Localization and function of the renal calcium-sensing receptor

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Abstract

The ability to monitor changes in the ionic composition of the extracellular environment is a crucial feature that has evolved in all living organisms. The cloning and characterization of the extracellular calcium-sensing receptor (CaSR) from the mammalian parathyroid gland in the early 1990s provided the first description of a cellular, ion-sensing mechanism. This finding demonstrated how cells can detect small, physiological variations in free ionized calcium (Ca2+) in the extracellular fluid and subsequently evoke an appropriate biological response by altering the secretion of parathyroid hormone (PTH) that acts on PTH receptors expressed in target tissues, including the kidney, intestine, and bone. Aberrant Ca2+ sensing by the parathyroid glands, as a result of altered CaSR expression or function, is associated with impaired divalent cation homeostasis. CaSR activators that mimic the effects of Ca2+ (calcimimetics) have been designed to treat hyperparathyroidism, and CaSR antagonists (calcilytics) are in development for the treatment of hypercalciuric disorders. The kidney expresses a CaSR that might directly contribute to the regulation of many aspects of renal function in a

PTH-independent manner. This Review discusses the roles of the renal CaSR and the potential

impact of pharmacological modulation of the CaSR on renal function

Main

Parathyroid hormone (PTH) is the major determinant of serum calcium ion (Ca2+) levels, and serum levels of PTH are regulated by the extracellular calcium-sensing receptor (CaSR) that is located in parathyroid glands1. The CaSR can detect minor changes in serum Ca2+ levels, and the amount of Ca2+ influx as a result of CaSR activation sub- sequently feeds back to alter the level of PTH secretion. Serum PTH levels are inversely associated with the extra- cellular Ca2+ level. Serum Ca2+ <1.1–1.2 mmol/l promotes PTH release, which results in reabsorption of Ca2+ by the kidney, Ca2+ absorption in the intestine (via 1α-hydroxyl- ation of 25(OH)D3 into 1,25(OH)2D3 by the kidney prox- imal tubule), and Ca2+ resorption from bone2. Serum Ca2+ levels >1.2 mmol/l have the opposite effect; Ca2+ binding at the CaSR elicits a Gq G-protein cascade that activates the phospholipase C pathway and prevents exocytosis of PTH. This event is accompanied by an increase in cyto-solic Ca2+ that has been released from intracellular stores1,2. Defective Ca2+ sensing by the parathyroid glands, due to altered CaSR expression or function, results in altered divalent cation homeostasis, which can lead to notable, long-lasting morbidity in patients if left untreated2.

Pharmacological modulators of the CaSR have been developed to correct both hyperparathyroidism and hypoparathyroidism (BOX 1). Calcimimetic drugs (such as cinacalcet) have been developed to rectify abnormal CaSR expression or function and have been successfully administered to patients for >10 years to treat hyperparathyroidism secondary to kidney failure3. AMG416 is a long-acting peptide that functions as a CaSR agonist and is suitable for intravenous administra- tion. Promising data was obtained from a phase II clin- ical trial that evaluated AMG416 for the treatment of secondary hyperparathyroidism in patients undergoing haemodialysis4. A phase III trial is now complete and met its primary and secondary end points, but the data are yet to be published5. CaSR antagonists (calcilytics) are also being investigated for the treatment of auto- somal dominant hypocalcaemia with hypercalciuria due to genetic mutations that result in hyperactivation of the CaSR6.

The combined use of detection methods with enhanced sensitivity (such as branched DNA in situ hybridization or proximity ligation assay), the develop- ment of pharmacological CaSR modulators (calcilytics and calcimimetics), and tissue-specific gene ablation studies have markedly enhanced our understanding of the role of the CaSR in the kidney. This Review will address the intrarenal distribution of the CaSR, the potential roles of this receptor in the kidney, and impli- cations of the use of pharmacological CaSR modulators on renal function.

The hypothesis that the CaSR might mediate the sensitivity of cyclic adenosine monophosphate (cAMP)-linked hormones to adenylyl cyclase type 6 (ADCY6) will also be discussed.

Renal control of extracellular Ca2+

The kidney has an important role in the homeostasis of extracellular Ca2+ levels, and urinary Ca2+ excretion is proportional to the filtered Ca2+ load. An increase in serum Ca2+ results in an increase in urinary Ca2+ excre- tion — an effect that can occur in the absence of the Ca2+-regulating hormones PTH and active vitamin D (1,25(OH)2D3). A number of studies have shown that the kidney is a calcium-sensing organ owing to its ability to sense changes in both urine and serum Ca2+ levels, and a direct role for the CaSR has been proposed in renal Ca2+ handling7. Familial hypocalciuric hypercalcaemia (FHH) is an autosomal dominant disease caused by mutations that inactivate the CASR gene and manifests as hyper- calcaemia, parathyroid hyperplasia, and inappropriately normal or even low urinary Ca2+ (REF. 8). A seminal study that preceded the molecular identification of the CaSR investigated the effects of PTH on urinary Ca2+ excre- tion in patients with FHH with hypoparathyroidism as a result of parathyroidectomy, and compared their physio- logic response following Ca2+ infusion to that elicited by hypoparathyroid patients without FHH9. Ca2+ clearance in those with FHH was lower than that recorded in those without FHH upon intravenous Ca2+ infusion, and this difference was retained in the presence of acetazolamide
— a diuretic that inhibits carbonic anhydrase in the proximal tubule, resulting in suppression of proximal tubule solute transport. Interestingly, a greater calciuric response was observed in patients with FHH compared to those without FHH upon administration of etacrynic acid, a loop diuretic. These data suggest that the thick ascending limb (TAL) of the loop of Henle is the predominant site for abnormal renal Ca2+ transport in patients with FHH9. Another study investigated the effect of acute and chronic CaSR inhibition on serum Ca2+ levels in thyroparathyroidectomized rats in the presence or absence of PTH infusion. Data showed that the CaSR directly participates in the control of extracellular Ca2+ levels via TAL-mediated regulation of paracellular Ca2+ transport Specifically, chronic CaSR inhibition increased tubular Ca2+ reabsorption and increased blood Ca2+ concentra- tion without affecting intestinal Ca2+ reabsorption or bone resorption10. Evidence also suggests that the CaSR can directly regulate Ca2+ and Mg2+ permeability via several members of the claudin family (CLDN) of tight junction proteins, including CLDN14 (REFS 11,12), CLDN16, and CLDN19 (REF. 13). In addition to the TAL, various cell types of the proximal tubule,

collecting duct, and juxtaglomer- ular apparatus also reportedly exhibit Ca2+ sensitivity14, as will be discussed in more detail below.

