Renal tubular calcium reabsorption is a critical determinant of extracellular fluid (ECF) calcium concentration; for the need of constancy of ECF calcium concentration, the renal tubular handling of calcium is tightly controlled in order to match renal calcium excretion to the net amount of calcium entering the ECF. Both parathyroid hormone (PTH) and vitamin D metabolites are involved in the control of renal tubular calcium reabsorption and ECF calcium concentration [1]. Besides this hormonal control, it has been recognized recently that ECF calcium is able to regulate its own reabsorption by the mammalian tubule. Indeed, a large body of evidence supports the view that ECF calcium exerts this action by activating the calcium/polyvalent cation-sensing receptor (CaSR) located in the plasma membrane of many tubular cell types. First, increasing ECF calcium concentration elicits a marked increase in urinary calcium (and magnesium) excretion [2,3] and this occurs independently of any change in the calcium-regulating hormones [2,3]. Second, the inhibitory effect of ECF calcium on its own reabsorption is shared by other CaSR agonists, e.g. magnesium [4]. Third, the relationship between ECF calcium and urinary calcium excretion is altered in patients bearing mutations of the CASR gene: renal tubular calcium reabsorption is enhanced in patients with inactivating mutations [5,6] and decreased in patients with activating mutations. Therefore, there is abundant evidence that renal tubular CaSR plays a role in the control of divalent cations reabsorption under both normal and pathological conditions.

Keywords: calcium-sensing receptor; cortical thick ascending limb; renal tubular cation handling
Localisation of the extracellular CaSR

Transcripts of the CASR gene are expressed in many nephron segments of rat kidney, extending from glomeruli to the inner medullary collecting duct (IMCD) [7]. The CaSR protein is expressed in the proximal tubule, medullary and cortical thick ascending limb (TAL) segments, macula densa cells, distal convoluted tubule (DCT) and type-A intercalated cells in the distal tubule and cortical collecting duct [8] and in inner medullary collecting duct cells [9]. The polarity of expression varies from segment to segment, the protein being expressed in the apical membrane of proximal tubule and IMCD cells and in the basolateral membrane of TAL and DCT cells [8,9]. Interestingly, the highest density of protein expression has been observed in the cortical TAL (cTAL), which is known to reabsorb calcium and magnesium in a regulated manner.

CaSR under physiological conditions

Consistent with its polarized plasma membrane localization, CaSR has been shown to be involved in the control of TAL calcium and magnesium reabsorption. In the mouse and rat TAL, both calcium and magnesium are reabsorbed selectively in the cortical portion (cTAL) [10] and this reabsorption is passive along an electrical gradient through the paracellular pathway [10,11]. The electrical gradient is related to transcellular NaCl reabsorption. The first step is NaCl entry into the cell via the electroneutral apical Na-K-2Cl co-transporter BSC1 (NKCC2). Subsequently, most of the potassium recycles back to the lumen, through an apical potassium channel, which is necessary to maintain NaCl absorption via BSC1 (NKCC2). In the absence of recycling, NaCl absorption is inhibited because of the low availability of potassium in luminal fluid. In addition, potassium recycling hyperpolarizes the apical membrane. Chloride exits the cell across the basolateral membrane mainly via the CLC-Kb channel, which depolarizes the basolateral membrane. The overall consequence is a lumen-positive transepithelial voltage that drives calcium, magnesium and also sodium through the paracellular pathway. The pathway permeability for calcium and magnesium requires the presence of a specific protein, paracellin-1 (also known as claudin-16), which is co-expressed with occludin in the tight junctions of TAL [12]. Inactivating mutations of the paracellin-1 gene cause a specific decrease in cTAL calcium and magnesium reabsorption and renal loss of both cations without renal sodium loss, which is the landmark of an inherited disease referred to as hypercalcicuric hypomagnesaemia with nephrocalcinosis [4].

