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ROLE OF THE Ca^{2+}-SENSING RECEPTOR IN DIVALENT MINERAL ION HOMEOSTASIS

STEVEN C. HEBERT[1,*], EDWARD M. BROWN[2] AND H. WILLIAM HARRIS[3]

[1]*Renal Division and* [2]*Endocrine–Hypertension Division, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA* and [3]*Renal Section, Children's Hospital and Harvard Medical School, Boston, MA, USA*

Summary

The divalent mineral cations Ca^{2+} and Mg^{2+} play many and diverse roles both in the function of cells and in extracellular processes. The metabolism of these cations is a complex process involving the coordinated function of several organ systems and endocrine glands. A recently cloned G-protein-coupled receptor responds to extracellular calcium concentration (Ca^{2+}_o-sensing receptor, CaSR) and mediates several of the known effects of Ca^{2+}_o on parathyroid and renal function. The CaSR, which is also expressed in a number of other tissues including thyroidal C-cells, brain and gastrointestinal tract, may function as a Ca^{2+}_o sensor in these tissues as well. Thus, Ca^{2+}_o is a first messenger (or hormone) which, *via* CaSR-mediated activation of second messenger systems (e.g. phospholipases C and A_2, cyclic AMP) leads to altered function of these cells. Several mutations in the human CaSR gene have been identified and shown to cause three inherited diseases of calcium homeostasis, clearly implicating the CaSR as an important component of the homeostatic mechanism for divalent mineral ions.

Ca^{2+} and Mg^{2+} losses from the body are regulated by altering the urinary excretion of these divalent cations. The localization of the CaSR transcripts and protein in the kidney not only provides a basis for a direct Ca^{2+}_o (or Mg^{2+}_o) -mediated regulation of Ca^{2+} (and Mg^{2+}) excretion but also suggests a functional link between divalent mineral and water metabolism. In the kidney, the thick ascending limb of Henle (TAL) plays crucial roles in regulating both divalent mineral reabsorption and urine concentration. Recent studies have suggested models whereby extracellular Ca^{2+}, *via* the CaSR expressed in the TAL as well as in the collecting duct system, modulates both Ca^{2+}_o and Mg^{2+}_o as well as water reabsorbtion. When taken together, these studies suggest that the CaSR not only provides the primary mechanism for Ca^{2+}_o-mediated regulation of parathyroid hormone secretion from parathyroid glands but also for direct modulation of renal divalent mineral excretion and urinary concentrating ability. These latter functions may furnish a mechanism for integrating and balancing water and divalent cation losses that minimizes the risk of urinary tract stone formation. This mechanism can explain hypercalcemia-mediated polyuria (diabetes insipidus).

Key words: homeostasis, mineral ion, Ca^{2+}, Mg^{2+}, kidney, diabetes insipidus, receptor, G-protein-coupled.

Introduction

The concept of an extracellular Ca^{2+}-sensing receptor

The kidney plays key roles in Ca^{2+} and Mg^{2+} homeostasis by providing the major route for mineral cation excretion from the body. Regulating the tubular reabsorption of these divalent cations from the glomerular filtrate is crucial to divalent mineral ion homeostasis. The cellular mechanisms mediating mineral ion transport across nephron segments from proximal tubule to collecting duct have been reviewed in detail elsewhere (De Rouffignac and Quamme, 1994). Traditional views of renal mineral ion handling have focused on the important roles played by the calciotropic hormones, parathyroid hormone (PTH) and calcitonin, as well as vitamin D (Kurokawa, 1994; Rouse and Suki, 1995; Parfitt and Kleerkoper, 1980; Aurbach *et al.* 1985; Stewart and Broadus, 1987). Urinary calcium excretion (U_{Ca}) increases steeply with rising circulating Ca^{2+} concentrations (P_{Ca}) beyond a certain threshold (Fig. 1) (see Kurokawa, 1987, 1994, for reviews). A similar steep inverse sigmoidal relationship exists between increasing extracellular [Ca^{2+}] and PTH secretion from parathyroid cells and has been modeled to suggest the possible cooperative interactions of at least three calcium ions with the cation-sensing mechanism (Brown, 1991). The relationship between U_{Ca} and P_{Ca} can be modulated by both PTH and vitamin D; the absence of either (or both) calciotropic factor significantly shifts the *threshold* for the curve to the left such that urinary Ca^{2+} loss is observed at lower circulating Ca^{2+} concentrations (Kurokawa, 1994; Fig. 1). The *steepness* of the relationship between U_{Ca} and P_{Ca} is, however, not lost even when both PTH and vitamin D are absent, indicating that some additional factor contributes to determining urinary Ca^{2+}

