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NEPHROLOGY FORUM

Extracellular calcium-sensing receptor: Implications for calcium and magnesium handling in the kidney

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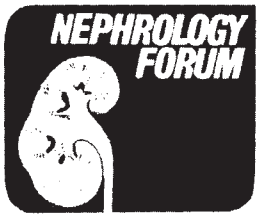

Case presentation

A 48-year-old man presented with recurrent nephrolithiasis. He had passed two stones over the past seven years; a stone passed three years ago required ureterolithotomy. At that time, the serum calcium was 10.6 mg/dl (normal, 8.8–10.6 mg/dl) and the serum phosphorus was 2.9 mg/dl (normal, 2.5–4.5 mg/dl). Crystallographic analysis of that stone showed that it contained a predominance of calcium oxalate with a small amount of calcium phosphate. Since then the patient has generally felt well while taking no medications. He had noticed that he had been drinking more water over the previous few months and had had occasional nocturia. He had no family history of nephrolithiasis.

On physical examination he was alert and in no distress. Weight was 75 kg; blood pressure, 145/92 mm Hg; the rest of the examination was unremarkable. Serum chemistries were: calcium, 11.1 mg/dl; phosphorus, 3.2 mg/dl; alkaline phosphatase, 75 U/liter (normal, 15–95 U/liter); BUN, 15 mg/dl; creatinine, 1.0 mg/dl; uric acid, 7.5 mg/dl (normal, 2.6–8.1 mg/dl); sodium, 139 mEq/liter; potassium, 4.1 mEq/liter; chloride, 102 mEq/liter; and bicarbonate, 25 mEq/liter. A 24-hour urine collection revealed a total volume of 2750 ml; creatinine clearance, 80 ml/min; calcium, 350 mg/day; and uric acid, 600 mg/day (normal, < 750 mg/day). The serum intact PTH was 70 pg/ml (normal, 10–55 pg/ml), 25-hydroxyvitamin D was normal, and serum $1,25(OH)_2D$ was 65 pg/ml (normal, 10–60 pg/ml). Maximal urinary concentration after overnight water deprivation was 550 mOsm/kg H_2O. An adenoma weighing 2 g was removed from the right parathyroid gland.

Discussion

DR. STEVEN C. HEBERT (*Director, Laboratory of Molecular Physiology and Biophysics, Renal Division, Department of Medicine, Brigham & Women's Hospital, and Professor of Medicine, Harvard Medical School, Boston, Massachusetts, USA*): In addition to providing a new target for the development of pharmaceutical agents that could be effective in altering PTH secretion, the molecular cloning of the extracellular Ca^{2+}-sensing G-protein-coupled receptor from parathyroid, kidney, and brain (variously referred to as BoPCaR, RaKCaR, HuPCaR, HuKCaSR, and CaSR), which will be abbreviated in this discussion to CaR [1–6], has significantly expanded our understanding of calcium and magnesium metabolism. New insights into the roles played by this receptor in several organs, including kidney and brain, soon will be forthcoming. Some of this new information may well suggest hitherto unsuspected roles for the CaR and extracellular Ca^{2+} in the various functions of these organ systems. In this discussion, I will focus on the proposed function of the CaR in Ca^{2+}, Mg^{2+}, and water metabolism. Today's patient, who had primary hyperparathyroidism, illustrates some of the effects that increases in extracellular calcium can have on renal function [see Refs. 7, 8 for reviews].

The kidney provides the major route for mineral ion excretion from the body by adjusting the tubular reabsorption of divalent cations from the glomerular filtrate. The kidney therefore plays a key role in Ca^{2+} and Mg^{2+} homeostasis. The cellular mechanisms mediating mineral ion transport across nephron segments from proximal tubule to collecting duct have been reviewed elsewhere [9, 10] and will not be covered here in detail. Traditional views of renal mineral ion handling have focused on the important roles played by the calciotropic hormones, PTH and calcitonin, as well as vitamin D [9, 11–16]. As discussed by Kurokawa in a previous Nephrology Forum a decade or so ago [16], urinary calcium excretion [U_{Ca}] increases steeply when circulating Ca^{2+} concentrations [P_{Ca}] rise beyond a certain threshold (Fig. 1; see Ref. 16 for a recent review). The inverse sigmoidal relationship between increasing extracellular Ca^{2+} and PTH secretion from parathyroid cells is also quite steep; this relationship suggests the possible cooperative interactions of three or more Ca^{2+} ions with the cation-sensing mechanism [8]. Both PTH and vitamin D are important modulators of the relationship between U_{Ca} and P_{Ca}, and the absence of either calciotropic factor (or both) significantly shifts the threshold (or "set point") for the curve to the left, such that urinary Ca^{2+} loss is observed at lower circulating Ca^{2+} concentrations (Fig. 1) [11]. The steepness of the relationship between U_{Ca} and P_{Ca} is not lost, however, even when both PTH and vitamin D are absent. This relationship indicates that one or more additional factors contribute to renal Ca^{2+} excretion (Fig.

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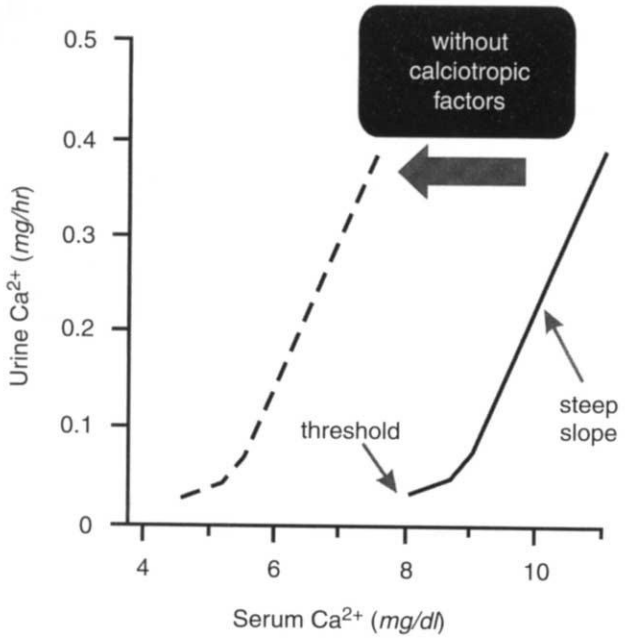

Fig. 1. *Relationship between urinary Ca^{2+} excretion and total serum Ca^{2+} concentration.* Urinary Ca^{2+} increases steeply in proportion to serum Ca^{2+} beyond a threshold concentration. The curves in the presence (*solid line*) or absence (*dashed line*) of the calciotropic factors vitamin D and PTH are shown. Note that absence of either calciotropic factor shifts the set point to the left without affecting the slope of the curves. (Adapted from Ref. 16.)