CaSR expression in the kidney

Determining the exact intrarenal distribution of the CaSR is crucial to our understanding of its functional role in the kidney, but the data accumulated thus far has been controversial and hindered by the lack of suitably sensi- tive methods required for detecting low levels of CaSR expression. Although CaSR expression in cells of the parathyroid glands or the TAL of the kidney is unrefuted, CaSR expression in other tubular and glomerular cells of the kidney has been a subject of debate. Inconsistent find- ings have been ascribed to differences in the model sys- tems used, methodological approach, and molecular tools employed. One study performed immunohistochemistry using commercial and custom antibodies directed against the amino terminus, carboxy-terminus and the entire CaSR protein to investigate the intrarenal distribution and physiology of the CaSR in the human, rat and mouse kid- ney15. Sensitive and specific detection methods (namely branched DNA in situ hybridization and proximity liga- tion assays) were used to determine the exact cellular localization of the CaSR mRNA and protein (TABLE 1). The study found that the CaSR is expressed across the entire length of the nephron, with the highest expression within the TAL. Expression was also found in cells of the collecting duct, but with differences in the strength of the immunoreactivity signal and in the cellular polarity (apical, basolateral, or intracellular). Intrarenal CaSR pro- tein expression was not detected in mice with targeted ablation of CaSR in the kidney tubules. Proximity ligation assays in the mouse and rat kidney confirmed the expres- sion of the CaSR within the glomerulus and the proximal tubule, which were previously two sites of major contro- versy. This expression pattern was confirmed across the three species studied, but marked variation in the overall expression levels and in the nature of the biochemical species (CaSR monomer versus dimer) was observed. These observations have helped clarify the intrarenal dis- tribution of the CaSR, which will not only enable 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.

The glomerulus

As mentioned above, expression of the CaSR in the glo- merulus has been reported with inconsistent findings16,17. The proximity ligation assay described above demon- strated

low, but above background expression in mouse, rat, and human glomeruli, in both podocytes and mesan- gial cells15. Marked differences in the expression levels were observed even within adjacent glomeruli of the same kidney, which could be ascribed to differences in the func- tional status of the cells15. These observations, together with the very low CaSR expression levels, could account for some of the discrepant reports of CaSR expression in the glomerulus.

Evidence for a role for the CaSR in the regulation of glomerular function has derived from studies in which glomerular damage was induced in mice and rats using puromycin aminonucleoside (PAN)17. Expression of the CaSR was found in cultured murine podocytes, where calcimimetics prevented PAN-induced apoptosis and cytoskeletal damage in vitro18. Administration of a calci- mimetic to rats also prevented PAN-induced proteinuria and glomerulosclerosis18. Other studies have shown that calcimimetics can decrease the progression of renal dam- age by preventing podocyte loss and interstitial fibrosis in uninephrectomized, apolipoprotein E-deficient mice19. The effects of the calcimimetic were observed in the absence of changes in serum PTH or systemic blood pres- sure, suggesting a direct involvement of the glomerular CaSR.

In cultured human mesangial cells, CaSR activa- tion leads to intracellular Ca2+ mobilization by both Gq protein-linked activation of inositol trisphosphate and extracellular Ca2+ influx via the short transient recep- tor potential channels 3 and 6 (TRPC3, TRPC6)17. Aberrant Gq protein signalling causes glomerular damage via activation of TRPC6 in murine models of PAN-induced nephrosis and diabetic nephropathy20, but whether direct CaSR activation in mesangial cells neg- atively affects glomerular function via Gq protein-linked opening of TRPC6 is unknown. Further studies are nec- essary to investigate the existence of a potential crosstalk between the CaSR and TRPC6 in mesangial cells and the impact of long term, clinical use of pharmacological CaSR modulators on glomerular function.

Juxtaglomerular apparatus

Numerous stimuli (such as a reduction in blood pressure or reduced luminal NaCl concentration) can activate cells of the juxtaglomerular apparatus to secrete renin, largely by increasing the intracellular cAMP pool21. Acute activation of the CaSR with a calcimimetic can inhibit renin secretion, as shown in vitro in cultured mouse primary juxtaglomerular cells, and in vivo22. A rise in intracellular Ca2+ generally promotes exocytosis in secretory cells, whereas an increase in extracellular Ca2+ in cells of the juxtaglomerular apparatus suppresses renin secretion via CaSR-mediated activation of Gq

protein, which results in intracellular Ca2+ mobilization — a phe- nomenon known as the ‘calcium paradox’ (REF. 23). An increase in extracellular Ca2+ also leads to suppression of the intracellular cAMP pool by activating calcium- regulated adenylyl cyclase type 5 (ADCY5), ADCY6, and phosphodiesterases24.

In contrast to what has been observed in acute set-

tings, chronic calcimimetic treatment had no effect on either baseline or stimulated plasma renin activity in rats in vivo22. If these observations were to translate to humans, they would suggest that long-term calcimimetic treatment would be unlikely to markedly affect renin plasma levels.

Proximal tubule

PTH, acting through the parathyroid hormone/ parathyroid hormone-related peptide 1 receptor25, has a crucial role in regulating inorganic phosphate (Pi) transport in the proximal tubule. The CaSR is expressed apically in the proximal tubules of the mouse, rat and human kidney7,15,26, and CaSR expression was retained in conditionally immortalized proximal tubu- lar epithelial cells obtained from the urine of a healthy individual27.

In addition to the well established systemic effects of calciotropic hormones, accumulating evidence sug- gests the existence of a renal feedback loop that inte-

grates the actions of the CaSR, PTH, and 1,25(OH) D 2-3

to provide rapid and local control of Ca2+ and Pi homeostasis (FIG. 1a). Data gathered from studies performed in isolated S3 segments of the proximal tubule suggest that activation of the CaSR can directly and rapidly blunt the phosphaturic action of PTH, thereby modu- lating the inhibitory effect of PTH on Pi absorption26. On the other hand, chronic high dietary Pi intake or acute PTH administration in vivo can suppress prox- imal tubule CaSR expression28. In turn, 1,25(OH)2D3 increases CaSR expression and serum Ca2+ levels by enhancing Ca2+ absorption from the distal convoluted tubule (DCT). In addition, 1α-hydroxylation of 25(OH) D3 into 1,25(OH)2D3 was inhibited by high Ca2+ levels in cultured HKC-8 human-derived renal proximal tubular cells29 and studies performed in mice lacking both the CaSR and Pth genes showed that the CaSR mitigates the calcaemic response to 1,25(OH)2D3 in vivo30. As these studies were carried out in the context of Pth gene deficiency, it was concluded that the exaggerated cal- caemic response to 1,25(OH)2D3 in CaSR−/−;Pth−/−mice could only be explained by direct, PTH-independent effects of the CaSR on the kidney30.

Pharmacologic (calcimimetic) and genetic (CaSR- knockout mice) studies have

demonstrated that acti- vation of the CaSR by Ca2+ or by calcimimetics leads to increased luminal acidification31. These data were demonstrated by varying the luminal Ca2+ concentration in vivo following micropuncture of the mouse proximal tubule as well as in vitro in isolated and cultured mouse proximal tubules that were perfused with various luminal Ca2+ concentrations31.

The proximal tubule is responsible for ~65% of fluid reabsorption via transcellular and paracellular routes. The sodium–hydrogen exchanger 3 (SLC9A3) is crucial for fluid reabsorption and proton secretion. Activation of SLC9A3 leads to interstitial Na+ accumulation, with a local increase in its osmolarity. This process drives paracellular water reabsorption, as well as reabsorption of solutes (including Ca2+) in a process known as sol- vent drag. CaSR activation in the proximal tubule drives Na+ transport, which is likely to occur by activation of SLC9A3; this effect would result in an increase in fluid and solute reabsorption. PTH also induces natriure- sis and diuresis by promoting the internalization of SLC9A3 and the sodium-dependent phosphate trans- port protein 2A (SLC34A1), via cAMP–protein kinase A (PKA) stimulation32. Activation of the apical CaSR could counteract these actions, which would result in increased Ca2+ and fluid reabsorption. These obser- vations suggest that in the proximal tubule, the CaSR can directly fine tune the actions of PTH, and thereby directly control Ca2+ and Pi reabsorption — an effect that is independent of systemic changes in calciotropic hormones (FIG. 1a). These actions of the CaSR can be over-ridden by systemic changes in serum PTH or Pi, which selectively downregulate CaSR expression in the proximal tubule28.