*e-mail: shebert@bustoff.bwh.harvard.edu.

excretion (Fig. 1). The recent observations demonstrating that extracellular Ca^{2+} itself, interacting with the newly cloned CaSR, provides an important component of this regulatory function will be reviewed below.

The extracellular Ca^{2+}-sensing receptor

Modulation of PTH secretion by Ca^{2+}_o involves interaction of this cation with a specific cell-surface receptor. Over the last decade, indirect evidence had accumulated suggesting the existence of such an ion-sensing receptor since raising $[Ca^{2+}]_o$ activated a number of second messenger systems in parathyroid cells in a fashion similar to that for other G-protein-coupled receptors. For example, activation of phospholipase C (PLC) leads to accumulation of inositol 1,4,5-trisphosphate (Brown *et al.* 1987), which in turn leads to release of Ca^{2+} from intracellular stores (Nemeth and Scarpa, 1986; for reviews, see Brown, 1991, 1992; Nemeth, 1995). With these observations as a guide, Brown *et al.* (1993) used expression in *Xenopus laevis* oocytes to clone the complementary DNA (cDNA) encoding the $[Ca^{2+}]_o$-sensing receptor from bovine parathyroid gland (BoPCaSR). Subsequently, the receptor was cloned from human parathyroid gland (Garrett *et al.* 1995), rat (Riccardi *et al.* 1995) and human (Aida *et al.* 1995) kidney, and rat brain (Ruat *et al.* 1995). Expression of these receptors in *Xenopus* oocytes (by injecting them with synthetic mRNA transcribed from the cDNAs) gives rise to Ca^{2+}_o-sensing behavior in injected oocytes which is pharmacologically similar to that of the native Ca^{2+}_o-sensing receptor of the parathyroid gland (Garrett *et al.* 1995; Brown *et al.* 1993; Riccardi *et al.* 1995): the CaSR is activated by the same di- and trivalent cations and even polycations (e.g. neomycin) as the native receptor (Ridefelt *et al.* 1992; Brown *et al.* 1990, 1991; Nemeth, 1990). Thus, this CaSR is a G-protein-coupled, cell surface receptor that recognizes an inorganic ion, as opposed to an organic molecule, as its ligand (Conklin and Bourne, 1994).

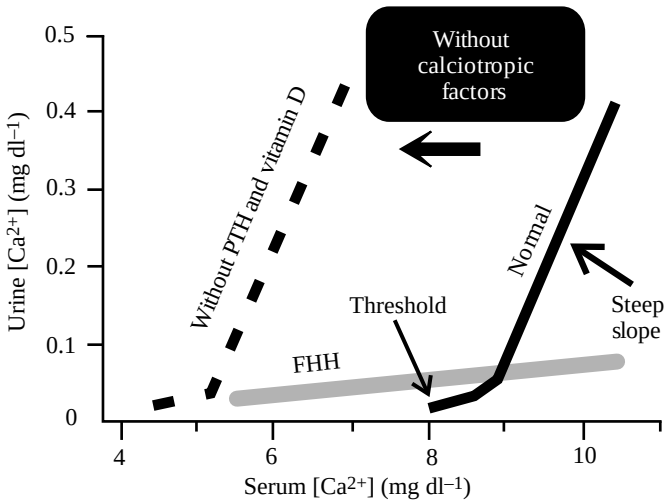

Fig. 1. Relationship between urinary Ca^{2+} excretion and total serum Ca^{2+} concentration. Urinary Ca^{2+} concentration increases steeply as serum Ca^{2+} concentration rises beyond a threshold concentration. The curves in the presence (solid line) or absence (dashed line) of calciotropic factors, vitamin D and parathyroid hormone (PTH), are shown. Also indicated are the effects of Ca^{2+} load on urinary Ca^{2+} excretion in hypoparathyroid familial hypocalciuric hypercalcemia (FHH) individuals (adapted from Kurokawa, 1987). Note the flat line (light solid line) in these individuals indicating near complete absence of a response (adapted from Attie *et al.* 1983).