1). As I will discuss in this Forum, recent evidence suggests that extracellular Ca^{2+} itself, interacting with the newly cloned CaR in the kidney, provides a major part of this regulatory function.

The CaR plays crucial roles in regulating renal divalent mineral transport processes by both direct and indirect mechanisms. Parathyroid cells recognize remarkably small perturbations in the circulating concentration of Ca^{2+} (or Mg^{2+}) and then respond by altering the secretion of PTH. In fact, most of the "sensing" of extracellular Ca^{2+} in parathyroid cells occurs over changes in free Ca^{2+} concentration of approximately 0.25 mM; that is, PTH secretion versus $[Ca^{2+}]_o$ is quite steep, with a $K_{1/2} \approx 1.2$ mM; [8]. Recent molecular and genetic evidence has demonstrated that the cloned CaR, which is expressed on the surface of parathyroid cells, provides the principal mechanism for extracellular Ca^{2+} "sensing" by the parathyroid gland [reviewed in Ref. 17]. In turn, PTH modulates the renal reabsorption of Ca^{2+} and Mg^{2+} as well as PO_4^{3-}. Thus the CaR, by providing a sensor for extracellular Ca^{2+}-mediated modulation of calciotropic hormones, *indirectly* regulates renal divalent mineral handling. Moreover the kidney, like the parathyroid, is able to respond directly (that is, independently of changes in calciotropic hormones) to alterations in extracellular Ca^{2+} (or Mg^{2+}) with the resultant modulation of mineral ion transport [see Refs. 8, 18–21 for reviews]. The cloning of the CaR from rat [2] and human [5] kidney and the expression of the CaR in renal epithelial cells [22] is consistent with a mechanism whereby extracellular calcium participates *directly* in the regulation of its own reabsorption through local, receptor-mediated actions of Ca^{2+} (and/or Mg^{2+}) on the kidney. Evidence supporting this view will be described in the following sections.

The homeostatic adjustments in urinary excretion of mineral ions provided by calciotropic factors (mainly PTH and vitamin D) and the CaR are not without potential consequences on renal function. With increased loads of calcium (for example, from enhanced bone turnover or absorption from the intestinal tract, or from abnormalities of mineral ion reabsorption along the nephron), urinary calcium 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, because mineral ions can reach supersaturation levels in the terminal collecting duct; this supersaturation in turn increases the risk of nephrolithiasis, nephrocalcinosis, or both. Recently we have 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, especially during periods of increased mineral ion excretion [23]. The renal CaR appears to provide the crucial "sensing" mechanism in the distal nephron and collecting duct for integrating and balancing water and divalent mineral loss. Direct interactions of extracellular Ca^{2+} with the renal CaR could explain in large part the disordered water metabolism (that is, nephrogenic diabetes insipidus) observed during pathologic states of hypercalcemia (for example, primary hyperparathyroidism or certain malignancies) [24, 25].

The extracellular Ca^{2+}-sensing receptor

Extracellular Ca^{2+} modulates both PTH secretion and renal tubular divalent mineral and water transport processes by interacting with a specific receptor, the CaR. A wealth of indirect evidence has suggested the existence of a Ca^{2+}-responsive, receptor-like mechanism in parathyroid cells; for instance, raising extracellular Ca^{2+} activated a number of second messenger systems in a fashion similar to that for other G-protein-coupled receptors, for example, activation of phospholipase C (PLC) with consequent accumulation of inositol 1,4,5-trisphosphate (IP_3) [26] and release of Ca^{2+} from intracellular stores [27] [see Refs. 7, 8, 18 for reviews]. Moreover, second messenger systems in several nephron segments also are modulated by changes in extracellular Ca^{2+} [28–32]. The ability to express exogenous receptors in oocytes of the frog *Xenopus laevis*, and then to detect function of phosphoinositide (PI)-coupled receptors through electrophysiologic measurement of intracellular Ca^{2+}-activated chloride channels, enabled Brown and coworkers to clone the complementary DNA (cDNA) encoding the Ca^{2+}-sensing receptor from bovine parathyroid gland (BoPCaR) [1]. Subsequently, this receptor also was cloned from human parathyroid [3], rat [2] and human [5] kidney, and rat brain [4]. When these receptors were expressed in oocytes (by injecting the oocytes with synthetic mRNA transcribed from the cloned cDNAs), they behaved like the native Ca^{2+}-sensing receptor of the parathyroid gland [1–3]: the cloned CaR was activated by the same di- and trivalent cations and even polycations (for example, neomycin) as the native receptor [33–36]. This CaR represents the first example of a G-protein-coupled, cell-surface receptor in mammalian species that recognizes an inorganic ion rather than a molecule as its ligand [37]. The availability of these cloned genes and of antibodies against the receptor (produced from knowledge of the deduced amino acid sequences of the receptor proteins) has permitted studies of receptor localization, function, and regulation as well as identification of mutations of this receptor that produce inherited disorders of calcium metabolism. Much of this work is in progress, and over the next few years we can expect additional