Thick ascending limb

The TAL is responsible for the reabsorption of ~25% of filtered Ca2+, largely by the cortical TAL (CTAL) but also to some extent by the medullary TAL; the reabsorption of Ca2+ is coupled with the reabsorption of NaCl33. PTH stimulates renal tubular Ca2+ reabsorption in the TAL by increasing transcellular and paracellular Ca2+ per- meability10,34. In the CTAL, an increase in peritubular Ca2+ suppresses a PTH-induced increase in intracellular cAMP35. CaSR activation in the CTAL also decreases hormone-stimulated (calcitonin, glucagon, vasopressin) intracellular cAMP accumulation by inhibiting ADCY6 (REF. 36). Paracellular Ca2+ permeability is reliant upon the uptake of NaCl across the luminal membrane of this segment, which occurs via the solute carrier family 12 member 1 (SLC12A1) — a bumetanide-sensitive, kid- ney specific Na-K-Cl symporter. K+ ions that are trans- ported into the cell by SLC12A1 are recycled back into the lumen through the apical

70-pS K+ channel, of which the renal outer medulla K+ (ROMK) channel is a com- ponent. Intracellular Na2+ and Cl− are extruded basolat- erally via the Na-K-ATPase and voltage-gated chloride channel protein (CLCNKB)37. Together, these processes establish a positive transepithelial potential difference, which provides the driving force for passive reabsorption of monovalent and divalent cations (Na+, Ca2+, Mg2+) via the paracellular route.

The 70-pS K+ channel is formed of ROMK2 (a 30-pS channel) and other subunits that remain to be defined38. An increase in peritubular Ca2+ or Mg2+ activates the baso- lateral CaSR, which has been shown in microdissected TALs from the rat to directly inhibit the 70-pS apical K+ channel via cytochrome p450 and cause an increase in 20-hydroxyeicosatetraenoic acid production39 (FIG. 1b). Whether CaSR activation also leads to inhibition of api- cal K+ entry via ROMK2 is currently unknown. Because K+ recycling is the rate-limiting step for SLC12A1 activity, such an effect would be expected to result in an abrogation of the driving force for paracellular cation reabsorption. In addition, CaSR activation in isolated rat kidney TALs inhibits the basolateral 50-pS ATP-sensitive inward rec- tifier K+ channel 10 (KCNJ10), which results in inhibi- tion of basolateral K+ recycling and transepithelial cation transport40. Evidence that such a mechanism occurs in vivo is, however, currently lacking.

CaSR regulation of paracellular permeability.

Direct effects of the CaSR on paracellular permeability have been postulated as calcimimetics and calcilytics affect PTH-stimulated Ca2+ reabsorption in the absence of changes in the transepithelial membrane potential41. Hypercalcaemia-mediated polyuria (that occurs dur- ing nephrogenic diabetes insipidus) mimics the effects caused by loop diuretics that target SLC12A1, and affected patients exhibit a Bartter-like phenotype with hypokalaemia, hyper-reninaemia with secondary hyper- aldosteronism, and metabolic alkalosis42. Indeed, type 5 Bartter syndrome can also be present in a subset of indi- viduals with autosomal dominant hypocalcaemia with hypercalciuria caused by activating CaSR mutations43. This condition shares many of the phenotypic hallmarks of Bartter syndrome types 1 and 2, which are caused by mutations in the genes encoding SLC12A1 and ROMK, respectively44,45 (FIG. 1b).

Tissue-specific gene ablation and pharmacological studies, as well as observations in patients carrying activating or inactivating mutations in the CaSR gene, have unanimously demonstrated a primary role for the CaSR renal in the control of urinary Ca2+ excretion41. Paracellular permeability in the TAL is controlled by CLDN14, CLDN16, and CLDN19 — all

of which have been associated with hypercalciuria, nephrolithiasis, and reduced bone mineral density in humans11–13. CLDN16 and CLDN19 heterodimerize to form a CLDN14-gated pore, through which divalent cations can be reabsorbed. CLDN14 expression increases with high dietary Ca2+ intake or calcimimetic treatment in vivo, which results in decreased paracellular divalent cation permeabil- ity46. Studies have demonstrated that CLDN14 is acti- vated via a calcineurin–nuclear factor of activated T cell (NFATc1)–microRNA (miR)-based transcriptional mechanism, as well as through chromatin remodelling. These events occur downstream of CaSR activation in the TAL. The CaSR controls the transcription of miR-9 and miR-374, which target the 3′-untranslated region of CLDN14 (REF. 13). Ciclosporin, a calcineurin inhibitor, prevents CaSR-dependent regulation of CLDN14 and urinary Ca2+ excretion13 (FIG. 1b). Physiologically, the CaSR–CLDN14 axis in the TAL prevents Ca2+ overload in the presence of elevated serum Ca2+ levels, but distur- bances in this process could contribute to pathological hypercalciuria and nephrocalcinosis, as will be described in more detail below. Furthermore, analysis of a CaSR- specific deletion in the entire murine kidney tubule found that loss of renal CaSR expression results in hypo- calciuria that is not accompanied by notable changes in serum and urinary Mg2+ excretion47. These knockout mice exhibited normal serum Ca2+ and PTH levels, indicating that the predominant role of the renal CaSR is to inhibit Ca2+ reabsorption in a PTH-independent fashion. This effect is accounted for, at least in part, by increased contribution of the paracellular pathway, as evidenced by concomitant downregulation of CLDN14 and activation of SLC12A1 (REF. 47). Calcimimetic drugs would be expected to increase urinary Ca2+ output via PTH-dependent and PTH-independent (renal CaSR-mediated) actions, and could potentially increase the risk of Ca2+ stone formation. While it is known that a single dose of a calcimimetic can increase urinary Ca2+ excre- tion in renal transplant patients with secondary hyper- parathyroidism48 and that clinical use of calcimimetics does not seem to elicit polyuria or renal Na+ wasting, the effect of calcimimetics or calcilytics on nephrolithiasis remains to be investigated.

Distal convoluted tubule

The DCT is a short segment of the nephron that has a key role in fine-tuning Ca2+ and Mg2+ reabsorption. Apical Ca2+ reabsorption occurs through the transient receptor potential cation channel subfamily V mem- ber 5 (TRPV5), and is transported across the basolateral membrane by the plasma membrane Ca2+-transporting ATPase, ATP2B2, and the sodium–calcium exchanger 1, SLC8A1 (REFS 49,50). In the DCT, PTH stimulates trans-

cellular Ca2+ reabsorption by cAMP-mediated activation of TRPV5 (REF. 49) (FIG. 1c). Studies have reported that the CaSR is expressed on the basolateral membrane and api- cal surface of the DCT, as well as in vesicles that express TRPV5 (REFS 7,15,51–53). Interestingly, activation of the CaSR in HEK-293 human embryonic kidney cells stably transfected with human CaSR increases Ca2+ reabsorp- tion via TRPV5 (REF. 51). As Na+ and Ca2+ reabsorption are closely associated with each other in the TAL, a natriu- retic stimulus would be expected to increase Ca2+ delivery to the DCT. The functional interaction between TRPV5 and the CaSR would faciliate removal of Ca2+ from the pre-urine without affecting Na+ reabsorption, thus min- imizing renal calcium wasting. However, this concerted interplay is unlikely to prevent the hypercalciuric effect of loop diuretics, or the effect of activating CaSR muta- tions. Moreover, the CaSR has been shown to interact with the K+ channel KCNJ10 that is expressed on the basolateral membrane of the DCT; this interaction causes inactivation of KCNJ10, which in turn could reduce K+ recycling through the basolateral Na–K-ATPase thereby contributing to the inhibition of Na+ reabsorption in this segment52 (FIG. 1c).