The deduced amino acid sequence of the CaSR shows the characteristic seven-membrane-spanning helical signature found in all G-protein-coupled receptors (GPRs; Fig. 2; Jackson, 1991; Bockaert, 1991). The CaSR has a low, but significant, amino acid sequence similarity (21–26 % identity) only with the metabotropic glutamate receptors, mGluRs, expressed in the central nervous system (Nakanishi, 1992). Conklin and Bourne (1994) have suggested that the extracellular ligand-binding domains of the CaSR and the mGluRs have an overall structural organization which is similar to that of bacterial periplasmic nutrient-binding proteins (Tam and Saier, 1993; O'Hara *et al.* 1993). These bacterial proteins recognize for cellular uptake (*via* permeases) a variety of extracellular solutes, including organic nutrients as well as inorganic ions such as phosphate and nickel (Tam and Saier, 1993). Thus, it is plausible that the extracellular Ca^{2+}-sensing receptor may have evolved from an ancient family of cell-surface proteins binding essential extracellular solutes.

As expected from the pharmacology of the native parathyroid Ca^{2+}_o-sensing receptor, the cloned CaSR is unusual for a GPR in that it responds to its natural ligand, in this case Ca^{2+}, only in the millimolar ion concentration range that is the physiologically relevant Ca^{2+} concentration for extracellular fluid and cation sensing by parathyroid (and kidney). In this regard, the large extracellular domain does not contain any of the known high-affinity Ca^{2+}-binding motifs, but instead has several regions rich in negatively charged (acidic) amino acids. These residues probably mediate the low-affinity binding of cationic receptor agonists (e.g. Ca^{2+}, Mg^{2+}, Gd^{3+}, neomycin) in a fashion similar to the acidic domains found on low-affinity Ca^{2+}-binding proteins such as calsequestrin (Fliegel *et al.* 1987). These negatively charged sites could provide for binding of multiple calcium ions on each receptor molecule and may provide for cooperative cation interactions and the steep activity curve shown in Fig. 1.

Inherited human diseases of Ca^{2+}_o-sensing demonstrate the relevance of the CaSR in divalent mineral ion homeostasis

Two rare hypercalcemic disorders, familial hypocalciuric hypercalcemia (FHH; Marx *et al.* 1981*a*; Law and Heath III, 1985) and neonatal severe hyperparathyroidism (NSHPT), result from inactivating mutations (Pollak *et al.* 1993) when present in the heterozygous and homozygous ('knockout' equivalent) states, respectively (Pollak *et al.* 1994*b*). In addition, one form of autosomal dominant hypocalcemia (Estep *et al.* 1981) results from a mutation in the CaSR gene

(Pollak *et al.* 1994*a*) leading to expression of an overactivated receptor ('transgenic' equivalent).

The FHH gene had already been localized to the long arm of chromosome 3 in most (Heath III *et al.* 1993; Chou *et al.* 1992) but not all (Trump *et al.* 1993; Heath III *et al.* 1993) families with FHH and NSHPT when the CaSR was cloned. Pollak *et al.* (1993) quickly demonstrated point mutations (i.e. single base changes) within the receptor gene in three families with FHH mapping to chromosome 3q, and these results were subsequently confirmed by Heath III *et al.* (1994) and Pearce *et al.* (1994). Mutations are scattered throughout the predicted protein (Fig. 2; Heath III *et al.* 1994; Pearce *et al.* 1994; Pollak *et al.* 1993) and apparently modify the structure and/or ligand-binding properties of the CaSR.