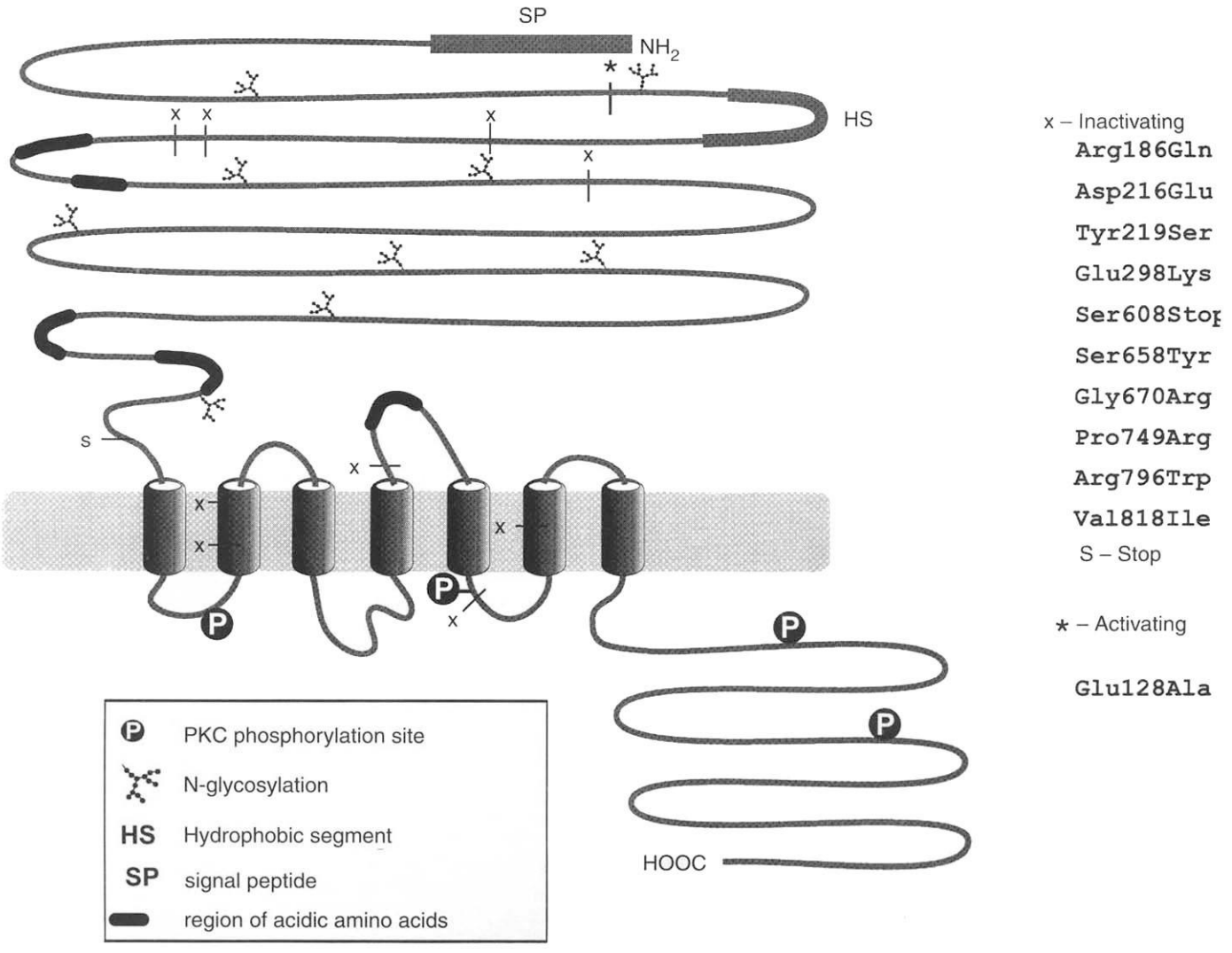

Fig. 2. *Topologic features of the predicted CaR.* Symbols are given in the key. Locations of known "inactivating" (x) and "activating" (*) mutations are indicated.

information, as well as probably some surprises, on the roles of this receptor in endocrine glands, kidney, intestine, brain, and possibly other tissues and cells [see Refs. 23, 38].

As expected, the deduced amino acid sequence of the CaR shows the classic seven-transmembrane-spanning helical domain thus far found on all G-protein-coupled receptors (GPRs; Fig. 2) [39, 40]. The CaR is a novel receptor, however, in that it has a significant (but low) amino acid sequence similarity only with the G-protein-coupled (metabotropic) glutamate receptors, mGluRs, expressed in the central nervous system [41]. Conklin and Bourne have suggested that the extracellular ligand-binding domains of the CaR and the mGluRs have an overall structural organization [37] similar to that of bacterial periplasmic nutrient-binding proteins [42, 43]. These bacterial cell-surface proteins bind a variety of extracellular solutes (thus enabling cellular uptake), including organic nutrients as well as inorganic ions such as phosphate and nickel [43]. Thus the extracellular Ca^{2+}-sensing receptor might have evolved from an ancient family of cell-surface proteins binding essential extracellular solutes. This similarity also suggests the possibility of additional ion (solute)-sensing receptors.

The pharmacology of this CaR is unusual for a GPR in that it responds to its natural ligand, in this case Ca^{2+} (and Mg^{2+}), only in the millimolar ion concentration range (recall that the pharmacologically relevant concentrations of other first messengers are usually in the picomolar to micromolar range). The former, however, is the physiologically relevant Ca^{2+} concentration range (0.75–2.0 mM free Ca^{2+}) for extracellular fluid, as opposed to the Ca^{2+} cytosolic range [10^{-9}–10^{-6} M]. In this regard, the large extracellular domain does not exhibit any of the known high-affinity Ca^{2+}-binding motifs. Instead it contains several regions rich in negatively charged (acidic) amino acids that probably mediate the low-affinity binding of cationic receptor agonists (for example, Ca^{2+}, Mg^{2+}, Gd^{3+}, neomycin) in a fashion similar to the acidic domains found on low-affinity calcium-binding proteins such as calsequestrin [44]. These negatively charged sites could

bind multiple Ca^{2+} ions on each receptor molecule, which might provide for cooperative cation interactions and the steep activity curve (Fig. 1).

Inherited human diseases of extracellular Ca^{2+} sensing

Elimination of a gene (and therefore the function of the protein encoded by the gene) through gene "knockouts," or overexpression of genes via production of transgenic animals, has provided powerful tools for defining the biologic role of a variety of genes. In certain cases, human genetic diseases have yielded examples of naturally occurring "knockouts" or "transgenics" by exhibiting mutations that inactivate or activate, respectively, the gene itself or the function provided by the encoded protein. Indeed, the clinical and physiologic relevance of the CaR has been established by the demonstration that mutations in it cause three inherited diseases of calcium metabolism. Two rare hypercalcemic disorders, familial hypocalciuric hypercalcemia (FHH) [45, 46] and neonatal severe hyperparathyroidism (NSHPT) result from inactivating mutations [47] when present in the heterozygous and homozygous ("knockout" equivalent) states, respectively [48]. One form of an autosomal dominant hypocalcemia [49] also results from a mutation in the CaR gene [50] and leads to expression of an overactivated receptor ("transgenic" equivalent).

The FHH gene had already been localized to the long arm of chromosome 3 in most [51, 52] but not all [51–54] families with FHH and NSHPT when the CaR was cloned. This CaR was an obvious candidate gene for FHH. Indeed, Pollak and coworkers demonstrated that the CaR gene mapped to the FHH disease locus and that point mutations (that is, single base changes) within this receptor gene were responsible for FHH in three families [47]. Other groups subsequently confirmed these results [54–56]. Mutations are scattered throughout the predicted protein (Fig. 2) [47, 55, 56] and apparently modify the structure and/or ligand-binding properties of the CaR. In one family, the mutation produces a stop codon that results in translation of a truncated and presumably biologically inactive receptor (Fig. 2) [56]. The genes involved in the families with the FHH phenotype not mapping to chromosome 3q are unknown [54].