Collecting duct

CaSR expression is found along the entire length of the collecting duct, from the cortex to the medulla14,15. The CaSR is expressed in intercalated cells and princi- pal cells of the cortical collecting duct14,15 (FIG. 1d). The CaSR exhibits a predominant apical distribution in the intercalated cells and it has been suggested from studies performed in dissected mouse outer medullary collecting ducts that high activation of the CaSR due to high extracellular Ca2+ promotes urine acidification through stimulation of the luminal H+- ATPase54. Data suggest that formation of alkaline urine predisposes to urolithiasis55,56. Of note, Trpv5−/− mice are hypercalciuric and polyuric with increased urinary acid excretion and therefore, do not exhibit renal calcification because of this marked urine acidification54. When acidification is prevented by targeted gene ablation of the collect- ing duct-specific B1 subunit of the renal H+–ATPase in Trpv5−/− mice, notable tubular precipitation of cal- cium phosphate crystals occurs and animals die prema- turely of hydronephrosis, likely due to the presence of renal stones54.

The CaSR is localized to the apical membrane of the principal cells and is colocalized with the aquaporin 2 (AQP2) water channel in intracellular vesicles14,57,58. Several in vitro, in vivo, and human observational studies have suggested that CaSR signalling inhibits vasopressin-induced trafficking and expression of AQP2 (REFS 59–67). A postulated

mechanism for this process is that the actions of vasopressin to promote water reabsorption from the lumen causes an increase in urinary Ca2+ concentration as a consequence of urine concentration, which in turn activates the CaSR located on the apical membrane of the principal cells. The activation of the CaSR reduces the vasopressin-stimulated insertion of AQP2 into the plasma membrane and the rate of water reabsorption, leading to the formation of dilute urine, and conse- quently a reduced risk of Ca2+ supersaturation62,65,68. Studies performed in cultured renal cells and micro- dissected collecting ducts have demonstrated that the inhibitory effect of CaSR signalling on AQP2 traffick- ing to the plasma membrane is mainly due to a strong reduction in cAMP-induced AQP2 phosphorylation at serine 256 and AQP2 trafficking, resulting in blunt- ing of the osmotic water-permeability response66. Although evidence is building for the effects of extra- cellular Ca2+ or calcimimetics (either luminal or baso- lateral) on renal physiology (TABLE 2), further studies are necessary to determine the relevance of the inter- play between the CaSR and AQP2 in humans and the possibility of using CaSR modulators to rectify severe hypercalciuria and nephrocalcinosis69.

Ca2+-mediated inhibition of cAMP

CaSR and ADCY6 co-expression

Evidence that the CaSR and ADCY6 are co-expressed in the same cells in defined nephron segments has led to the hypothesis of a functional interplay between CaSR sig- nalling and inhibition of intracellular cAMP content in response to an increase in extracellular Ca2+. Specifically, functional expression of ADCY6 has been demonstrated in juxtaglomerular cells20, the proximal tubule70, TAL, and collecting duct71. As previously discussed, antagonizing the effects of PTH in the kidney results in CaSR signalling that affects Ca2+ and Pi metabolism and regulates tubu- lar transport of Mg2+, Na+, K+, Cl−, and water excretion41. These effects are modulated by distinct hormones that act on selected tubular segments where the CaSR is expressed. Previous observations in renal epithelial cells demon- strated that CaSR activation (as a result of an increase in extracellular Ca2+), and modulation of its function by pH and nutrients, results in adjustment of intracellular cAMP levels36,66,72; these data suggest that the CaSR converts an extracellular Ca2+ stimulus into a negative feedback signal on Gs protein-linked hormones that act via cAMP. Given the co-expression of the CaSR and ADCY6 in the same cells of defined nephron segments, it can be speculated that the negative feedback of CaSR signalling on hormones act- ing

through the Gs–ADCY6–cAMP pathway confers high sensitivity of hormone effects to changes in Ca2+.

Co-expression in the juxtaglomerular apparatus.

Activation of the CaSR in renal juxtaglomerular cells stimulates intracellular Ca2+-mediated decreases in cAMP content and inhibition of renin release through activa- tion of ADCY5 and ADCY6 (REFS 24,73). In the proxi- mal tubule, PTH increases Pi excretion by stimulating the internalization of SLC34A1 and solute carrier family 23 member 3 (SLC23A3) Na+-dependent Pi transport- ers from the apical plasma membrane via activation of ADCY6 through PTH type 1 receptors associated with the cAMP–PKA signalling pathway70. Evidence that ADCY6 is the adenylyl cyclase isoform that mediates this effect is based on data derived from Adcy6−/− mice, which dis- played Pi wasting and no response to PTH in terms of Pi excretion and urinary cAMP excretion70. These data sug- gest that in the proximal tubule, ADCY6 modulates acute PTH-stimulated cAMP formation and urinary Pi excre- tion. In this scenario, CaSR in the proximal tubule could represent a major regulator of PTH action by antago- nizing its effects on Pi excretion through downregulation of the Gs–ADCY6–cAMP pathway (FIGS 1a,2).

Co-expression in the TAL.

As discussed, the main function of the CaSR in the TAL is the regulation of urinary Ca2+ excretion, which has a crucial effect on Ca2+ balance. Here, SLC12A1 and ROMK work in concert to establish lumen-positive transepithelial potential differences that enable Ca2+ reabsorption via the paracellular pathway14. The trafficking and activity of SLC12A1 is positively reg- ulated by phosphorylation at serine 126 under vasopressin action74,75. Studies performed in Adcy6−/− mice identified ADCY6 as the isoform that mediates the vasopressin- induced phosphorylation of SLC12A1 at serine 126, which is not phosphorylated in knockout mice71.

Other studies performed in microdissected CTAL have shown that the CaSR and ADCY6 are co-expressed within the same cells and that activation of the CaSR by extracellular Ca2+ induces a dose-dependent inhibition of intracellular cAMP content that results from both stimulation of cAMP hydrolysis and inhibition of cAMP syn- thesis36. This effect on intracellular cAMP associated with CaSR signalling in the TAL indicates that CaSR activation impairs vasopressin action in this region, which results in changes in the transepithelial potential due to changes in ion transport as well as regulation of the

expression of proteins that compose the tight junctions, including clau- dins. The CaSR would be expected to counteract PTH- induced Ca2+ reabsorption in the TAL through a similar signal transduction pathway. Antagonizing the action of vasopressin and PTH through impairment of the Gs–ADCY6–cAMP pathway results in a reduction in Ca2+ reabsorption and an increase in Ca2+ excretion (FIGS 1b,2).