Abnormal parathyroid and renal Ca^{2+}_o-sensing in FHH (Khosla *et al.* 1993) and NSHPT (Cooper *et al.* 1986; Marx *et al.* 1986) has been demonstrated as expected from an absent or abnormally functioning Ca^{2+}_o sensor. Parathyroid cells either show reduced sensitivity (FHH) or lack any PTH secretion responses (NSHPT) to increases in extracellular $[Ca^{2+}]$. As shown in Fig. 1, abnormal renal Ca^{2+}_o-sensing is suggested by the following observations: (i) despite hypercalcemia, individuals with these disorders show reduced fractional renal clearance of Ca^{2+} and Mg^{2+} (Attie *et al.* 1983; Marx *et al.* 1981*b*; Law and Heath III, 1985) and often exhibit frank hypocalciuria; (ii) individuals with FHH or NSHPT who have undergone parathyroidectomy continue to show markedly reduced renal Ca^{2+} clearance and a complete loss of the steep relationship between U_{Ca} and P_{Ca} (see Fig. 1 and note that the response is almost flat in these FHH individuals). Thus, the hypocalciuria observed in hypercalcemic FHH is clearly PTH-independent, indicating an intrinsic alteration in renal handling of Ca^{2+} somewhere along the nephron. Attie *et al.* (1983) also showed that the loop diuretic ethacrynic acid increased renal Ca^{2+} clearance, with these FHH individuals exhibiting an exaggerated response. This result raises the possibility that one nephron segment involved in the increased renal Ca^{2+} reabsorption in FHH is the thick ascending limb, a site of avid divalent mineral ion reabsorption and loop-diuretic action (see Fig. 3).

The renal clearance of Mg^{2+} is also reduced in patients with FHH, suggesting the possibility that the CaSR in kidney may also function in $[Mg^{2+}]_o$ sensing. The apparent affinity of the CaSR for Mg^{2+}, however, is too low for normal variations in the circulating Mg^{2+} concentration to influence this receptor (Brown, 1991). Nevertheless, it is possible that the basolateral