In accord with the genetics, in-vivo and in-vitro studies have shown abnormal parathyroid and renal Ca^{2+} sensing in FHH and NSHPT. Parathyroid cells either show reduced sensitivity (FHH) or lack any PTH secretion responses (NSHPT) to increases in extracellular Ca^{2+} [57–59]. Abnormal renal Ca^{2+} sensing is suggested by the finding that, despite hypercalcemia, individuals with these disorders have reduced fractional renal clearance of Ca^{2+} and Mg^{2+} [45, 46, 60] and often exhibit frank hypocalciuria, that is, well under 100 mg/day. Moreover, individuals with FHH or NSHPT who have had parathyroidectomy, and thus have reduced or absent circulating PTH, continue to show markedly reduced renal calcium clearance, even during the intravenous infusion of sufficient calcium to induce frank hypercalcemia [60]. Figure 3 illustrates a comparison of urinary calcium (U_{Ca}) as a function of serum calcium levels (P_{Ca}) in hypoparathyroid "normal" and FHH individuals. Another feature of hypoparathyroid FHH shown in Figure 3 is the complete loss of the steep relationship between U_{Ca} and P_{Ca} (compare Figs. 1 and 3 and note that the curve is almost flat in the FHH individuals). These studies clearly demonstrate that the hypocalciuria observed in hypercalcemic FHH is PTH independent and secondary to an intrinsic alteration in renal handling of Ca^{2+} somewhere along the nephron. More

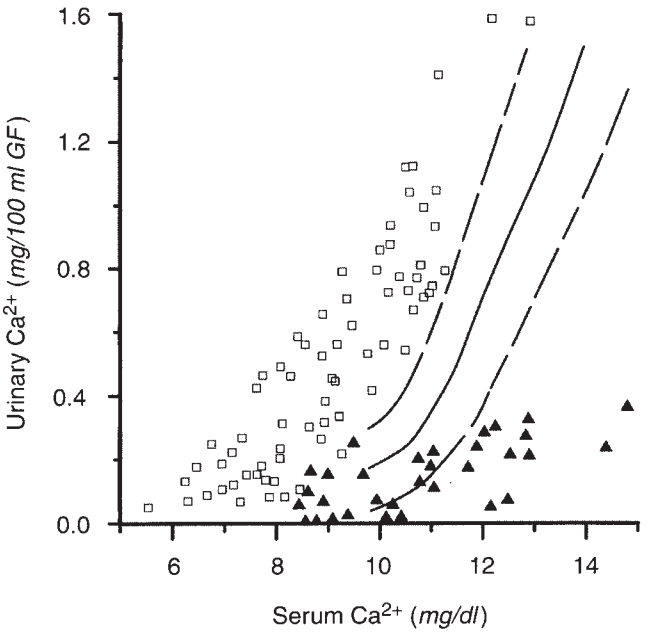

Fig. 3. *Effect of calcium load on urinary calcium excretion in hypoparathyroid FHH patients (▲) and hypoparathyroid controls (□) during infusions of calcium.* Urinary excretion of calcium is plotted as a function of total serum Ca^{2+} concentration. In the subjects receiving calcium infusion, creatinine clearance did not change during the study. The *solid line* and *dashed lines* represent mean and 95% confidence intervals, respectively, from normoparathyroid control individuals. (Adapted from Refs. 60 and 90.)

than a decade ago, Attie et al suggested a defect in the ascending limb of the loop of Henle, because people with FHH exhibited an exaggerated calciuric response to the loop diuretic ethacrynic acid [60]. This response is consistent with the hypothesis that the CaR, which is decreased in FHH, influences Ca^{2+} reabsorption in the thick ascending limb (Fig. 4).

The renal clearance of Mg^{2+}, like that of Ca^{2+}, also is reduced in patients with FHH, and some individuals with NSHPT have overt hypermagnesemia even with a normal creatinine clearance [45, 46]. This finding suggests that the CaR in kidney also functions as a Mg^{2+} sensor. The apparent affinity of the CaR for Mg^{2+}, however, is too low for normal variations in the circulating Mg^{2+} concentration to influence this receptor [8]. Nevertheless, it is possible that the basolateral concentration of Mg^{2+}, to which the receptor might be exposed in the thick ascending limb (where Ca^{2+} and Mg^{2+} regulate their own reabsorption [10, 20, 61] and where both Ca^{2+} and Mg^{2+} are reabsorbed [10, 62] in the absence of water [63]), is actually higher than that in blood. In this regard, Attie and colleagues found that hypoparathyroid individuals with FHH and NSHPT exhibited a reduced Mg^{2+} clearance comparable to the reduced clearance observed for Ca^{2+} [60].

Finally, unlike patients with hypercalcemia due to other causes, who commonly develop an ADH-resistant polyuria [25, 64–68], hypercalcemic individuals with FHH do not have polyuria and show normal maximal urinary concentrating ability after dehydration [24]. Their Ca^{2+} "resistance," which results from a reduced number of normal Ca^{2+}-sensing receptors, clearly diminishes the impact of hypercalcemia on loop of Henle or collecting duct functions, which are responsible for water handling.