Co-expression in the collecting duct.

Although a minimal amount of Ca2+ is transported in this segment, the extent of water reabsorption and urine acidification can profoundly modify Ca2+ solubility and, therefore, can increase the risk of Ca2+ stone formation. Both ADCY3 and ADCY6 are expressed in collecting duct principal cells and have both been suggested to con- tribute to the overall rise in cAMP during vasopressin stimulation76; however, several lines of evidence suggest that ADCY6 is the predominant enzyme that modulates vasopressin-regulated water reabsorption77–79. Collecting duct-specific knockout of Adcy6 in mice results in a uri- nary concentration defect associated with reduced vaso- pressin-stimulated cAMP accumulation80; therefore, Ca2+ sensing in the collecting duct by the CaSR might sensi- tize vasopressin to luminal Ca2+, which would result in an attenuation of the antidiuretic response to limit water reabsorption (FIGS 1d,2). In line with this view, data from cultured renal cells and microdissected mouse collecting ducts showed that the negative feedback of CaSR signal- ling on vasopressin action is primarily due to a reduction in the hormone-dependent generation of cAMP and possibly a reduction in cAMP hydrolysis66.

Overall, the emerging concept is that within the nephron, co-expression of CaSR and Ca2+-inhibitable ADCY6 elicits a negative feedback on the hormones that act through the Gs–ADCY6–cAMP pathway to confer high sensitivity of hormone effects to the extracellular Ca2+ concentration. This negative feedback mainly relies on inhibition of intracellular cAMP levels as a result of CaSR activation by extracellular Ca2+ and consequent inhibition of cAMP synthesis by ADCY6 (FIG. 2).

CaSR and nephrolithiasis

The CaSR in the TAL inhibits paracellular Ca2+ reabsorp- tion by activating CLDN14 transcription, which blocks the paracellular cation channel that is composed of CLDN16 and CLDN19 and is crucial for Ca2+ and Mg2+ reabsorption11. The CaSR also decreases the activity of SLC12A1, which is usually required for sustaining the electrochemical gradient and drives paracellular divalent cation reabsorption46. CaSR activation by serum Ca2+ in

the TAL, therefore, promotes calciuria. Conversely, acti- vation of the CaSR in the proximal tubule antagonizes the phosphaturic action of PTH and promotes proton secretion through SLC9A326,31, whereas in the collect- ing duct, CaSR activation decreases water reabsorption by inhibiting the tubular response to vasopressin and increases proton excretion by stimulating proton pump activity54,66. CaSR activity in the proximal tubule and collecting duct can, therefore, counterbalance the risk of calcium phosphate precipitation that is associated with the ability of the CaSR to enhance Ca2+ excretion in the TAL.

Based on these described findings, both the CaSR and CLDN14 have been investigated as two impor- tant loci for kidney stone disease. Allelic variants in both the CaSR and CLDN14 have a known associa- tion with nephrolithiasis81–86, but data obtained by several groups have produced contrasting results. In seven French families with familial calcium nephro- lithiasis87, and in a large group of 359 Canadian sibling pairs88, no evidence was found that linked mutations in the CaSR to an increased risk of renal stones. Other researchers, however, have shown that single- nucleotide polymorphisms (SNPs) in the CaSR gene are associated with nephrolithiasis and kidney stones. An association between the CaSR Arg990Gly poly- morphism and primary hypercalciuria was found to be more frequent in hypercalciuric patients that formed calcium stones compared to normocalciuric patients who formed stones or healthy individuals89. Subsequent in vitro data indicated that the Arg990Gly polymor- phism results in a gain-of-function of the CaSR89.

Other investigators, however, have found no associa- tion between this polymorphism and indices of serum Ca2+ homeostasis90.

Calcium nephrolithiasis was also found to be associ- ated with the rs6776158 SNP that is located within the first promoter region of the CaSR gene91. In vitro studies showed that the minor G allele at rs67780158 caused a decrease in the transcriptional efficiency of the first pro- moter in two renal cell lines91. Moreover, data indicate that the simultaneous presence of both rs1501899 and the Arg990Gly polymorphism might potentiate the risk of forming kidney stones in patients with primary hyperpar- athyroidism, despite their apparently opposite effects on CaSR function in the kidney92. Carriers of the Arg990Gly polymorphism exhibit increased Ca2+ excretion, which is expected to predispose to intratubular precipitation of calcium phosphate crystals89. Conversely, the rs6776158 SNP is associated with decreased CaSR expression and might, therefore, improve tubular efficiency to reabsorb Ca2+. This effect could lead to an increase in Ca2+ concen- tration in the interstitium, which would increase the risk of hydroxyapatite precipitation in the Randall plaque fol- lowed by precipitation of urinary calcium oxalate and for- mation of calcium-

oxalate stones. Deficient expression of the CaSR might also impair CaSR-associated urine acidifi- cation and dilution in the collecting duct, and promote Pi retention in the proximal tubule. This effect would expose patients to the risk of calcium phosphate precipitation in the tubular fluid. More recently, a genome-wide associa- tion study of kidney stone disease identified an interest- ing association with rs762468[A] in the first intron of the CaSR gene93. Based on these data, CaSR polymorphisms should be considered to be risk factors that predispose individuals to form kidney stones and might be provide potential genetic markers to identify patients at risk of developing calcium nephrolithiasis.

Claudins are a key component of the paracellular pathway. Studies investigating the physiological func- tion of claudins have highlighted a functional interaction between CaSR signalling and claudin gene expression, suggesting a role for claudins in nephrolithiasis. Defects in claudin function have been implicated in patients with hypercalciuria and nephrolithiasis85. In the TAL, the CLDN16 channel provides cation permeability in the tight junction that is essential for Ca2+ and Mg2+ reabsorption driven by a lumen-positive transepithelial potential gradient85. Conversely, CLDN14 blocks the para- cellular cation channel that is composed of both CLDN16 and CLDN19 (REF. 11). The identification that stimulation of CaSR signalling in the TAL downregulates miR-9 and miR-374 to cause an increase in CLDN14 levels, which in turn suppresses the permeability of the CLDN16 and CLDN19 channels and promotes Ca2+ excretion, strongly supports a role for claudins in nephrolithiasis85.

Conclusions

Numerous studies have shown that the CaSR is expressed along all segments of the nephron, from the glomerulus to the inner medullary collecting ducts.

Interestingly, notable differences in the level of CaSR expression along the nephron have been identified, with the highest expression in the TAL and the lowest in the glomerulus and proximal tubule. Accordingly, direct roles for the CaSR in the kidney have been proposed in these regions and seem to correlate with the expression levels of this receptor. In the TAL, the CaSR is a major determinant of urinary Ca2+ excre- tion. In other regions the CaSR acts to fine-tune and integrate multiple stimuli that derive from Na+, pH and mineral ion metabolism, thus setting the sensi- tivity threshold for several cAMP-coupled hormones to extracellular Ca2+.

