ATF936 and AXT914) differentially mitigate excessive signalling of calcium-sensing receptor mutants causing Bartter syndrome Type 5 and autosomal dominant hypocalcemia. *PLoS One* **9**, e115178 (2014).
6. Marx, S.J. et al. Circulating parathyroid hormone activity: familial hypocalciuric hypercalcemia versus typical primary hyperparathyroidism. *J Clin Endocrinol Metab* **47**, 1190-7 (1978).
7. Attie, M.F. et al. Urinary calcium excretion in familial hypocalciuric hypercalcemia. Persistence of relative hypocalciuria after induction of hypoparathyroidism. *J Clin Invest* **72**, 667-76 (1983).
8. Loupy, A. et al. PTH-independent regulation of blood calcium concentration by the calcium-sensing receptor. *J Clin Invest* **122**, 3355-67 (2012).
9. Gong, Y. et al. Claudin-14 regulates renal Ca(+)(+) transport in response to CaSR signalling via a novel microRNA pathway. *EMBO J* **31**, 1999-2012 (2012).
10. Gong, Y. & Hou, J. Claudin-14 underlies Ca(+)(+)-sensing receptor-mediated Ca(+)(+) metabolism via NFAT-microRNA-based mechanisms. *J Am Soc Nephrol* **25**, 745-60 (2014).
11. Gong, Y., Himmerkus, N., Plain, A., Bleich, M. & Hou, J. Epigenetic regulation of microRNAs controlling CLDN14 expression as a mechanism for renal calcium handling. *J Am Soc Nephrol* **26**, 663-76 (2015).
12. Riccardi, D. & Brown, E.M. Physiology and pathophysiology of the calcium-sensing receptor in the kidney. *Am J Physiol Renal Physiol* **298**, F485-99 (2010).
13. Graca, J.A.Z. et al. Comparative expression of the extracellular calcium sensing receptor in mouse, rat, and human kidney. *Am J Physiol Renal Physiol* (2015, in press).
14. Kwak, J.O. et al. The extracellular calcium sensing receptor is expressed in mouse mesangial cells and modulates cell proliferation. *Exp Mol Med* **37**, 457-65 (2005).
15. Meng, K. et al. Calcium sensing receptor modulates extracellular calcium entry and proliferation via TRPC3/6 channels in cultured human mesangial cells. *PLoS One* **9**, e98777 (2014).
16. Oh, J. et al. Stimulation of the calcium-sensing receptor stabilizes the podocyte cytoskeleton, improves cell survival, and reduces toxin-induced glomerulosclerosis. *Kidney Int* **80**, 483-92 (2011).
17. Gut, N. et al. The calcimimetic R-568 prevents podocyte loss in uninephrectomized ApoE-/- mice. *Am J Physiol Renal Physiol* **305**, F277-85 (2013).
18. Wang, L. et al. Gq signaling causes glomerular injury by activating TRPC6. *J Clin Invest* **125**, 1913-26 (2015).
19. Churchill, P.C. Second messengers in renin secretion. *Am J Physiol* **249**, F175-84 (1985).

20. Atchison, D.K., Ortiz-Capisano, M.C. & Beierwaltes, W.H. Acute activation of the calcium-sensing receptor inhibits plasma renin activity in vivo. *Am J Physiol Regul Integr Comp Physiol* **299**, R1020-6 (2010).
21. Grunberger, C., Obermayer, B., Klar, J., Kurtz, A. & Schweda, F. The calcium paradoxon of renin release: calcium suppresses renin exocytosis by inhibition of calcium-dependent adenylate cyclases AC5 and AC6. *Circ Res* **99**, 1197-206 (2006).
22. Beierwaltes, W.H. The role of calcium in the regulation of renin secretion. *Am J Physiol Renal Physiol* **298**, F1-F11 (2010).
23. Murer, H., Hernando, N., Forster, I. & Biber, J. Molecular aspects in the regulation of renal inorganic phosphate reabsorption: the type IIa sodium/inorganic phosphate co-transporter as the key player. *Curr Opin Nephrol Hypertens* **10**, 555-61 (2001).
24. Riccardi, D. et al. Localization of the extracellular Ca2+/polyvalent cation-sensing protein in rat kidney. *Am J Physiol* **274**, F611-22 (1998).
25. Ba, J., Brown, D. & Friedman, P.A. Calcium-sensing receptor regulation of PTH-inhibitable proximal tubule phosphate transport. *Am J Physiol Renal Physiol* **285**, F1233-43 (2003).
26. Di Mise, A. et al. Conditionally immortalized human proximal tubular epithelial cells isolated from the urine of a healthy subject express functional calcium-sensing receptor. *Am J Physiol Renal Physiol* **308**, F1200-6 (2015).
27. Riccardi, D. et al. Dietary phosphate and parathyroid hormone alter the expression of the calcium-sensing receptor (CaR) and the Na+-dependent Pi transporter (NaPi-2) in the rat proximal tubule. *Pflugers Arch* **441**, 379-87 (2000).
28. Bland, R., Walker, E.A., Hughes, S.V., Stewart, P.M. & Hewison, M. Constitutive expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in a transformed human proximal tubule cell line: evidence for direct regulation of vitamin D metabolism by calcium. *Endocrinology* **140**, 2027-34 (1999).
29. Egbuna, O. et al. The full-length calcium-sensing receptor dampens the calcemic response to 1alpha,25(OH)2 vitamin D3 in vivo independently of parathyroid hormone. *Am J Physiol Renal Physiol* **297**, F720-8 (2009).
30. Capasso, G. et al. The calcium sensing receptor modulates fluid reabsorption and acid secretion in the proximal tubule. *Kidney Int* **84**, 277-84 (2013).
31. Zhang, Y. et al. In vivo PTH provokes apical NHE3 and NaPi2 redistribution and Na-K-ATPase inhibition. *Am J Physiol* **276**, F711-9 (1999).
32. Friedman, P.A. Codependence of renal calcium and sodium transport. *Annu Rev Physiol* **60**, 179-97 (1998).
33. Wittner, M., Mandon, B., Roinel, N., de Rouffignac, C. & Di Stefano, A. Hormonal stimulation of Ca2+ and Mg2+ transport in the cortical thick ascending limb of Henle's loop of the mouse: evidence for a change in the paracellular pathway permeability. *Pflugers Arch* **423**, 387-96 (1993).
34. Takaichi, K. & Kurokawa, K. High Ca2+ inhibits peptide hormone-dependent cAMP production specifically in thick ascending limbs of Henle. *Miner Electrolyte Metab* **12**, 342-6 (1986).
35. de Jesus Ferreira, M.C. et al. Co-expression of a Ca2+-inhibitable adenylyl cyclase and of a Ca2+-sensing receptor in the cortical thick ascending limb cell of the rat kidney. Inhibition of hormone-dependent cAMP accumulation by extracellular Ca2+. *J Biol Chem* **273**, 15192-202 (1998).
36. Welling, P.A. & Ho, K. A comprehensive guide to the ROMK potassium channel: form and function in health and disease. *Am J Physiol Renal Physiol* **297**, F849-63 (2009).