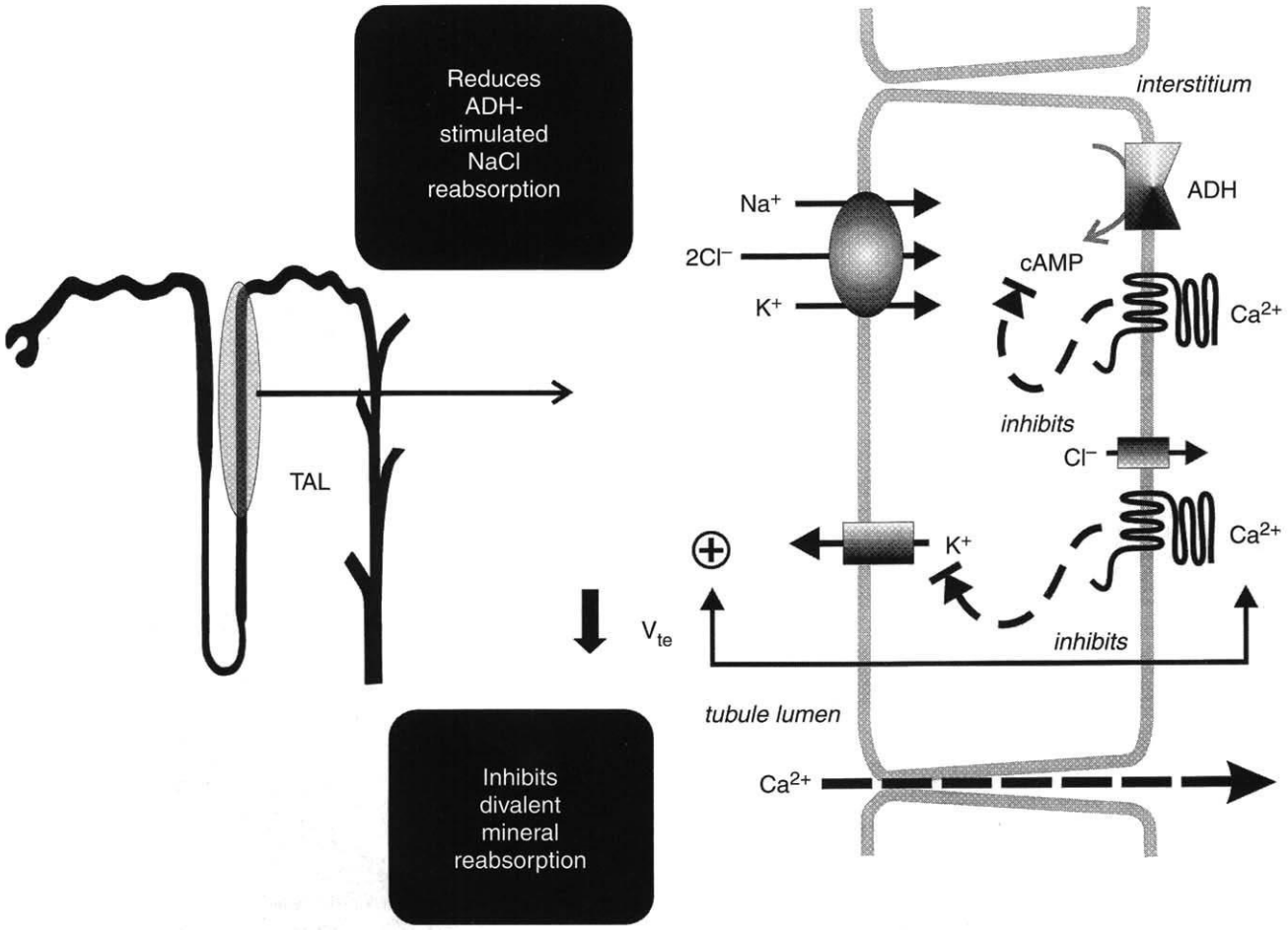

Fig. 4. *Model for the role of the extracellular Ca^{2+}-sensing receptor in the regulation of NaCl and Ca^{2+} (Mg^{2+}) reabsorption in the thick ascending limb (TAL).* Increases in peritubular Ca^{2+} activate the CaR and reduce hormone-stimulated cyclic AMP production, which reduces net NaCl absorption and the lumen-positive transcpithelial voltage, V_{te}. Activation of the CaR also reduces activity of the apical K^+ channels and thereby reduces potassium recycling. The net effect of both CaR-mediated processes is a reduction in divalent mineral (Ca^{2+} and Mg^{2+}) transport (reabsorption) via the paracellular pathway.

CaR in the kidney

The Ca^{2+}-sensing receptor has been localized within several segments of the rat nephron that subserve functions that are directly regulated by extracellular calcium. The receptor is most heavily expressed in the cortical thick ascending limb but also is present in the proximal tubule, medullary thick ascending limb, distal convoluted tubule, and along the entire collecting duct ([2], Riccardi D, unpublished observations). Previously described effects of extracellular Ca^{2+} (and Mg^{2+}) on these segments of the nephron include the following: inhibition of the 1-hydroxylation of 25-hydroxyvitamin D in the proximal tubule independent of PTH [69, 70; see Refs. 7, 8 for discussions]; inhibition of NaCl transport in the thick ascending limb [68]; reduction in Ca^{2+} and Mg^{2+} reabsorption in the medullary thick ascending limb [10, 20, 21, 61, 71, 72]; pertussis-toxin-sensitive decrease in hormone-stimulated cAMP accumulation in the medullary thick ascending limb and cortical thick ascending limb [29, 31, 73]; inhibition of ADH's action in the collecting duct [28, 32, 74]; and the direct effect of Ca^{2+} on calbindin-D_{28k} mRNA in the distal convoluted tubule (DCT) [75]. Studies are currently underway in several laboratories to assess the role of the CaR as the mediator of the effects of extracellular Ca^{2+} on several aspects of renal function.

The model for the thick ascending limb shown in Figure 4 proposes that elevated levels of peritubular Ca^{2+} (or Mg^{2+}) reduce NaCl reabsorption and the magnitude of the transepithelial voltage, and hence Ca^{2+} and Mg^{2+} reabsorption, via a Ca^{2+}-sensing, receptor-dependent mechanism. In the thick ascending limb (TAL), the lumen positive potential, V_{te}, is the driving force for Ca^{2+} and Mg^{2+} transport via the paracellular pathway [76–79]. In turn, V_{te} is directly related to the magnitude of net NaCl absorption [63], which is stimulated by the integrated action of several hormones including ADH and the calciotropic hormones PTH and calcitonin [62, 63, 80]. Preliminary results from my own laboratory suggest that the CaR is expressed on basolateral membranes of TAL cells (unpublished observations). Thus peritubular Ca^{2+}-mediated activation of the CaR could influence divalent mineral reabsorption either by reducing hormone-stimulated cyclic AMP production or by directly modulating the activity of the salt transporters themselves. In fact, increases in extracellular Ca^{2+} mediate pertussus-toxin-sensitive