Calcium and magnesium reabsorption in the cTAL is tightly regulated. Micropuncture studies have shown that peptide hormones, such as PTH, arginine vasopressin, calcitonin and glucagon, stimulate NaCl as well as calcium and magnesium reabsorption in the loop of Henle of the rat nephron and decrease their excretion in final urine [13]. Furthermore, PTH, the most important peptide hormone for the stimulation of renal calcium transport, elicits an increase in calcium and magnesium reabsorption in the mouse [14] and rabbit [15–17] cTAL perfused in vitro. Of note, Wittner et al. [14] have demonstrated that the stimulating effect of PTH on calcium and magnesium transport involves an increase in paracellular pathway permeability.

More recently, it has become clear that the activation of CaSR also affects a number of intracellular events in TAL cells and modulates transport processes along the cTAL epithelium. Activating CaSR increases intracellular free calcium concentration in the mouse [18] and rat [19] cTAL, DCT and cortical as well as outer medullary collecting duct. This also decreases hormone-dependent cAMP accumulation in the rat cTAL by inhibition of type-6 adenyl cyclase [20], increases inositol phosphate formation in the rat cTAL [21] and elicits an increase in phospholipase A2 activity and in intracellular cellular production of 20-hydroxyeicosatetraenoic acid [22].

Regarding the transport processes themselves, available data are relatively scarce. CaSR activation leads to a decrease in apical 70-pS K channel activity via a cytochrome P-450-dependent mechanism in the rat TAL [23]. However, no data are available to suggest that activating CaSR also decreases the activity of the small conductance apical ROMK channel. To date, based on studies showing that loss-of-function of ROMK channel can lead to Bartter’s syndrome [24], only the ROMK channel is thought to control the activity of the apical Na-K-2Cl co-transporter. Direct measurements of the effect of CaSR activation on transepithelial NaCl fluxes have yielded apparently conflicting results. Desfleurs et al. [21] have shown that increasing peritubular calcium concentration up to 5 mM decreases transepithelial calcium and magnesium reabsorption, but does not affect the transepithelial potential difference or the rate of sodium chloride reabsorption in the mouse cTAL perfused in vitro. More recently, similar results have been obtained by another laboratory in the mouse cTAL perfused in vitro [25]: type-I and -II agonists of the CaSR (gadolinium and NPS R-467) inhibit the PTH-stimulated calcium reabsorption, but do not change the transepithelial potential difference or the rate of sodium chloride reabsorption. Consistently, in vivo microperfusion experiments of Henle’s loop in rats, a selective increase in peritubular magnesium concentration markedly inhibits divalent cation but not NaCl reabsorption [26]. In humans, acute magnesium infusion increases urinary calcium excretion but not urinary NaCl excretion and does not alter the natriuretic response to furosemide [4]. Taken together, these studies provide convincing evidence that activating basolateral CaSR reduces the rate of net calcium and magnesium reabsorption in the cTAL without affecting NaCl reabsorption and transepithelial voltage. Therefore, it could be considered that CaSR might selectively regulate the paracellular pathway permeability. In contrast, in the rat cTAL perfused in vitro,
both peritubular calcium and neomycin (another potent agonist of CaSR) reduce basal and AVP-stimulated net chloride reabsorption [27]. In vivo microperfusion of Henle’s loop in thyroparathyroidectomized rats has shown that an increase in the peritubular plasma calcium concentration induces a small but significant reduction (8%) in NaCl reabsorption with a modest increase in urinary NaCl excretion [3]. A graded calcium infusion in healthy men, under a PTH clamp protocol and rigorously controlled NaCl balance, induces a modest increase in urinary NaCl excretion with a saturation phenomenon at high plasma calcium concentration [2]. Calcium is a more potent CaSR activator than magnesium [28]; it is therefore possible that the degree of inhibition of NaCl reabsorption in the cTAL is a function of the magnitude of CaSR activation. It remains uncertain at present whether activating CaSR inhibits NaCl reabsorption in the cTAL or not. Two recent reports described patients who showed characteristics of both autosomal dominant hypocalcaemia and Bartter’s syndrome [29,30]. These patients had activating mutations in the CASR gene, resulting in a marked gain-of-function of the protein [29,30]. This suggests that, in humans, marked activation of the CaSR may result in a combined decrease in calcium and NaCl reabsorption in the TAL of Henle’s loop. However, a yet unresolved issue is whether the NaCl transport alteration occurs in the cortical or in the medullary part of the TAL. In fact, the role of CaSR expression in the medullary TAL, a segment that is not involved in divalent cation transport, has not yet been assessed.