37. Kieferle, S., Fong, P., Bens, M., Vandewalle, A. & Jentsch, T.J. Two highly homologous members of the ClC chloride channel family in both rat and human kidney. *Proc Natl Acad Sci U S A* **91**, 6943-7 (1994).
38. Wang, W., Lu, M., Balazy, M. & Hebert, S.C. Phospholipase A2 is involved in mediating the effect of extracellular Ca2+ on apical K+ channels in rat TAL. *Am J Physiol* **273**, F421-9 (1997).
39. Kong, S. et al. Stimulation of Ca2+-sensing receptor inhibits the basolateral 50-pS K channels in the thick ascending limb of rat kidney. *Biochim Biophys Acta* **1823**, 273-81 (2012).
40. Toka, H.R., Pollak, M.R. & Houillier, P. Calcium Sensing in the Renal Tubule. *Physiology (Bethesda)* **30**, 317-26 (2015).
41. Vargas-Poussou, R. et al. Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. *J Am Soc Nephrol* **13**, 2259-66 (2002).
42. Vezzoli, G. et al. Autosomal dominant hypocalcemia with mild type 5 Bartter syndrome. *J Nephrol* **19**, 525-8 (2006).
43. Simon, D.B. et al. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K+ channel, ROMK. *Nat Genet* **14**, 152-6 (1996).
44. Simon, D.B. et al. Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na-K-2Cl cotransporter NKCC2. *Nat Genet* **13**, 183-8 (1996).
45. Dimke, H. et al. Activation of the Ca(2+)-sensing receptor increases renal claudin-14 expression and urinary Ca(2+) excretion. *Am J Physiol Renal Physiol* **304**, F761-9 (2013).
46. Toka, H.R. et al. Deficiency of the calcium-sensing receptor in the kidney causes parathyroid hormone-independent hypocalciuria. *J Am Soc Nephrol* **23**, 1879-90 (2012).
47. Courbebaisse, M. et al. Effects of cinacalcet in renal transplant patients with hyperparathyroidism. *Am J Nephrol* **35**, 341-8 (2012).
48. de Groot, T. et al. Parathyroid hormone activates TRPV5 via PKA-dependent phosphorylation. *J Am Soc Nephrol* **20**, 1693-704 (2009).
49. de Groot, T., Bindels, R.J. & Hoenderop, J.G. TRPV5: an ingeniously controlled calcium channel. *Kidney Int* **74**, 1241-6 (2008).
50. Topala, C.N. et al. Activation of the Ca2+-sensing receptor stimulates the activity of the epithelial Ca2+ channel TRPV5. *Cell Calcium* **45**, 331-9 (2009).
51. Huang, C. et al. Interaction of the Ca2+-sensing receptor with the inwardly rectifying potassium channels Kir4.1 and Kir4.2 results in inhibition of channel function. *Am J Physiol Renal Physiol* **292**, F1073-81 (2007).
52. Yang, T. et al. Expression of PTHrP, PTH/PTHrP receptor, and Ca(2+)-sensing receptor mRNAs along the rat nephron. *Am J Physiol* **272**, F751-8 (1997).
53. Renkema, K.Y. et al. The calcium-sensing receptor promotes urinary acidification to prevent nephrolithiasis. *J Am Soc Nephrol* **20**, 1705-13 (2009).
54. Tessitore, N. et al. Renal acidification defects in patients with recurrent calcium nephrolithiasis. *Nephron* **41**, 325-32 (1985).
55. Buckalew, V.M., Jr. Nephrolithiasis in renal tubular acidosis. *J Urol* **141**, 731-7 (1989).
56. Sands, J.M. et al. Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited water permeability in rat kidney inner medullary collecting duct. *J Clin Invest* **99**, 1399-405 (1997).
57. Brown, E.M. Physiology and pathophysiology of the extracellular calcium-sensing receptor. *Am J Med* **106**, 238-53 (1999).