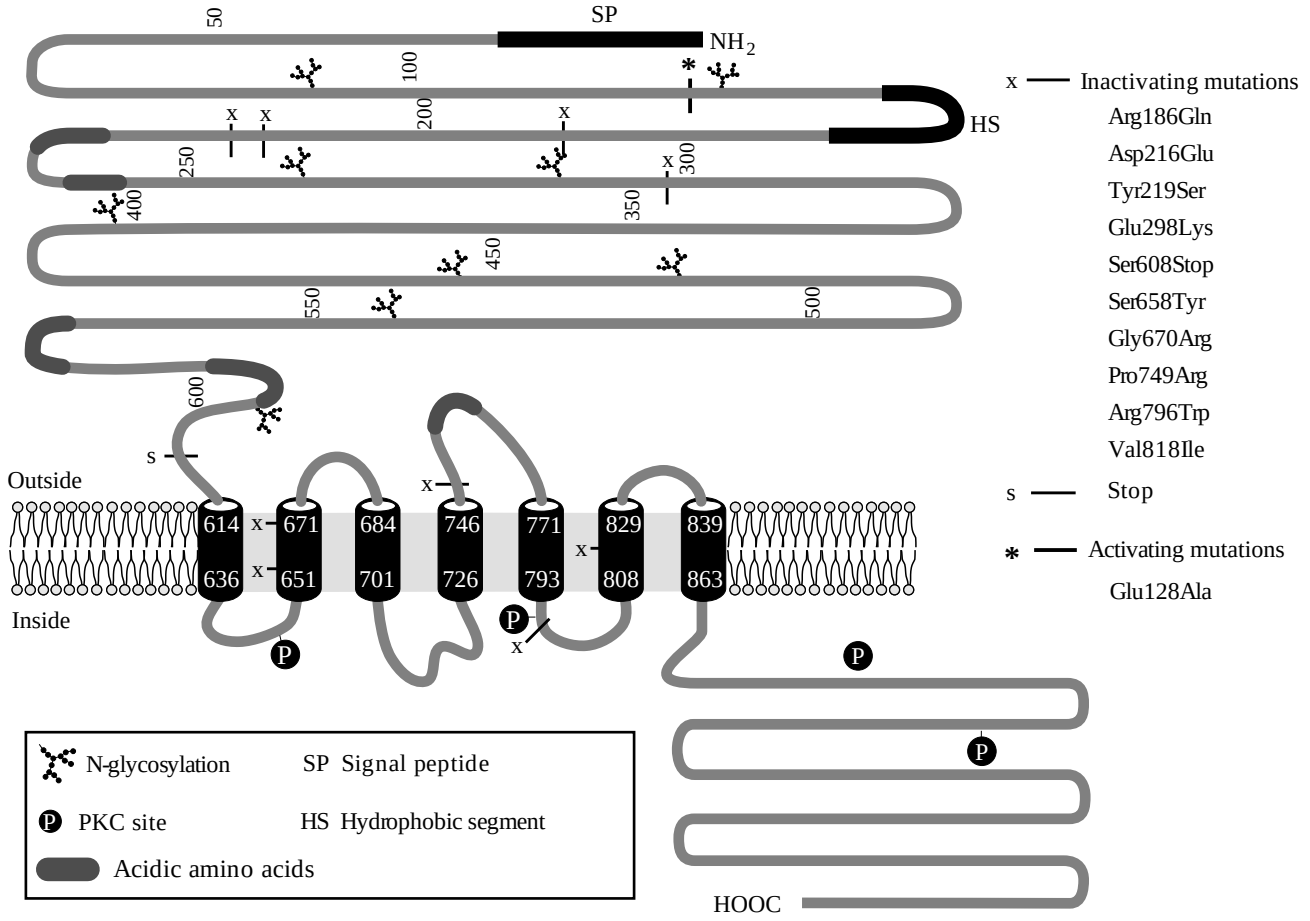

Fig. 2. Schematic representation of the principal structural features of the predicted BoPCaSR1 protein. Symbols are given in the key. Locations of known 'inactivating' and 'activating' mutations are indicated. See text for discussion. PKC, protein kinase C.

concentration of Mg^{2+} to which the receptor may be exposed in the thick ascending limb, where Ca^{2+} and Mg^{2+} regulate their own reabsorption (De Rouffignac and Quamme, 1994; Quamme, 1989; Quamme and Dirks, 1980*a*,*b*) and where both Ca^{2+} and Mg^{2+} are reabsorbed (De Rouffignac and Quamme, 1994; De Rouffignac *et al.* 1991) in the absence of water (Hebert and Andreoli, 1984), is actually higher than that in blood.

Finally, unlike patients with hypercalcemia due to other causes, who commonly develop an antidiuretic hormone-resistant polyuria (Suki *et al.* 1969; Beck *et al.* 1959, 1974; Gill and Bartter, 1961; Manitius *et al.* 1960; Guignard *et al.* 1970), hypercalcemic FHH individuals have no polyuria and show normal maximal urinary concentrating ability with dehydration (Marx *et al.* 1981*b*). Their Ca^{2+} 'resistance', which results from a reduced number of normal Ca^{2+}-sensing receptors, must diminish the impact of hypercalcemia on loop of Henle or collecting duct functions which are responsible for water handling.

Roles for the CaSR in renal handling of divalent minerals and water

The $Ca^{2+}{}_o$-sensing receptor is localized within several regions of the kidney that are directly regulated by extracellular Ca^{2+} concentration (Riccardi *et al.* 1995). The receptor transcript is most heavily expressed in the cortical thick ascending limb (CTAL) in the rat kidney, as well as in the proximal tubule, medullary thick ascending limb (MTAL), distal convoluted tubule and along the entire collecting duct (Riccardi *et al.* 1995). The actions of $[Ca^{2+}]_o$ (and $[Mg^{2+}]_o$) on renal functions that reside within these segments of the nephron include the following: inhibition of NaCl transport in the thick ascending limb (Suki *et al.* 1969); reduction in Ca^{2+} and Mg^{2+} reabsorption in the MTAL (Quamme, 1982, 1989; Shareghi and Agus, 1982; De Rouffignac and Quamme, 1994; Quamme and Dirks, 1980*a*,*b*); pertussis-toxin-sensitive diminution of hormone-stimulated cyclic AMP accumulation in the MTAL and CTAL (Takaichi and Kurokawa, 1986, 1988; Takaichi *et al.* 1986); and inhibition of antidiuretic hormone action in the collecting duct (Dillingham *et al.* 1987; Jones *et al.* 1988). The effects of $[Ca^{2+}]_o$ on NaCl and water transport in the TAL and collecting duct could be mediated by inhibition of antidiuretic hormone (or other hormone)-stimulated cyclic AMP accumulation or by $[Ca^{2+}]_i$-dependent signaling mechanisms (Teitelbaum and Berl, 1994; Breyer, 1991) and, thereby, account for the reduced concentrating ability (nephrogenic diabetes insipidus) associated with hypercalcemic states. What is the evidence that these actions are mediated by the $[Ca^{2+}]_o$-sensing receptor recently cloned from the parathyroid and kidney?