**CaSR under pathological conditions**

That the renal CaSR indeed plays a significant role in renal tubular handling of divalent cation metabolism is supported by additional data obtained from pathophysiological studies conducted in mice and humans. For example, patients with a heterozygous inactivating mutation of the CASR gene display, independently of the altered control of PTH secretion, an increase in tubular calcium reabsorption [5]. Consequently, their urinary calcium excretion is lower than that measured in patients with primary hyperparathyroidism and an identically elevated serum calcium concentration [6]. This probably explains that the occurrence of calcium stone disease is considerably less frequent in patients with familial hypercalcaemia with hypocalciuria (FHH) than in those with primary hyperparathyroidism. In addition, the increased renal tubular calcium reabsorption that occurs in FHH has been specifically localized in the TAL, since it disappears in the presence of furosemide [6]. The opposite change in renal calcium handling has been described in patients with a heterozygous activating mutation of the CASR gene. These patients have a higher urinary calcium excretion than hypoparathyroid patients with a similarly low serum calcium concentration [31], demonstrating a lower renal tubular calcium reabsorption in patients with autosomal dominant hypocalcaemia than in those with hypoparathyroidism and are prone to develop nephrocalcinosis and calcium nephrolithiasis.

Two recent studies in homozygous CaSR-deficient mice rescued by either Gcm2 or PTH deficiency yielded data on the specific, PTH-independent role of CaSR in the regulation of calcium metabolism [32,33]. Both studies showed that CaSR did not directly (i.e. independently of its control of PTH secretion) determine mean (equilibrium) serum calcium concentration. However, one study reported that individual serum calcium values were much more scattered in CASR−/−, Pth−/− than in CASR−/−, Pth+/+ mice, suggesting that CaSR might play a role in the fine-tuning of serum calcium around its equilibrium value [32]. Regarding the specific effect of CaSR in the control of renal calcium handling, one study clearly demonstrated, as expected, that CaSR deficiency markedly decreased urinary calcium excretion [33]. The other, however, failed to demonstrate any significant difference in urinary calcium excretion between CASR−/− and CASR+/+ mice, perhaps because of a major scattering in individual urinary calcium values in the former [32]. Of note, the dramatic bone consequences observed in CASR−/−, Pth+/+ mice are lacking in the CASR−/− rescued mice, suggesting that the bone CaSR does not play any essential, non-redundant role in regulating chondrogenesis or osteogenesis [33].

Because of its important role in the renal handling of calcium, several studies have looked for a possible involvement of CaSR in the pathogenesis of idiopathic hypercalciuria. Genetic variants of the CASR gene were found not to be associated with idiopathic hypercalciuria or calcium nephrolithiasis [34]. Similarly, we did not find any point mutation in the CaSR gene in families with idiopathic hypercalciuria [35]. We failed to observe any relationship between CaSR gene polymorphisms (either A986S or R990G) and the value of urinary calcium excretion in a large series of patients with calcium stone disease (unpublished data). Recently, however, another group reported that a single polymorphic variant at codon 990 of the CASR gene (AGG→CGG) was associated with a significantly increased relative risk to be hypercalciuric [36]. To date, no explanation for these discrepant results is available, except the possible role of unapparent population stratification.

In conclusion, a large body of evidence supports the view that CaSR is a major regulator of calcium and magnesium reabsorption in the cTAL and, consequently, of overall tubular divalent cation handling. However, several issues remain unresolved. It is still unclear whether CaSR activation in the cTAL decreases NaCl reabsorption in this segment or not. The mechanism through which CaSR activation could alter the function of paracellin-1 and the paracellular pathway permeability also remains unsettled. Finally, the role of CaSR in the medullary part of TAL should be investigated: a CaSR-dependent inhibition of NaCl reabsorption could explain at least part of the polyuria that accompanies hypercalcaemic states.
Conflict of interest statement. None declared.

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