58. Earm, J.H. et al. Decreased aquaporin-2 expression and apical plasma membrane delivery in kidney collecting ducts of polyuric hypercalcemic rats. *J Am Soc Nephrol* **9**, 2181-93 (1998).
59. Valenti, G. et al. Urinary aquaporin 2 and calciuria correlate with the severity of enuresis in children. *J Am Soc Nephrol* **11**, 1873-81 (2000).
60. Valenti, G. et al. Low-calcium diet in hypercalciuric enuretic children restores AQP2 excretion and improves clinical symptoms. *Am J Physiol Renal Physiol* **283**, F895-903 (2002).
61. Procino, G. et al. Extracellular calcium antagonizes forskolin-induced aquaporin 2 trafficking in collecting duct cells. *Kidney Int* **66**, 2245-55 (2004).
62. Bustamante, M. et al. Calcium-sensing receptor attenuates AVP-induced aquaporin-2 expression via a calmodulin-dependent mechanism. *J Am Soc Nephrol* **19**, 109-16 (2008).
63. Renkema, K.Y. et al. TRPV5 gene polymorphisms in renal hypercalciuria. *Nephrol Dial Transplant* **24**, 1919-24 (2009).
64. Procino, G. et al. Calcium-sensing receptor and aquaporin 2 interplay in hypercalciuria-associated renal concentrating defect in humans. An in vivo and in vitro study. *PLoS One* **7**, e33145 (2012).
65. Ranieri, M. et al. Negative feedback from CaSR signaling to aquaporin-2 sensitizes vasopressin to extracellular Ca2. *J Cell Sci* **128**, 2350-60 (2015).
66. Tamma, G. et al. A decrease in aquaporin 2 excretion is associated with bed rest induced high calciuria. *J Transl Med* **12**, 133 (2014).
67. Procino, G. et al. Aquaporin 2 and apical calcium-sensing receptor: new players in polyuric disorders associated with hypercalciuria. *Semin Nephrol* **28**, 297-305 (2008).
68. Bergsland, K.J., Coe, F.L., Gillen, D.L. & Worcester, E.M. A test of the hypothesis that the collecting duct calcium-sensing receptor limits rise of urine calcium molarity in hypercalciuric calcium kidney stone formers. *Am J Physiol Renal Physiol* **297**, F1017-23 (2009).
69. Fenton, R.A. et al. Renal phosphate wasting in the absence of adenylyl cyclase 6. *J Am Soc Nephrol* **25**, 2822-34 (2014).
70. Rieg, T. et al. Adenylyl cyclase 6 enhances NKCC2 expression and mediates vasopressin-induced phosphorylation of NKCC2 and NCC. *Am J Pathol* **182**, 96-106 (2013).
71. Nearing, J. et al. Polyvalent cation receptor proteins (CaRs) are salinity sensors in fish. *Proc Natl Acad Sci U S A* **99**, 9231-6 (2002).
72. Ortiz-Capisano, M.C., Reddy, M., Mendez, M., Garvin, J.L. & Beierwaltes, W.H. Juxtaglomerular cell CaSR stimulation decreases renin release via activation of the PLC/IP(3) pathway and the ryanodine receptor. *Am J Physiol Renal Physiol* **304**, F248-56 (2013).
73. Gimenez, I. & Forbush, B. Short-term stimulation of the renal Na-K-Cl cotransporter (NKCC2) by vasopressin involves phosphorylation and membrane translocation of the protein. *J Biol Chem* **278**, 26946-51 (2003).
74. Gunaratne, R. et al. Quantitative phosphoproteomic analysis reveals cAMP/vasopressin-dependent signaling pathways in native renal thick ascending limb cells. *Proc Natl Acad Sci U S A* **107**, 15653-8 (2010).
75. Hoffert, J.D., Chou, C.L., Fenton, R.A. & Knepper, M.A. Calmodulin is required for vasopressin-stimulated increase in cyclic AMP production in inner medullary collecting duct. *J Biol Chem* **280**, 13624-30 (2005).