The following results suggest a model for the action of extracellular Ca^{2+} on thick ascending limb function shown in Fig. 3. Elevated levels of extracellular Ca^{2+}(or Mg^{2+}) reduce NaCl reabsorption, and hence Ca^{2+} and Mg^{2+} reabsorption, by the TAL *via* a $[Ca^{2+}]_o$-sensing receptor-dependent mechanism. The net rate of NaCl absorption by the TAL is regulated by $G\alpha_s$-adenylate-cyclase-dependent generation of cyclic AMP

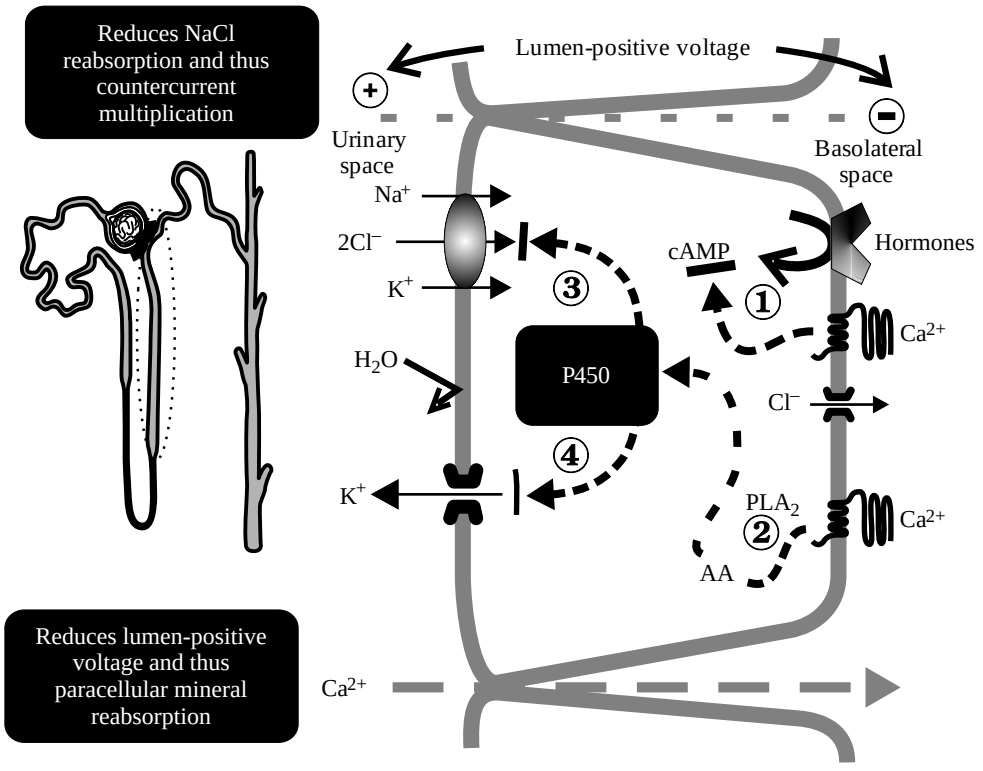

Fig. 3. Model for the role of the extracellular Ca^{2+} ($Ca^{2+}{}_o$)-sensing receptor (CaSR) in regulating NaCl and Ca^{2+} (Mg^{2+}) reabsorption in the kidney thick ascending limb (TAL). Ca^{2+}-mediated activation of the CaSR leads to a reduction in hormone-stimulated NaCl absorption *via* pertussis-toxin-sensitive inhibition of cyclic AMP (cAMP) (1); Ca^{2+}-induced activation of the CaSR leads to production of arachidonic acid (AA) *via* stimulation of phospholipase A_2 (PLA_2) (2); P450 metabolite of arachidonic acid, probably 20-HETE, inhibits the 'loop'-diuretic-sensitive $Na^+/K^+/2Cl^-$ cotransporter in apical membranes (3); P450 metabolite of arachidonic acid, also 20-HETE, inhibits apical K^+ channels (4). See text for further discussion.