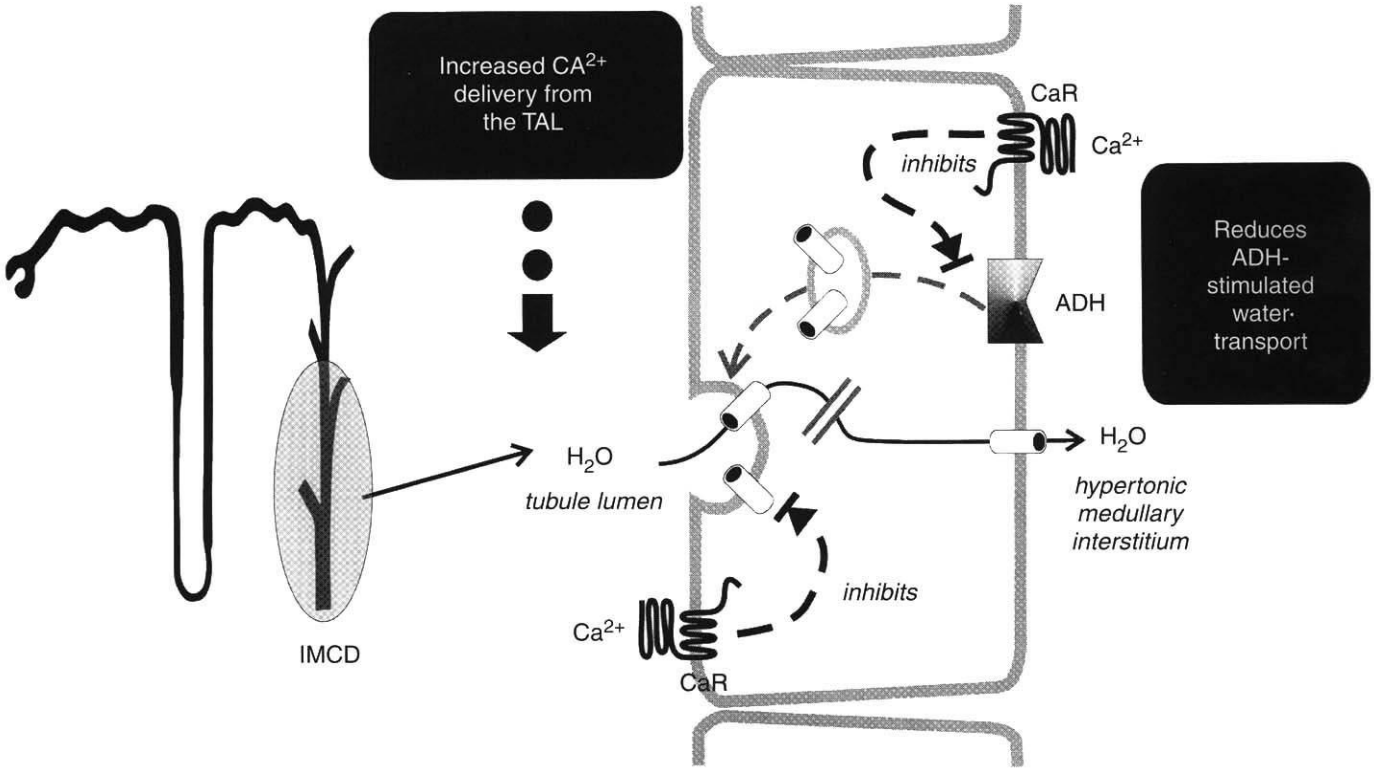

Fig. 5. *Role of the extracellular Ca^{2+}-sensing receptor in the regulation of water reabsorption in the terminal collecting duct.* Peritubular Ca^{2+} can influence ADH-stimulated cAMP production. In addition, luminal Ca^{2+} reduces ADH's ability to stimulate the activity of aquaporin-2 (APQ2) water channels in the apical membrane. The net effect of these CaR-mediated processes is a reduction in ADH-stimulated water permeability of the collecting duct.

reductions in cyclic AMP generated by these hormones [29, 31, 73], and the CaR likely mediates this effect. Could extracellular Ca^{2+} via the CaR also modulate any of the transporters involved in NaCl absorption by the TAL? This possibility was recently examined by Wang and coworkers [81, 82], who assessed the effects of extracellular Ca^{2+} on the 70 pS inwardly rectifying K^+ channel, which provides the major apical membrane potassium permeability of TAL cells [83]. Recycling of K^- via this channel not only supplies cation for Na^+-K^+-$2Cl^-$ cotransport but also is crucial to the generation of V_{te} [63, 83]. Wang et al showed that increasing extracellular Ca^{2+} (as well as neomycin, a polyvalent organic cation that is a potent agonist of the CaR [1, 2, 8]) reversibly reduced K^+ channel activity via a P450 oxygenase metabolite [82]. This reduction in the apical K^+ conductance of the TAL would be expected to reduce both NaCl and Ca^{2+} (or Mg^{2+}) reabsorption and markedly increase urinary Ca^{2+} excretion similar to that which occurs with the administration of furosemide [84, 85].

It is also possible that extracellular Ca^{2+} might modulate NaCl and/or Ca^{2+} transport in the DCT during hypercalcemia via a direct interaction of extracellular Ca^{2+} with the Ca^{2+}-sensing receptor. Thiazide-type diuretics, which block the apical Na^+-Cl^- cotransporter in the DCT, dissociate Na^+ and Ca^{2+} reabsorption by this nephron segment [86, 87]; this dissociation has been attributed to effects of cellular Na^+ uptake on either cellular Ca^{2+} entry [88] or exit [86] mechanisms. This inverse relationship between Ca^{2+} and Na^+ transport in DCT cells [88, 89] could account for reduced Ca^{2+} reabsorption by the DCT during hypercalcemia. In other words, the increased delivery of Na^+ to the DCT from the TAL would tend to decrease the ability of the DCT to reabsorb Ca^{2+} and hence help promote the calciuria. Clearly, this issue requires further study.

Several studies also suggest a potential role for the CaR in water handling by the collecting duct. These actions are depicted in Figure 5. Previous studies had suggested an effect of peritubular Ca^{2+} on modulating vasopressin-stimulated increases in water permeability of the cortical collecting duct [28, 32]. Moreover, a recent preliminary study also suggests an important role for tubular urine Ca^{2+} concentration in regulating the water permeability of the inner medullary collecting duct (IMCD) from rat kidney [74]. Baum and colleagues have shown that the CaR and aquaporin-2 co-localize to apical membranes of the IMCD and that raising the Ca^{2+} concentration in tubular fluid reversibly reduces the magnitude of ADH-stimulated water permeability of this terminal nephron segment. Thus, in the collecting duct there may be a Ca^{2+}-sensing function via the CaR from both basal (interstitial or circulating) and tubular urinary sides. More studies of the effects of extracellular Ca^{2+} on water and other solute transport mechanisms in the collecting duct are required before we can fully understand the extent of the regulatory roles played by the CaR in these processes.

Summary of the integrated effects of the CaR on divalent mineral and water excretion

Let me summarize the proposed role of the renal extracellular Ca^{2+}-sensing receptor in integrating divalent mineral and water handling by the kidney (Fig. 6). The net effect of increased