The CaSR can be the target for therapeutic interven- tion for a number of inherited and acquired conditions, whereby the level of expression and/or function of the CaSR is altered (BOX 1). Calcimimetics are effective in treating primary and secondary hyperparathyroidism. Calcilytics might be used in the future to treat hypopar- athyroidism and, in hypercalciuric patients that form calcium stones, to prevent nephrolithiasis and nephro- calcinosis. Evidence that clinical use of calcimimetics is not associated with either increased urine output or acidification is somewhat unexpected and remains to be fully understood. Immunolocalization studies of the renal CaSR have demonstrated that species differences cannot account for the discrepancies between observations made in humans with genetic CaSR mutations and their murine orthologues. Thus, while the direct contribution of the renal CaSR in preventing hypercalcaemia is undisputed, the precise role of the CaSR in Na2+ and urine outputs and urine acidification needs to be investigated further to discriminate between the CaSR-dependent and CaSR- independent effects of extracellular Ca2+. Finally, the effect of chronic calcimimetic and calcilytic treatment on neph- rolithiasis and nephrocalcinosis remains to be assessed. The next challenge will be to identify molecules that spe- cifically target the renal CaSR. Availability of these com- pounds will help with the development of CaSR-based therapeutics for treating hypercalciuric disorders and will provide a better understanding of the mechanisms that underpin abnormal Ca2+ handling by the kidney.

Figure legends

Figure 1 Localization of the calcium-sensing receptor (CaSR) in the kidney tubule. a | CaSR expression and function in the proximal tubule. The CaSR blunts the phosphaturic action of parathyroid hormone (PTH), inhibits the activity of CYP27B1 (25-hydroxyvitamin D3 1α-hydroxylase), and promotes acidification via the sodium– hydrogen exchanger SLC9A3, which in turns drives Na+ accumulation in the interstitium and solute reabsorption. b | CaSR in the thick ascending limb (TAL). Hypercalcaemia activates the CaSR in the TAL, which leads to inhibition of the renal outer medulla K+ (ROMK) channel, thereby preventing apical K+ recycling — the rate-limiting step for
Na-K-Cl cotransporter (SLC12A1) activity. This effect leads to a loss of the driving force for paracellular cation reabsorption. Thus, hypercalcaemia mimics the effects of loop diuretics and recapitulates Bartter syndrome types 1 and 2 phenotypes, ascribed to mutations in the genes encoding SLC12A1 and ROMK, respectively. This effect is also mimicked by activating mutations in the CaSR gene that causes type 5 Bartter syndrome with hypercalciuria, hypokalaemic alkalosis and hyper-reninaemic hyperaldosteronism. Bartter syndrome types 3 and 4 are caused by mutations in the genes encoding the basolateral Cl− channel, CLCKB, or its auxiliary subunit, Barttin, respectively. CaSR activation also directly regulates paracellular permeability via NFATc1–miRNA–CLDN14 signalling. c | CaSR in the distal convoluted tubule (DCT). At the apical membrane, the CaSR is biochemically associated with the 1,25(OH)2D3-regulated transient receptor potential cation channel subfamily V member 5 (TRPV5). Increased Ca2+ delivery to the DCT results in CaSR activation and increased apical Ca2+ entry via TRPV5. Intracellularly, calbindin–D28K (CALB1) delivers Ca2+ to the basolateral membrane where Ca2+ exits the cell via active mechanisms (ATP2B1 and SLC8A1). The CaSR also decreases the surface expression of the K+ channel KCNJ10, which results in inhibition of NaCl2 reabsorption.
d | CaSR in the collecting duct. In the presence of an antidiuretic stimulus, Ca2+ concentrations in the pre-urine can become super-saturating, potentially leading to Ca2+ stone formation. When the luminal Ca2+ concentration becomes critically high it activates the apical CaSR which, in principal cells, blunts vasopressin-mediated apical insertion of the aquaporin 2 (AQP2) water channel and the rate of water reabsorption. In intercalated cells, CaSR activation leads to luminal acidification. Overall, these two effects result in the production of a dilute, acidified urine, which reduces the risk of nephrolithiasis. 20-HETE, 20-hydroxyeicosatetraenoic acid; ATP2B1, plasma membrane calcium-transporting ATPase

1; AQP, aquaporin; AVP, arginine vasopressin; Cld, claudin; miRNA, microRNA; NFAT, nuclear factor of activated T cells; SLC8A1, sodium–calcium exchanger 1; SLC12A3, solute carrier family 12 member 3; PTH1R, parathyroid hormone 1 receptor; SLC4A4, sodium bicarbonate cotransporter 1.

Figure 2

The calcium-sensing receptor (CaSR) confers high sensitivity to extracellular Ca2+ concentration by reducing the effects of distinct hormones that selectively act in selected nephron segments via the Gs-coupled receptor–ADCY6–cAMP signalling pathway. The CaSR is expressed in renal juxtaglomerular cells and its activation stimulates Ca2+-mediated decreases in cAMP content and inhibits renin release through activation of adenylyl cyclase type 5 (ADCY5) and type 6 (ADCY6)24,73. In the proximal tubule, the CaSR is expressed on the luminal membrane and its activation impairs the action of parathyroid hormone (PTH) by antagonizing its effects on Pi excretion through downregulation of the Gs–ADCY6–cAMP pathway70. This effect reduces Pi excretion and thus limits the risk of formation of calcium–phosphate precipitates. In the thick ascending limb (TAL), CaSR signalling counteracts the action of vasopressin on activation of Na–K–Cl cotransporter (SLC12A1) phosphorylation, causing changes in transepithelial potential resulting in overall reduced Ca2+ reabsorption. In this segment, ADCY6 is activated by vasopressin71. Using a similar signal transduction pathway, the CaSR is expected to counteract PTH-induced Ca2+ reabsorption in the TAL. In the distal convoluted tubule CaSR might antagonize PTH-induced activation of Ca2+ reabsorption by TRPV5 via a cAMP–PKA cascade49. In the collecting duct ADCY6 is activated by vasopressin77,80. Stimulation of the CaSR by luminal Ca2+

reduces the vasopressin-stimulated insertion of aquaporin 2 (AQP2), which leads to the formation of dilute urine and favours the solubility of calcium–phosphate salts66. The negative feedback of the CaSR on hormones acting through the Gs–ADCY6–cAMP pathway is principally caused by the inhibition of cAMP synthesis by the Ca2+-inhibitable ADCY6 as a consequence of a CaSR-activated increase in intracellular Ca2+ concentration. Gs, Gs α subunit; TRPV5, transient receptor potential cation channel subfamily V member 5.

Key points

- A pivotal role for the renal calcium-sensing receptor (CaSR) in the control of divalent cation excretion in a parathyroid hormone-independent manner has been identified through tissue-specific CaSR ablation and pharmacological studies
- A functional interaction between the CaSR and Claudin-14 in the thick ascending limb permits regulation of paracellular Ca^{2+} reabsorption
- The CaSR fine tunes Ca^{2+}, Mg^{2+}, and Pi transport in the proximal tubule by integrating multiple inputs from divalent cation concentration, osmolarity, and urine acidification
- Calcimimetics would be expected to increase urinary Ca^{2+} 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
- The CaSR and adenylyl cyclase type 6 are co-expressed in the nephron and act to sensitize hormones to extracellular Ca^{2+} and counteract hormone-induced increases in cAMP

Box 1 | **Allosteric modulators of the calcium-sensing receptor (CaSR)**

Calcimimetics

Calcimimetics enhance CaSR sensitivity to extracellular Ca^{2+} and reduce serum parathyroid hormone (PTH) levels by activating the CaSR that is expressed in the parathyroid gland and the kidney. Two types 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 that has been approved for the treatment of advanced secondary hyper- parathyroidism, a common complication of end-stage renal disease. A phase III trial aimed at evaluating the novel calcimimetic peptide, AMG416 for the treatment of secondary hyperparathyroidism in patients with chronic kidney disease undergoing haemodialysis met its primary and secondary end points.