stimulated by the integrated action of several hormones (De Rouffignac *et al.* 1987, 1991; Hebert and Andreoli, 1984). $Ca^{2+}{}_o$-sensing receptor-$G\alpha_i$-mediated (pertussus-toxin-sensitive) reductions in levels of cyclic AMP generated by these hormones (Takaichi and Kurokawa, 1986, 1988; Takaichi *et al.* 1986) would result in reduced NaCl transport. Moreover, $Ca^{2+}{}_o$-sensing receptor-$G\alpha_q$-mediated increases in intracellular $[Ca^{2+}]$ or activation of protein kinase C may also contribute importantly to the inhibition of NaCl reabsorption. This 'loop'-diuretic-like action of $[Ca^{2+}]_o$ would not only reduce countercurrent multiplication, and hence urinary concentrating ability, but also decrease the lumen-positive potential (Hebert and Andreoli, 1984) that is the driving force for Ca^{2+} and Mg^{2+} transport *via* the paracellular pathway (Friedman, 1988; Bourdeau and Burg, 1979; Mandon *et al.* 1993; Di Stefano *et al.* 1993). Moreover, the effect of $[Ca^{2+}]_o$ (or possibly $[Mg^{2+}]_o$) on PTH-dependent cyclic AMP accumulation (Takaichi and Kurokawa, 1986) in the CTAL would be expected to diminish any PTH-mediated increase in the paracellular permeability to these divalent cations (Wittner *et al.* 1993). The net effect of increased basolateral (peritubular) $[Ca^{2+}]_o$ would be to reduce both NaCl and Ca^{2+} (or Mg^{2+}) reabsorption by the TAL and result in a marked increase in urinary Ca^{2+} excretion similar to that seen with administration of furosemide (Edwards *et al.* 1973). The associated decrease in countercurrent multiplication, and thereby urine concentration, would help ensure that the Ca^{2+} is excreted at a concentration below saturation. Clearly any effect of $[Ca^{2+}]_o$ to alter the antidiuretic-hormone-mediated increase in water permeability in the collecting duct (Dillingham *et al.* 1987; Jones *et al.* 1988) *via* a $[Ca^{2+}]_o$-sensing mechanism would add further to the reduction in urine concentration brought about by the effects of $[Ca^{2+}]_o$ in the TAL. During pathological or chronic hypercalcemia, this mechanism could account for nephrogenic diabetic insipidus commonly seen with this electrolyte disorder.

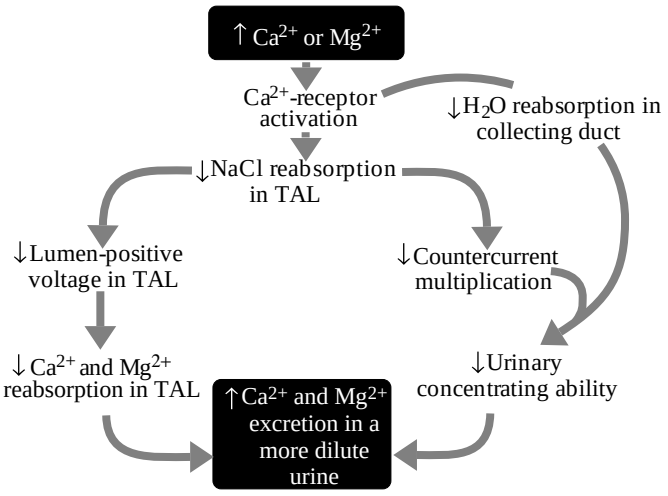

Fig. 4. Proposed model for integrating Ca^{2+}, Mg^{2+} and water handling by the kidney *via* the extracellular Ca^{2+}-sensing receptor (CaSR). With physiological transient hypercalcemia, the CaSR-mediated reduction in concentrating ability would provide a regulatory mechanism helping to promote excretion of the increased delivery of Ca^{2+} and Mg^{2+} from the thick ascending limb (TAL) to the collecting duct in a more dilute urine, thereby decreasing the risk of crystal/stone formation. A similar mechanism could account for the nephrogenic diabetes insipidus commonly observed in patients with pathological or chronic hypercalcemia.