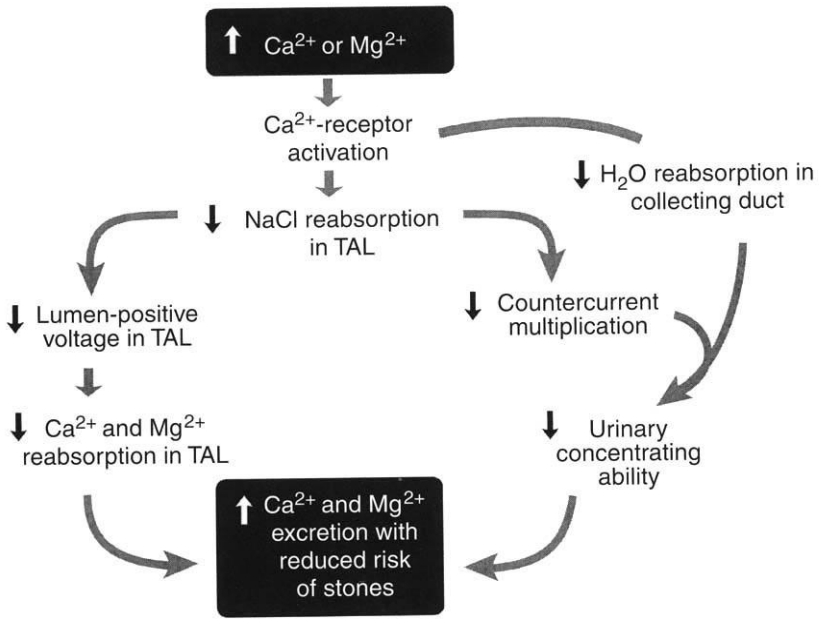

Fig. 6. *Proposed central role of the renal extracellular Ca^{2+}-sensing receptor* (CaR) *in integrating divalent mineral and water handling by the kidney.* With physiologic transient hypercalcemia, this CaR-mediated reduction in concentrating ability would provide a regulatory mechanism ensuring that the increased delivery of Ca^{2+} and Mg^{2+} from the thick ascending limb (TAL) to the collecting duct would be excreted in a more dilute urine, thereby decreasing the risk of crystal/stone formation. A similar mechanism could account for the nephrogenic DI commonly observed in patients with pathologic or chronic hypercalcemia.

basolateral (peritubular) Ca^{2+} on the TAL is a "loop"-diuretic-like action. This effect not only would reduce NaCl reabsorption and hence countercurrent multiplication, but also would decrease the lumen-positive V_{te} in TAL, which is the driving force for Ca^{2+} and Mg^{2+} transport via the paracellular pathway. An increased load of Ca^{2+} (and/or Mg^{2+}) then would be delivered to the collecting duct. Direct effects of extracellular Ca^{2+}, acting via the CaR to reduce the ADH-stimulated water permeability of the collecting ducts would add further to the reduction in urine concentration brought about by effects of extracellular Ca^{2+} in the TAL. Particularly important would be the potential direct influence of the tubular urine Ca^{2+} concentration itself, as it would help to integrate directly changes in distal Ca^{2+} delivery with the magnitude of water reabsorption in the terminal collecting duct. Thus the kidney has a built-in divalent mineral regulatory mechanism (that is, the CaR) that helps ensure that Ca^{2+} and Mg^{2+} are excreted at concentrations below saturation. During pathologic or chronic hypercalcemia this mechanism could account for the nephrogenic diabetes insipidus commonly seen with this electrolyte disorder [25]. As demonstrated by the case presentation, despite this integrated mechanism, stone formation still occurs under certain circumstances.

Questions and answers

DR. NICOLAOS E. MADIAS (*Chief, Division of Nephrology, New England Medical Center, Boston, Massachusetts*): Two other cations that come to mind in terms of possible interaction with the Ca^{2+}-sensing receptor are lithium and aluminum. Lithium is a monovalent cation that occasionally leads to hyperparathyroidism. On the other hand, in-vitro work indicates that aluminum, a trivalent cation, inhibits PTH release from parathyroid cells, and aluminum-related bone disease is often associated with decreased PTH. Do these cations bind to the receptor?

DR. HEBERT: Ed Brown showed years ago that lithium induces abnormal calcium-regulated release of PTH from dispersed bovine parathyroid cells. Since the calcium-sensing receptor mediates the regulation of PTH secretion, when you reduce calcium sensing, PTH secretion increases and hyperparathyroidism results. Some patients on lithium will have a disease like primary hyperparathyroidism, which is most often reversible by eliminating the lithium. In some limited studies we've begun to look at that. It's not surprising that monovalent cations have some effects on this calcium sensor. The binding of calcium to the receptor is thought to involve acidic negatively charged amino acids mainly located in the large extracellular domain, and other cations could alter calcium-binding interactions.

DR. MADIAS: Isn't it somewhat surprising that a system like calcium homeostasis that is so tightly regulated would have a receptor recognized by several ligands other than Ca^{2+} and exhibiting such a substrate promiscuity?

DR. HEBERT: The promiscuity may be a consequence of physiologically appropriate millimolar affinity of this receptor for calcium. This sensor responds to small variations in calcium in a virtual "sea" of calcium (and other ions). If it were a high-affinity calcium ion receptor, all calcium-binding sites would be fully saturated at normal circulating calcium concentrations, and the receptor would be continuously activated and unaffected by changes in circulating calcium. Multiple (low-affinity) calcium-ion-binding sites may function in a cooperative fashion to account, at least in part, for the steep relationship between extracellular ionic calcium and PTH release or renal calcium excretion. The nature of the calcium-binding sites thus might account for the ability of other ions to interact with these sites. Consequently, other cations (inorganic ions or organic drugs) could interfere with calcium sensing.

DR. BESS DAWSON-HUGHES (*USDA Human Nutrition Research Center on Aging, Tufts University School of Medicine, Boston*): Do you believe that the calcium sensor has anything to do with the hypertension that is associated with hyperparathyroidism?

DR. HEBERT: That's an interesting question, but we don't have an answer for it. If we had to speculate, one of the areas we would offer as playing a potential role would be the effects of the

receptor in the central nervous system. I discussed one locus, the subfornical organ, but the receptor is also expressed in other regions of the brain. Could there be a role for the calcium receptor in the central regulation of blood pressure? It would be premature to speculate too much other than to say that it's possible that there could be many effects of calcium on brain and possibly vascular function. Some of these cells might be responding to local changes in calcium that may be related to neuronal activity. Local changes in calcium could provide important signals to groups of functioning neurons in the central nervous system, including those involved in blood pressure control.

DR. JEFFREY TATRO (*Division of Endocrinology, New England Medical Center*): Is there evidence for other variants of the receptor, or is there only a single gene?

DR. HEBERT: So far, we think it's a single gene. There is another potential calcium-sensing molecule that has been identified by Lundgren et al, which is a member of the LDL receptor family, a GP330-like molecule that is quite interesting [91]. It's clear that from the FFH-NSHPT studies that the receptor that we have cloned is the primary sensor in the parathyroid gland. This does not exclude the possibility that associated molecules or other receptors can modulate (or provide) ion sensing in certain other tissues. We certainly can't exclude the possibility that other genes are involved in ion sensing. One could postulate sensors for potassium, phosphate, sodium, or protons, for example. Another aspect of this that makes such a speculation rational is the fact that if one looks at the potential structure of the extracellular domain, one can find a similarity between the extracellular domain of the metabotropic glutamate receptors and the calcium-sensing receptor. Several investigators have studied the structural homologies of the extracellular domain with other proteins. They identified significant similarities between the extracellular domain of the metabotropic glutamate receptors and a group of proteins that are called periplasmic nutrient-binding proteins found in bacteria with cell walls. These small molecules, many of which have been crystallized, have known structures. These proteins are free-floating in the periplasm between the bacterial cell membrane and the cell wall. As nutrients enter through pores in the cell wall, these specific proteins bind these nutrients and complex with bacterial permeases. The latter provides for transport of the nutrient into the bacterial cell. Many different bacterial nutrient-binding proteins are known, and several of the nutrients include ions. Thus, if these proteins and the calcium-sensing receptor have a common evolutionary origin, there could exist receptors in humans for other ions or nutrients.