Calcilytics

Calcilytics are allosteric antagonists of the CaSR that evoke a rightward shift in the Ca^{2+} concentration-response curve at the CaSR, such that higher than normal Ca^{2+} levels are required to suppress PTH release. Owing to their ability to evoke oscillating fluctuations in serum PTH (a known bone anabolic stimulus), calcilytics such as ronacaleret[94], and

JTT-305 (also known as encaleret)[95] were initially developed to treat age-related osteoporosis. Although their development has been halted due to lack of efficacy, they could be repurposed to treat hypercalciuric disorders caused by an overactive CaSR.

Adverse effects

The CaSR is expressed in numerous tissues, and therefore, 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 might increase urinary calcium excretion and the risk of renal calcium deposits and could alter renal graft function. Conversely, calcilytics are expected to reduce urinary calcium excretion, and might be used to treat gain-of-function variants of the CaSR associated with hypercalciuria and stone diseases. However, an undesired effect would be the stimulation of PTH release, which might worsen hypercalciuria.

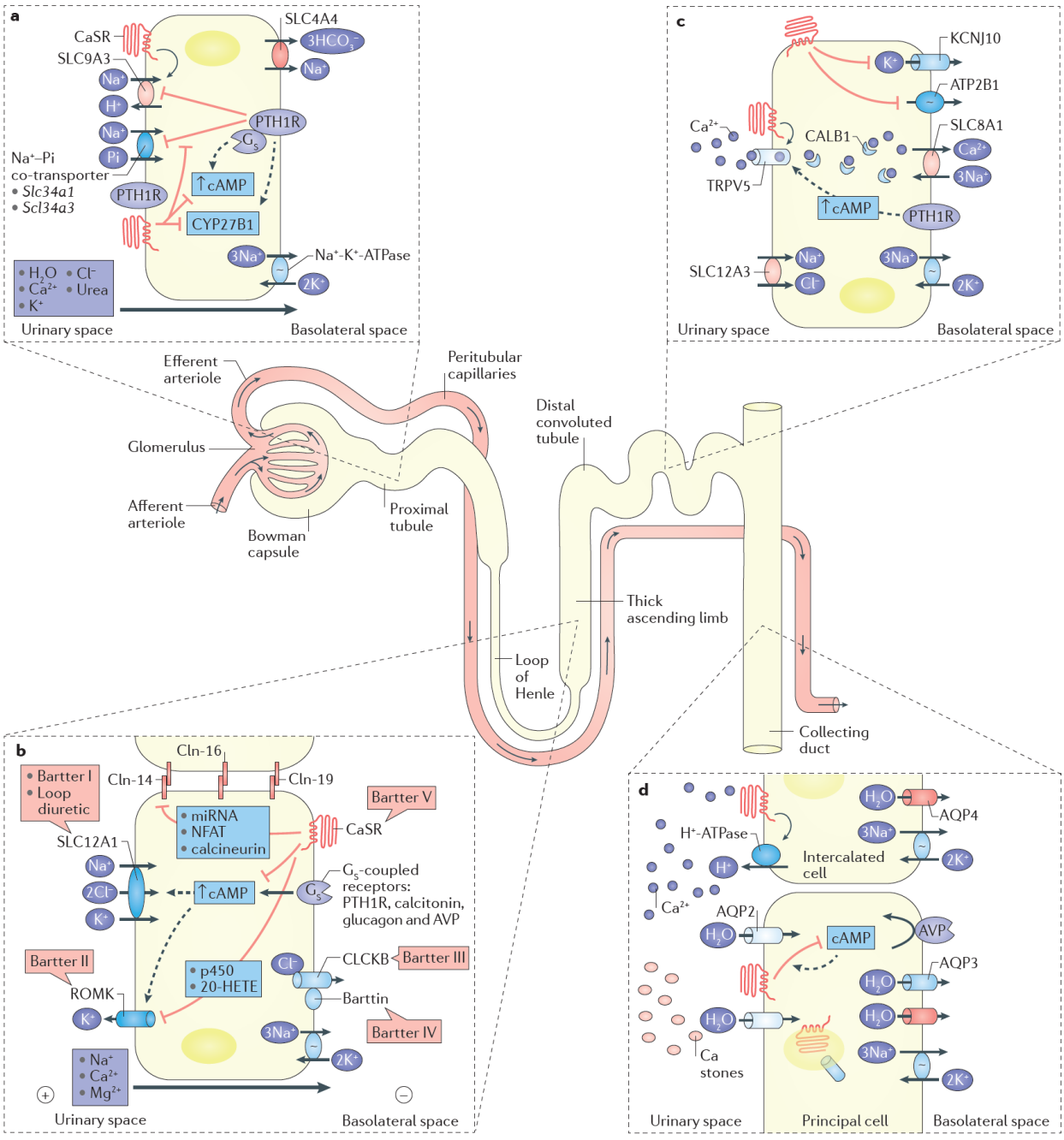
a
CaSR
SLC9A3
SLC4A4
3HCO3−
Na+
H+
PTH1R
Gs
Na+–Pi co-transporter
• Slc34a1
• Scl34a3
Pi
↑cAMP
CYP27B1
3Na+
Na+-K+-ATPase
2K+
• H2O • Cl−
• Ca2+ • Urea
• K+
Urinary space
Basolateral space
c
KCNJ10
K+
ATP2B1
Ca2+
CALB1
SLC8A1
TRPV5
↑cAMP
PTH1R
SLC12A3
Cl−
Urinary space
Basolateral space
Efferent arteriole
Peritubular capillaries
Distal convoluted tubule
Glomerulus
Afferent arteriole
Bowman capsule
Proximal tubule
Thick ascending limb
Loop of Henle
Collecting duct
b
Cln-16
Cln-14
Cln-19
• Bartter I
• Loop diuretic
Bartter V
SLC12A1
• miRNA
• NFAT
• calcineurin
CaSR
2Cl−
K+
↑cAMP
Gs-coupled receptors: PTH1R, calcitonin, glucagon and AVP
Bartter II
ROMK
• p450
• 20-HETE
Cl−
CLCKB
Bartter III
Barttin
Bartter IV
• Na+
• Ca2+
• Mg2+
Urinary space
Basolateral space
d
H+-ATPase
H2O
AQP4
3Na+
2K+
Intercalated cell
Ca2+
AQP2
cAMP
AVP
AQP3
Ca stones
Urinary space
Principal cell
Basolateral space