76. Rieg, T. et al. Adenylate cyclase 6 determines cAMP formation and aquaporin-2 phosphorylation and trafficking in inner medulla. *J Am Soc Nephrol* **21**, 2059-68 (2010).
77. Chabardes, D. et al. Localization of mRNAs encoding Ca2+-inhibitable adenylyl cyclases along the renal tubule. Functional consequences for regulation of the cAMP content. *J Biol Chem* **271**, 19264-71 (1996).
78. Helies-Toussaint, C., Aarab, L., Gasc, J.M., Verbavatz, J.M. & Chabardes, D. Cellular localization of type 5 and type 6 ACs in collecting duct and regulation of cAMP synthesis. *Am J Physiol Renal Physiol* **279**, F185-94 (2000).
79. Roos, K.P., Strait, K.A., Raphael, K.L., Blount, M.A. & Kohan, D.E. Collecting duct-specific knockout of adenylyl cyclase type VI causes a urinary concentration defect in mice. *Am J Physiol Renal Physiol* **302**, F78-84 (2012).
80. Vezzoli, G., Terranegra, A. & Soldati, L. Calcium-sensing receptor gene polymorphisms in patients with calcium nephrolithiasis. *Curr Opin Nephrol Hypertens* **21**, 355-61 (2012).
81. Kelly, C., Gunn, I.R., Gaffney, D. & Devgun, M.S. Serum calcium, urine calcium and polymorphisms of the calcium sensing receptor gene. *Ann Clin Biochem* **43**, 503-6 (2006).
82. Bushinsky, D.A. Nephrolithiasis: site of the initial solid phase. *J Clin Invest* **111**, 602-5 (2003).
83. Thorleifsson, G. et al. Sequence variants in the CLDN14 gene associate with kidney stones and bone mineral density. *Nat Genet* **41**, 926-30 (2009).
84. Hou, J. The role of claudin in hypercalciuric nephrolithiasis. *Curr Urol Rep* **14**, 5-12 (2013).
85. Arcidiacono, T. et al. Idiopathic calcium nephrolithiasis: a review of pathogenic mechanisms in the light of genetic studies. *Am J Nephrol* **40**, 499-506 (2014).
86. Lerolle, N. et al. No evidence for point mutations of the calcium-sensing receptor in familial idiopathic hypercalciuria. *Nephrol Dial Transplant* **16**, 2317-22 (2001).
87. Petrucci, M. et al. Evaluation of the calcium-sensing receptor gene in idiopathic hypercalciuria and calcium nephrolithiasis. *Kidney Int* **58**, 38-42 (2000).
88. Vezzoli, G. et al. R990G polymorphism of calcium-sensing receptor does produce a gain-of-function and predispose to primary hypercalciuria. *Kidney Int* **71**, 1155-62 (2007).
89. Harding, B. et al. Functional characterization of calcium sensing receptor polymorphisms and absence of association with indices of calcium homeostasis and bone mineral density. *Clin Endocrinol (Oxf)* **65**, 598-605 (2006).
90. Vezzoli, G. et al. Decreased transcriptional activity of calcium-sensing receptor gene promoter 1 is associated with calcium nephrolithiasis. *J Clin Endocrinol Metab* **98**, 3839-47 (2013).
91. Vezzoli, G. et al. Risk of nephrolithiasis in primary hyperparathyroidism is associated with two polymorphisms of the calcium-sensing receptor gene. *J Nephrol* **28**, 67-72 (2015).
92. Fitzpatrick, L.A. et al. The effects of ronacaleret, a calcium-sensing receptor antagonist, on bone mineral density and biochemical markers of bone turnover in postmenopausal women with low bone mineral density. *J Clin Endocrinol Metab* **96**, 2441-9 (2011).
93. Kimura, S. et al. JTT-305, an orally active calcium-sensing receptor antagonist, stimulates transient parathyroid hormone release and bone formation in ovariectomized rats. *Eur J Pharmacol* **668**, 331-6 (2011).

**Table 1. Summary of CaSR mRNA (in situ hybridisation) and protein (immunohistochemistry and proximity ligation assay) expression levels along the nephron.** The highest CaSR mRNA and protein expression levelswere observed in the TAL while only faint protein expression could be osberved in the glomerulus (from: Graca et al, AJP RP, 2015)[13].

| | **Glomerulus** | **PT** | **TAL** | **DCT** | **CNT** | **CCD** | **OMCD/IMCD** |
|---|---|---|---|---|---|---|---|
| **mRNA** | - | + | +++ | ++ | + | ++ | + |
| **Protein** | + | + | +++ | ++ | + | ++ | + |

## Table 2. Reported effects of CaSR activators and inhibitors on renal physiopathology

| Nephron segment | Modulated function | Experimental model | Evidence for specific CaSR involvement | References |
|---|---|---|---|---|
| Glomerulus | Regulation of glomerular function, possible role in preventing glomerulosclerosis | Cultured podocytes; toxin-induced glomerulosclerosis in rats; apolipoprotein E-deficient mice | Type II calcimimetic (R-568) | *Refs 16-17* |
| Juxtaglomerular apparatus | Inhibition of renin secretion | Primary cultures of mouse juxtaglomerular cells; *in vivo* experiments in rats | Type I calcimimetic (extracellular Ca) | *Ref 20* |
| Proximal tubule (apical) | Inhibition of PTH-dependent phosphate uptake | Microdissected mouse S3 segments; proximal tubule-like opossum kidney cells | Type I calcimimetics ($Gd^{3+}$ / extracellular Ca) | *Ref 25* |
| Proximal tubule (apical) | Increase in luminal acidification; increase in fluid reabsorption | *In vivo* micropuncture in rats; *in vitro* perfused mouse proximal tubules; proximal tubules isolated from CaSR knockout mice | Type II calcimimetic (cinacalcet)/type I calcimimetic (luminal Ca) | *Ref 30* |
| Thick ascending limb (basolateral) | Inhibition of PTH, vasopressin, calcitonin, glucagon, actions; decrease in Ca absorption. | Dissected mouse TAL; dissected rat TAL | Type I calcimimetic (extracellular Ca) | *Refs 34-35* |
| Thick ascending limb (basolateral) | Control of urinary Ca absorption; increased expression of Claudin-14 | *In vivo* treatment of mice; cell culture models; patients with autosomal dominant hypocalcemia (ADH) due to CaSR activating mutations | Type II calcimimetic (cinacalcet); calcilytic (NPS2143) | *Refs 10; 45* |
| Distal convoluted tubule (apical/basolateral) | Stimulation of Ca reabsorption | Cell culture models expressing a "dominant negative" CaSR (R185Q) | Type I calcimimetic (neomycin) | *Ref 50* |
| Collecting duct intercalated cells (apical) | Increase in luminal acidification | Dissected mouse outer medullary collecting ducts; gene ablation of the collecting duct-specific B1 subunit of H(+)-ATPase | Type I calcimimetic (luminal Ca) | *Ref 53* |
| Collecting duct principal cells (apical) | Decrease in AQP2-mediated water reabsorption, inhibition of vasopressin response | Hypercalcemic rats; dissected rat kidney inner medullary collecting duct; cell culture models; human bedrest; AQP2 isolated vesicles; hypercalciuric patients | Type I calcimimetics (neomycin, gadolinium)/type II calcimimetic (R-568) Type I calcimimetic (luminal Ca) | *Refs 56; 58-64* |