Conclusions

The CaSR plays crucial roles in the regulation of renal divalent mineral transport processes by both direct and indirect mechanisms. Parathyroid cells recognize remarkably small perturbations in the circulating concentration of Ca^{2+} (approximately 1–2 % changes in $[Ca^{2+}]$) and then respond by altering the secretion of PTH. Recent molecular and genetic evidence has demonstrated that the cloned CaSR which is expressed on the surface of parathyroid cells provides the principal mechanism for extracellular $[Ca^{2+}]$ 'sensing' by the parathyroid gland (reviewed in Brown *et al.* 1995). Moreover, the kidney, like the parathyroid, is able to respond directly (i.e. independently of changes in levels of calciotropic hormones) to alterations in extracellular Ca^{2+} (or Mg^{2+}) concentration, with the resultant modulation of mineral ion transport (see Quamme and Dirks, 1980*b*; Quamme, 1989; Lau and Bourdeau, 1995; Nemeth, 1995; Brown, 1991, for reviews). The cloning of the CaSR from rat (Riccardi *et al.* 1995) and human (Aida *et al.* 1995) kidney and the expression of the CaSR in renal epithelial cells provide evidence that is consistent with a mechanism whereby extracellular Ca^{2+} participates directly in the regulation of its own reabsorption through local, receptor-mediated actions of Ca^{2+} (and/or Mg^{2+}) on the kidney (see Fig. 3).

The homeostatic adjustments in urinary excretion of mineral ions provided by calciotropic factors (mainly PTH and vitamin D) and the CaSR are not without potential consequences on renal function. With increased loads of calcium (e.g. from enhanced bone turnover or absorption from the intestinal tract, or from abnormalities of mineral ion reabsorption along the nephron), urinary Ca^{2+} excretion can increase dramatically (Fig. 1). The continued formation of a concentrated urine during periods of increased urinary Ca^{2+} or Mg^{2+} loss could present a problem, since mineral ions may reach supersaturation levels in the terminal collecting duct which, in turn, enhances the risk of nephrolithiasis and/or nephrocalcinosis. We have recently suggested that a 'trade-off' of water conservation for Ca^{2+} or Mg^{2+} loss operates to minimize the risk of stone formation under normal circumstances during periods of enhanced mineral ion excretion (Brown and Hebert, 1995) (Fig. 4). Elevations in $[Ca^{2+}]_o$ activate the CaSR in the thick ascending limb of Henle and lead to reduced reabsorption of Ca^{2+} (and Mg^{2+}) and hence increased Ca^{2+} (and Mg^{2+}) excretion in the urine. The absolute concentration of these mineral cations is reduced during this period of high cation excretion by two mechanisms. First, reduced NaCl transport by the thick ascending limb diminishes

countercurrent multiplication and hence urinary concentrating power. In addition, the sensing of the increased Ca^{2+} concentration in the urine in the terminal collecting duct by CaSRs facing the urinary space would reduce antidiuretic-hormone-stimulated water reabsorption from urine to medullary interstitial fluid. The end result of these actions of Ca^{2+} on the CaSR would be to cause excretion of the load of Ca^{2+} (or Mg^{2+}) at a urinary concentration that would be below that needed for mineral salt crystal formation (i.e. reduced risk of stone formation). Thus, the renal CaSR appears to provide the crucial 'sensing' mechanism in the distal nephron for integrating and balancing water and divalent mineral losses. Direct interactions of extracellular Ca^{2+} with the renal CaSR could explain in large part the disordered water metabolism (i.e. nephrogenic diabetes insipidus) observed under pathological states of hypercalcemia (e.g. with primary hyperparathyroidism or associated with certain malignancies; Gill and Bartter, 1961; Marx *et al.* 1981*a*).

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