FIGURE 1

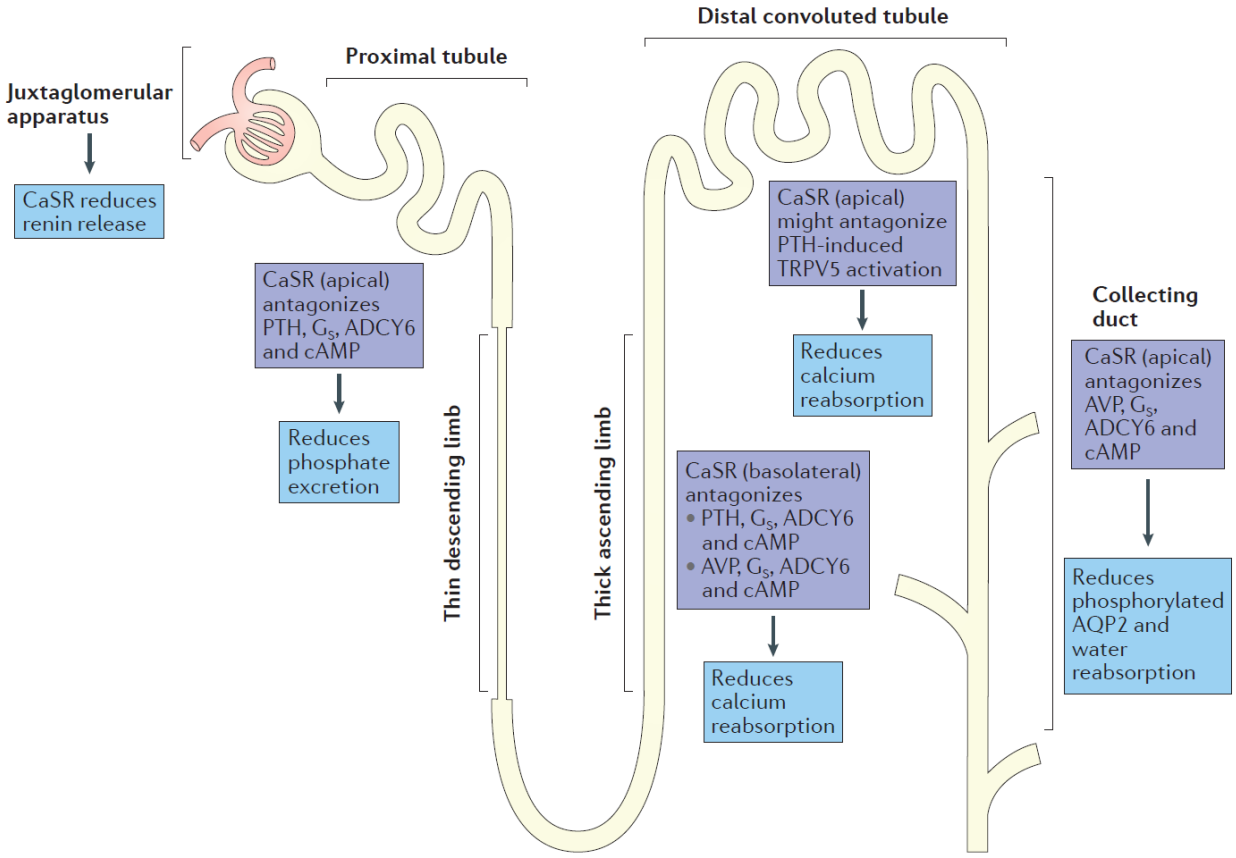
Distal convoluted tubule
Proximal tubule
Juxtaglomerular apparatus
CaSR reduces renin release
CaSR (apical) antagonizes PTH, G_s, ADCY6 and cAMP
Reduces phosphate excretion
Thin descending limb
Thick ascending limb
CaSR (apical) might antagonize PTH-induced TRPV5 activation
Reduces calcium reabsorption
CaSR (basolateral) antagonizes
• PTH, G_s, ADCY6 and cAMP
• AVP, G_s, ADCY6 and cAMP
Reduces calcium reabsorption
Collecting duct
CaSR (apical) antagonizes AVP, G_s, ADCY6 and cAMP
Reduces phosphorylated AQP2 and water reabsorption

FIGURE 2

Table 1 | **CaSR mRNA and protein expression levels along the nephron[15]**

Region	CaSR mRNA expression	CaSR protein expression
Glomerulus	—	+
Proximal tubule	+	+
Thick ascending limb	+++	+++
Distal convoluted tubule	++	++
Connecting tubule	+	+
Cortical collecting duct	++	++
Outer medullary and inner medullary collecting ducts	+	+

The highest mRNA and protein expression levels of the CaSR are found in the thick ascending limb, whereas weak protein expression is found in the glomerulus. –, negative/weak expression; +, mild expression; ++, moderate expression; +++, strong expression.

Table 2 | **Reported effects of CaSR activators (calcimimetics) and inhibitors (calcilytics) on renal physiopathology**

Nephron segment expressing CaSR	Experimental model(s) to test CaSR function	Modulated function(s)	Evidence for specific CaSR involvement*	Refs
Glomerulus	• Cultured podocytes • Toxin-induced glomerulosclerosis in rats • Apolipoprotein E-deficient mice	• Regulation of glomerular function • Possible role in preventing glomerulosclerosis	Type II calcimimetic (R-568)	18,19
Juxtaglomerular apparatus	• Primary cultures of mouse juxtaglomerular cells • *In vivo* experiments in rats	Inhibition of renin secretion	Type I calcimimetic (extracellular Ca^{2+})	22
Proximal tubule (apical)	• Microdissected mouse S3 segments • Proximal tubule-like opossum kidney cells	Inhibition of PTH-dependent phosphate uptake	Type I calcimimetics (Gd^{3+}/ extracellular Ca^{2+})	26
	• *In vivo* micropuncture in rats • *In vitro* perfused mouse proximal tubules • Proximal tubules isolated from CaSR-knockout mice	• Increase in luminal acidification • Increase in fluid reabsorption	• Type II calcimimetic (cinacalcet) • Type I calcimimetic (luminal Ca^{2+})	31
Thick ascending limb (basolateral)	• Dissected mouse TAL • Dissected rat TAL	• Inhibition of PTH, vasopressin, calcitonin, and glucagon activity • Decrease in Ca^{2+} absorption	Type I calcimimetic (extracellular Ca^{2+})	35,36
	• *In vivo* treatment of mice • Cell culture models • Patients with autosomal dominant hypocalcemia caused by CaSR-activating mutations	• Control of urinary Ca^{2+} absorption • Increased expression of claudin-14	• Type II calcimimetic (cinacalcet) • Calcilytic (NPS2143)	12,46
Distal convoluted tubule (apical/basolateral)	Cell culture models expressing a dominant negative CaSR mutation (R185Q)	Stimulation of Ca^{2+} reabsorption	Type I calcimimetic (neomycin)	51
Collecting duct intercalated cells (apical)	• Dissected mouse outer medullary collecting ducts • Gene ablation of the collecting duct-specific B1 subunit of H^+-ATPase	Increase in luminal acidification	Type I calcimimetic (luminal Ca^{2+})	54
Collecting duct principal cells (apical)	• Hypercalcaemic rats • Dissected rat kidney inner medullary collecting duct • Cell culture models • Human bedrest • AQP2 isolated vesicles • Hypercalciuric patients	• Decrease in AQP2-mediated water reabsorption • Inhibition of vasopressin response	• Type I calcimimetics (neomycin, gadolinium) • Type II calcimimetic (R-568) • Type I calcimimetic (luminal Ca)	57, 59-65

*Based on CaSR activators or inhibitors. AQP2, aquaporin 2; CaSR, calcium-sensing receptor; PTH, parathyroid hormone.

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Author contributions

Both authors researched data for the article, provided a sub- stantial contribution to discussions of the content, contrib- uted equally to writing the article and to review and/or editing of the manuscript before submission.

Competing interests statement

The authors declare no competing interests

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