**Figure 1A**

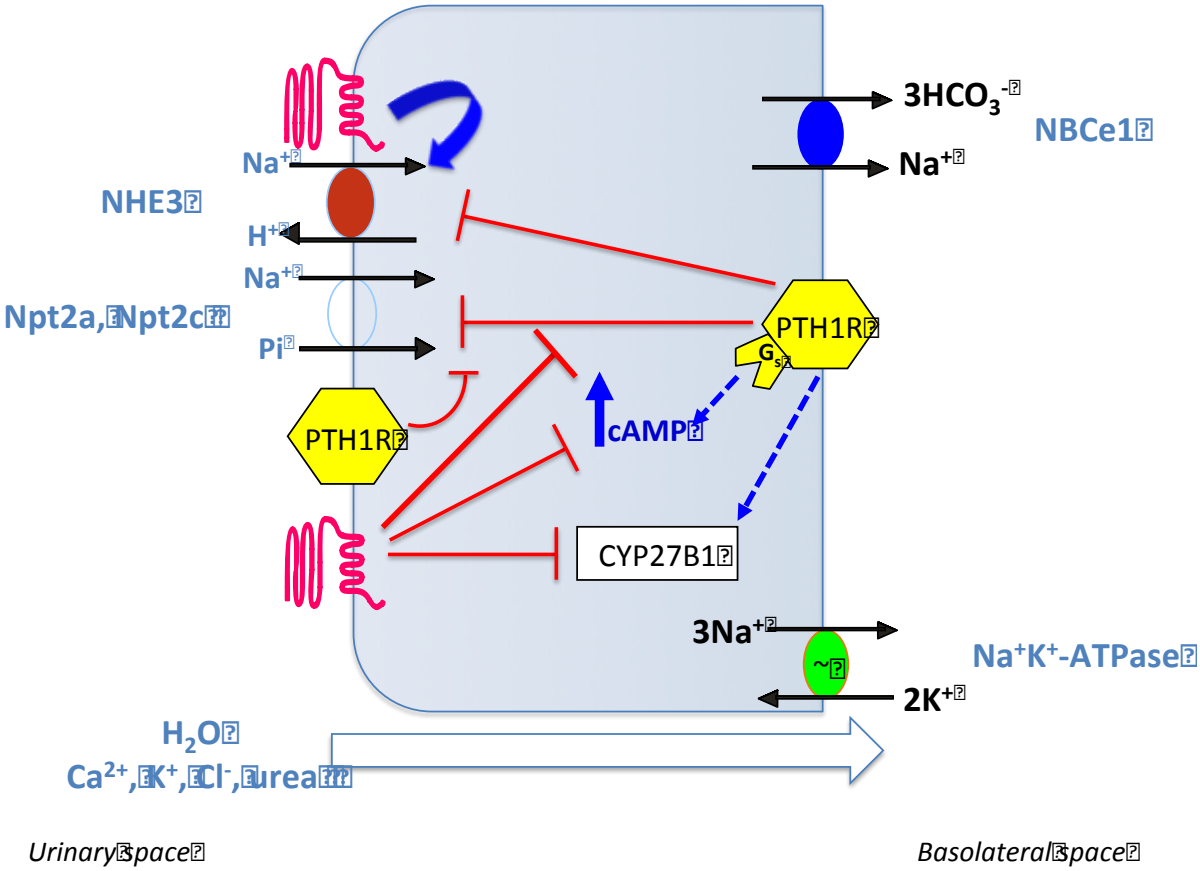

3HCO3-
NBCe1
Na+
Na+
NHE3
H+
Na+
Npt2a, Npt2c
Pi
PTH1R
Gs
PTH1R
cAMP
CYP27B1
3Na+
Na+K+-ATPase
2K+
H2O
Ca2+, K+, Cl-, urea
Urinary space
Basolateral space