DR. ANDREW J. KING (*Division of Nephrology, New England Medical Center*): A frequent clinical finding in patients who are hypercalcemic is severe volume depletion. Do you think the mechanism by which that occurs relates to inhibition of the potassium channel by the calcium receptor?

DR. HEBERT: Yes, we think that the calcium receptor will be proven to be a major regulator of salt, as well as of divalent mineral reabsorption, in the thick limb. Calcium-mediated activation of the receptor leading to inhibition of the apical potassium channel would produce an effect similar to the response to furosemide on thick limb function. To that extent, the patient would lose sodium. In fact, one can detect small increases in sodium excretion with transient hypercalcemia.

DR. KING: Is the receptor sensitive to monovalent cations such as sodium?

DR. HEBERT: Probably. As I said earlier, we expect that a number of monovalent ions alter calcium ion interactions with the receptor.

DR. MADIAS: In vitro, dispersed parathyroid cells exhibit substantial heterogeneity in their PTH response to changes in the Ca^{2+} concentration of the medium [92, 93]. Is it known whether the Ca^{2+}-sensing receptor is involved in this variability, for example due to different complement of receptors?

DR. HEBERT: You do see differences in staining among groups of cells in the parathyroid. This is particularly prominent if one looks at parathyroid tissue from primary and uremic secondary hyperparathyroidism [94]. In secondary hyperparathyroidism, the gland contains what looks like islands of cells that have a normal amount of receptor surrounded by many cells with a very low complement of receptor; this finding probably reflects the clonal nature of the cellular hypertrophy.

DR. AJAY K. SINGH (*Division of Nephrology, New England Medical Center*): You suggested that activation of the calcium-sensing receptor due to high prevailing plasma levels of calcium and/or magnesium modulates urinary excretion of the corresponding divalent cation. Could you elaborate on calcium-sensing receptor function when there is hypocalcemia with a normal, or perhaps even an elevated, magnesium level or the converse, a low magnesium level with a normal calcium?

DR. HEBERT: That's an important question. As I discussed, it takes a much higher concentration of magnesium than calcium to activate the receptor. That partially answers the question you asked, but it raises an additional question: is this receptor a magnesium sensor, at least in certain tissues? Can the calcium receptor sense normal concentrations of magnesium in the kidney? Quamme and others have shown over the last two decades that, in the thick ascending limb, hypermagnesemia in rats dramatically affects loop function with regard to sodium chloride reabsorption as well as magnesium and calcium reabsorption [20, 21]. Thus extracellular magnesium is clearly involved somehow in modulating thick ascending limb function. It is possible that in the thick ascending limb, where calcium and magnesium reabsorption normally occur, that is, ions moving from urinary space to the basal side of the cell in an epithelium where there is virtually no water movement, the calcium and/or magnesium concentration on the basal side of the cell increases to levels that could activate or influence the receptor. In this way, magnesium reabsorption by the thick limb could influence both calcium and magnesium reabsorption.

DR. MADIAS: I'm trying to think of some other clinical disorders in which the Ca^{2+}-sensing receptor might be dysregulated. One disorder that comes to mind is familial idiopathic hypercalciuria. Another is Bartter's syndrome. Could you speculate on these possibilities?

DR. HEBERT: We've thought along these lines as well and are currently investigating the possible involvement of receptor dysregulation in idiopathic hypercalciuria and Bartter's. Lifton's group at Yale has made significant advances in the identification of genes involved in autosomal recessive forms of Bartter's syndrome, including the Gitelman's variant of Bartter's syndrome [95, 96]; these forms are characterized by inherited hypokalemic alkalosis as well as alterations in divalent mineral excretion. Gitelman's disease is caused by mutations in the thiazide-sensitive Na^{+}-Cl^{-} cotransporter that presumably result in loss of function

of the cotransporter. We (unpublished observations) and Lemmink and coworkers [97] also have found linkage and/or mutations in the thiazide-sensitive Na^+-Cl^- cotransporter in other families with the Gitelman's phenotype, thus confirming the findings by Lifton's group. Gitelman's disease thus can be regarded as a dysfunction of salt transport in the distal convoluted tubule. Individuals with Gitelman's disease are characterized by hypomagnesemia (with hypermagnesuria) and hypocalciuria. Because Na^+-Cl^- cotransporter activity presumably is reduced in this disease, the reduced excretion of calcium can be explained by this functional defect that mimics thiazide use. More recently Lifton's group identified mutations in the loop-diuretic-sensitive Na^+-K^+-$2Cl^-$ cotransporter as the molecular basis of Bartter's syndrome in certain families, whose affected members are characterized by hypokalemic alkalosis, severe volume depletion, and hypercalciuria [96]. Thus, at least in some patients with familial Bartter's syndrome, the abnormal gene is the furosemide (bumetanide)-sensitive Na^+-K^+-$2Cl^-$ cotransporter in the thick ascending limb of Henle (TAL), and this disease can be thought of as a dysfunction of salt transport in the TAL. Loss of function of Na^+-K^+-$2Cl^-$ cotransport in the TAL would decrease calcium and magnesium reabsorption and increase their loss in the urine; this effect mimics the response to furosemide. It is possible that mutations in other genes may be identified in other families with the autosomal recessive form of Bartter's syndrome.

Although dysfunction in the Ca^{2+}-sensing receptor in the TAL would be expected to alter TAL function, I think that it is unlikely that the Ca^{2+}-sensing receptor gene is directly involved in the molecular basis of Bartter's syndrome, because one would expect not only abnormalities in divalent handling by the kidney but also altered parathyroid-PTH secretory function. The potential role of the Ca^{2+}-sensing receptor in familial idiopathic hypercalciuria is currently under investigation.

DR. MADIAS: Is any work being done on the regulation of the receptor, and are there any recognized hormonal effects on the receptor?

DR. HEBERT: To date, we have not seen any effector systems that have a significant impact or influence on receptor density.

DR. MADIAS: Is the Ca^{2+}-sensing receptor involved in other calcium-related phenomena, such as renal hemodynamics, renin release, calcitonin secretion, or bone resorption?

DR. HEBERT: This is a very interesting and important question. We and others are looking at these issues, and answers may be forthcoming.

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