Figure 1B

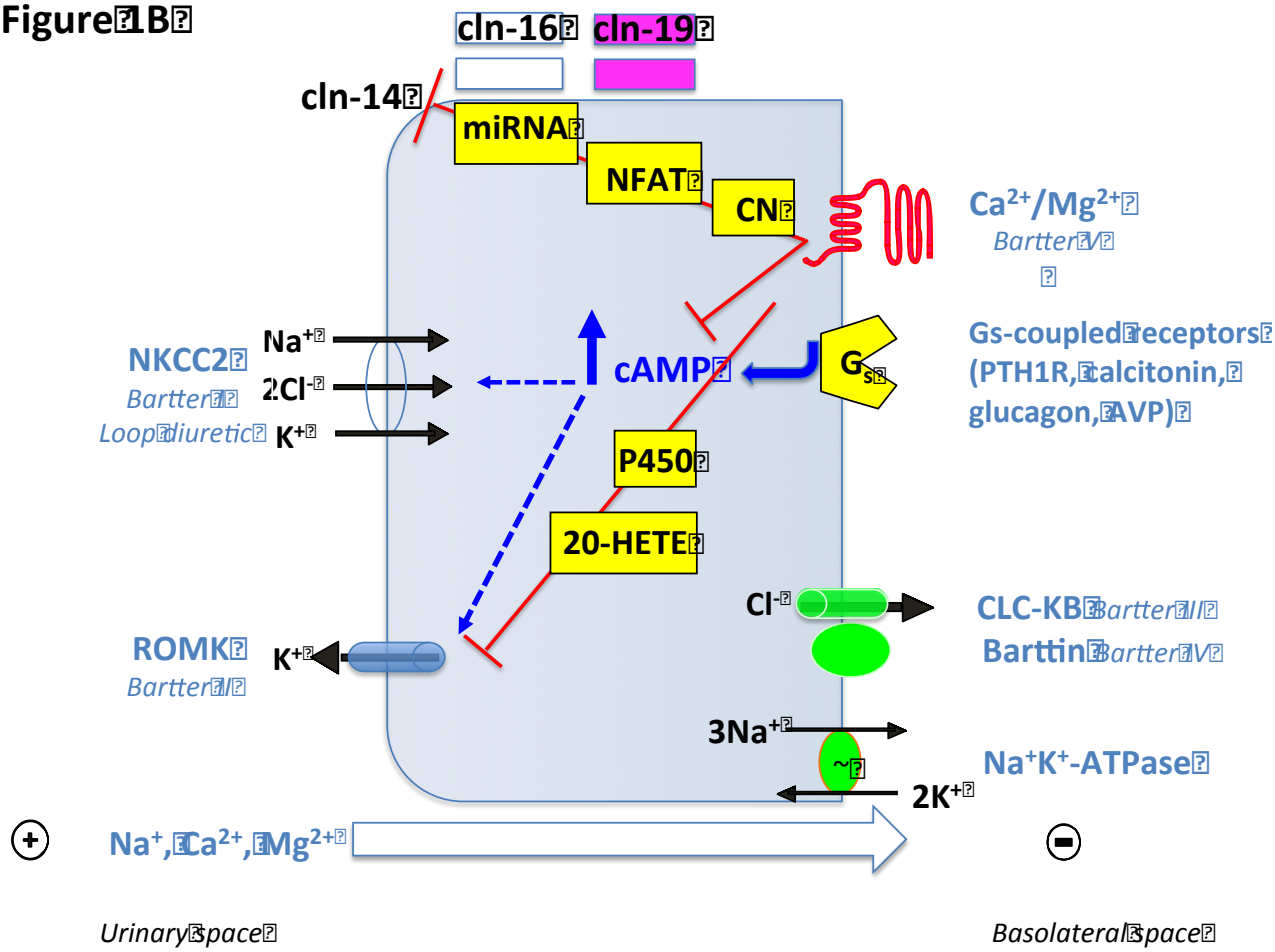

cln-16
cln-19
cln-14
miRNA
NFAT
CN
Ca2+/Mg2+
Bartter V
Gs-coupled receptors
(PTH1R, calcitonin,
glucagon, AVP)
NKCC2
Bartter I
Loop diuretic
Na+
2Cl-
K+
cAMP
Gs
P450
20-HETE
ROMK
Bartter II
K+
Cl-
CLC-KB Bartter III
Barttin Bartter V
3Na+
Na+K+-ATPase
2K+
Na+, Ca2+, Mg2+
Urinary space
Basolateral space

**Figure 1C**

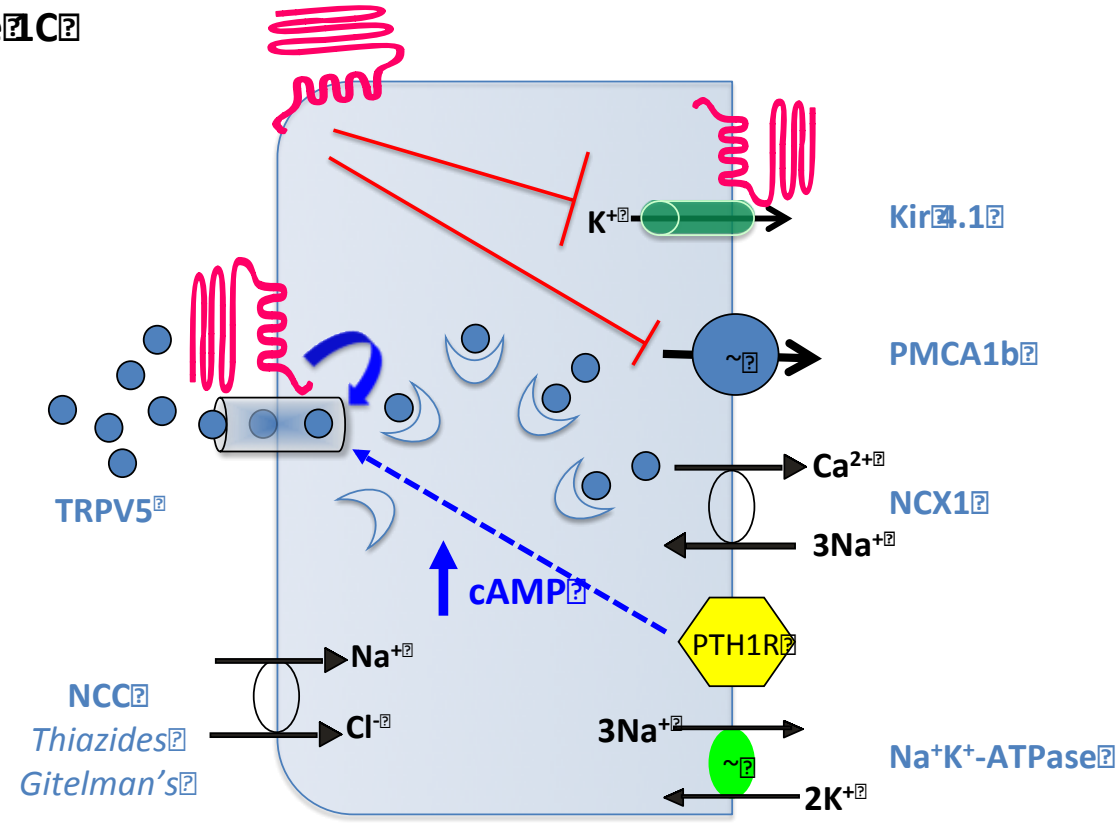

K+
Kir4.1
~
PMCA1b
Ca2+
NCX1
3Na+
TRPV5
cAMP
PTH1R
Na+
NCC
Thiazides
Gitelman's
Cl-
3Na+
Na+K+-ATPase
~
2K+
Urinary space
Basolateral space

**Figure 1D**

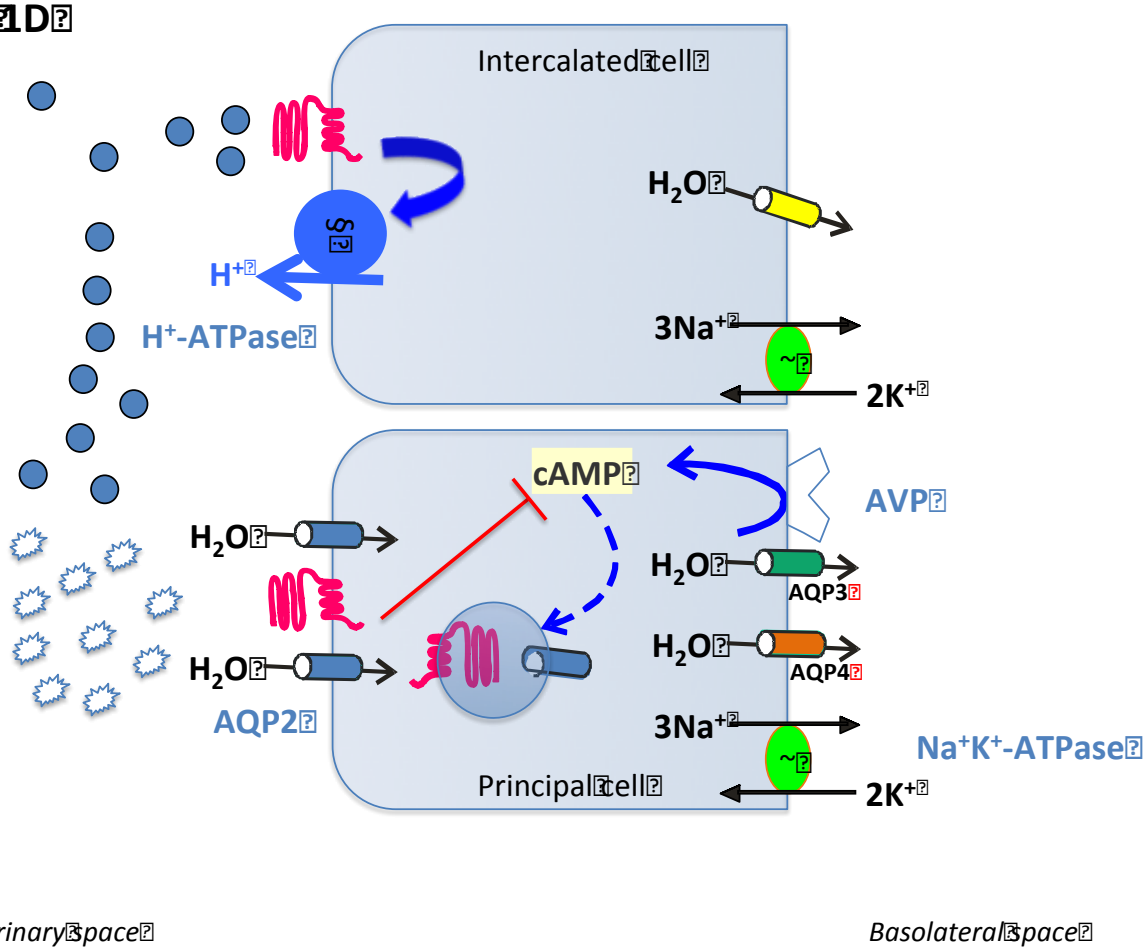
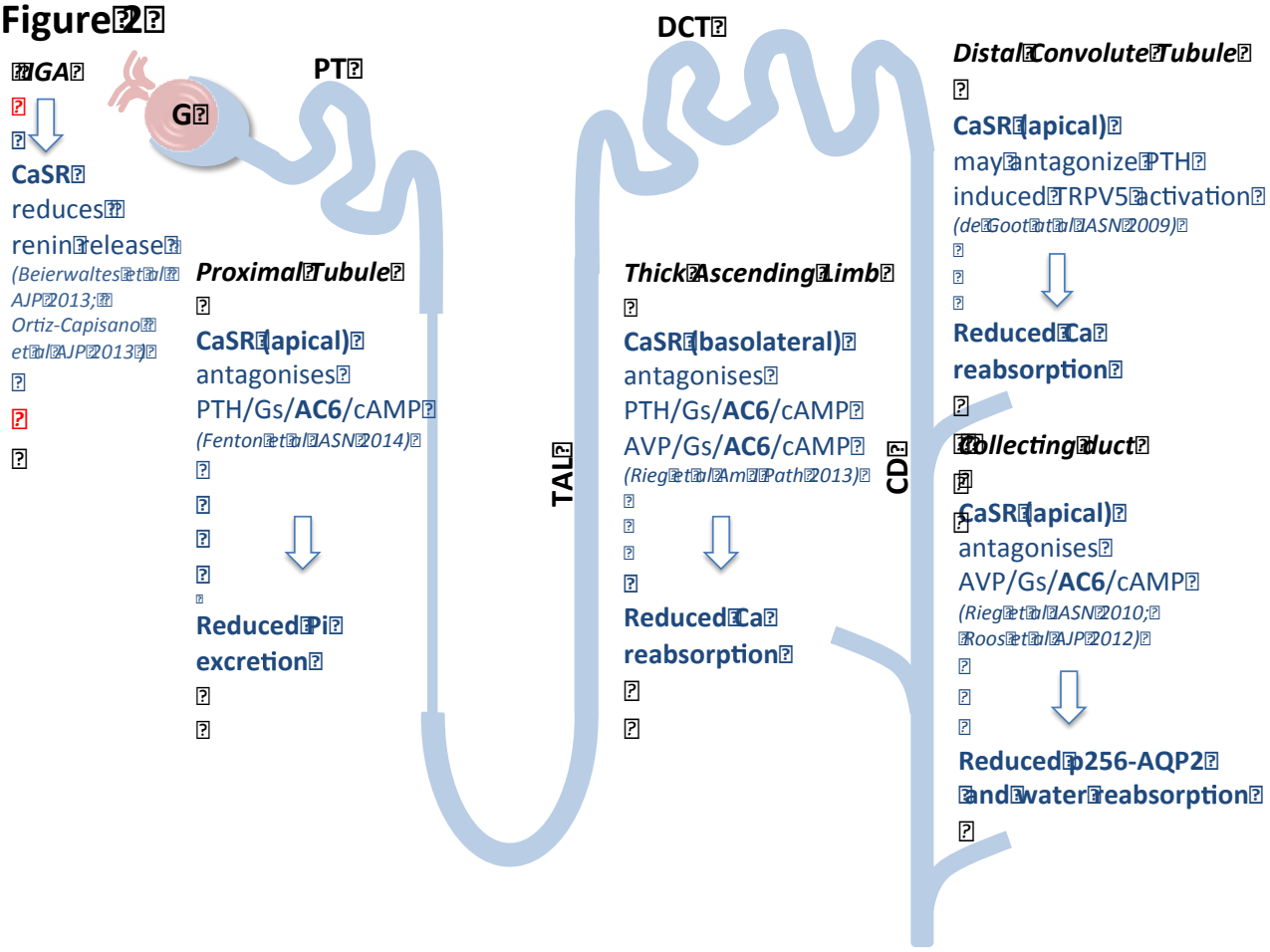

Intercalated cell
$H_2O$
$H^+$
$H^+$-ATPase
$3Na^+$
$2K^+$
cAMP
AVP
$H_2O$
$H_2O$
AQP3
$H_2O$
$H_2O$
AQP4
AQP2
$3Na^+$
$Na^+K^+$-ATPase
Principal cell
$2K^+$
*Urinary space*
*Basolateral space*

**Figure 2**


JGA
G
CaSR
reduces
renin release
(Beierwaltes et al AJP 2013;
Ortiz-Capisano et al AJP 2013)
PT
Proximal Tubule
CaSR (apical)
antagonises
PTH/Gs/AC6/cAMP
(Fenton et al ASN 2014)
Reduced Pi
excretion
TAL
Thick Ascending Limb
CaSR (basolateral)
antagonises
PTH/Gs/AC6/cAMP
AVP/Gs/AC6/cAMP
(Rieg et al Am J Path 2013)
Reduced Ca
reabsorption
DCT
CD
Distal Convolute Tubule
CaSR (apical)
may antagonize PTH
induced TRPV5 activation
(de Groot et al ASN 2009)
Reduced Ca
reabsorption
Collecting Duct
CaSR (apical)
antagonises
AVP/Gs/AC6/cAMP
(Rieg et al ASN 2010;
Roos et al AJP 2012)
Reduced p256-AQP2
and water reabsorption