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**REVIEW ARTICLE**

# The Many Roles of the Calcium-Sensing Receptor in Health and Disease

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The physiological relevance of calcium in many vital processes requires that its concentration in extracellular fluids be kept within a narrow range. The near-constancy of this parameter emphasizes the remarkable sensitivity of cells sensing changes in extracellular calcium concentration to minimal fluctuations (<2%) and the level of sophistication of the homeostatic system (1).The identification of a cell surface, $Ca^{2+}$(polyvalent cation)-sensing receptor (CaR), has shed considerable light on the molecular aspects of hypercalcemia on cell function (2). Activation of the receptor by calcium triggers an intracellular cascade of second messengers producing a variety of biological effects, many of which have yet to be understood. This suggests, for the first time, that $Ca^{2+}$ can exert its effects in a hormone-like fashion without crossing the plasma membrane. The demonstration that inherited genetic disorders of $Ca^{2+}$ homeostasis are associated with mutations that reduce or enhance responsiveness of the receptor to extracellular $Ca^{2+}$ concentration clearly proposes CaR as the main regulator of divalent mineral ion excretion (3). This hypothesis is confirmed by the assessment of the presence of the receptor in all regions involved in $Ca^{2+}$ homeostasis (e.g., parathyroid glands, kidney, calcitonin-secreting C cells, bone-derived cell lines, and intestine) (1,4–8). Recently, the receptor has also been found in regions not normally involved in mineral ion metabolism, such as the brain, eye, stomach, and pancreas (9–13). This clearly indicates a much broader relevance of CaR in the maintenance of local ionic homeostasis and, possibly, in the involvement in vital processes such as the regulation of cell fate. © 2000 IMSS. Published by Elsevier Science Inc.

*Key Words:* Extracellular calcium $[Ca^{2+}]_o$, Intracellular calcium $[Ca^{2+}]_i$, G protein-coupled receptor, Hypercalcemia, Mineral ion metabolism.

## Introduction

Extracellular calcium is essential for a number of vital processes, including bone mineralization, blood coagulation, regulation of enzymatic activity, and the modulation of permeability and excitability of plasma membranes. For these reasons, its concentration in extracellular fluids is under strict control by a complex homeostatic system that includes the parathyroid and thyroid glands, kidney, bones, and intestines (1). We have recently demonstrated that changes in the concentration of extracellular $Ca^{2+}$ ($[Ca^{2+}]_o$) are constantly monitored by a variety of specialized cells present throughout the body (2). In these cells, $Ca^{2+}$-sensing occurs through a membrane protein known as the extracellular $Ca^{2+}$ (polyvalent cation)-sensing receptor (CaR), using a molecular mechanism similar to that utilized by receptors for calciotropic hormones. Stimulation of the receptor evokes a variety of intracellular signal transduction pathways, which in turn produce diverse biological effects in different cell types. This review will report on the identification of the first cell-surface, ion-sensing receptor and the many roles identified of the receptor inside and outside the $Ca^{2+}$ homeostatic system (Tables 1 and 2).

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**Table 1.** Evidence for CaR expression in cells controlling systemic $Ca^{2+}$ homeostasis

| Tissue/cell | Reference |
|---|---|
| Parathyroid gland | |
| Bovine | (2) |
| Human | (31) |
| Kidney | |
| Rat | (4) |
| MDCK cells | (34) |
| Thyroid | |
| Calcitonin-secreting cells | (5) |
| Intestine | |
| Rat | (8) |
| $CaCo^2$ cells | (35) |
| T84, HT-29, CaCo2 | (77) |
| Bone and bone-derived cells | |
| MC3T3-E1 | (6) |
| ST2 | (7) |
| Mature osteoclasts | (78) |
| Placenta | (79) |
| Chondrocytes | (80) |

## Molecular Identification of the First Cell Surface, $Ca^{2+}$(Polyvalent Cation)-Sensing Receptor

Several observations were conducted as a prerequisite for the molecular identification of the first $Ca^{2+}$(polyvalent cation)-sensing receptor from bovine parathyroid and subsequently from rat kidney. First, it was known that inhibition of parathyroid hormone (PTH) secretion by increases in $[Ca^{2+}]_o$ in parathyroid cells was likely mediated by one or more G protein-coupled receptors, because stimulation of the putative receptor was able to activate membrane enzyme phospholipase C (PLC). This was to increase arachidonic acid production and reduce production of the second messenger, cyclic adenosine monophosphate (cAMP) (reviewed by Reference 1). PLC activation, in turn, resulted in degradation of membrane phospholipids with a subsequent increase in intracellular inositol 1,4,5-trisphosphate ($IP_3$) levels, diacylglycerols, and $Ca^{2+}$ concentration, released from internal stores (1). Second, *Xenopus laevis* oocytes have been successfully used to identify receptors coupled to the PLC-inositol triphosphate-induced rise in intracellular $Ca^{2+}$, because oocytes endogenously express $Ca^{2+}$-activated $Cl^-$ channels, whose activity is readily measured using standard electrophysiology methods (14). Using this expression system, Nemeth and Scarpa (15) recorded large, agonist-dependent $Ca^{2+}$-activated $Cl^-$ currents when oocytes were injected with messenger RNA extracted from bovine parathyroid glands. And thirdly, indirect observations on parathyroid cells reported that, in addition to $Ca^{2+}$, another divalent cation—$Mg^{2+}$—as well as trivalent cations of the lanthanide series (e.g., gadolinium, $Gd^{3+}$), were able to evoke intracellular responses virtually indistinguishable from those induced by high $[Ca^{2+}]_o$ (1). The latter became a priceless tool for cloning CaR, since the use of non-permeable agonists of the receptor (e.g., $Gd^{3+}$ and neomycin) allowed differentiation of pure, receptor-mediated responses from other indirect actions of $Ca^{2+}$, which could readily cross the membrane.

Given the previous observations, we therefore utilized *Xenopus laevis* oocytes as an expression system to isolate a complementary DNA (cDNA) encoding an extracellular CaR from bovine parathyroid glands (2). As in parathyroid glands, the isolated receptor expressed in oocytes can be activated by changes in a concentration of extracellular $Ca^{2+}$ $Mg^{2+}$, $Gd^{3+}$, or neomycin, with $IC_{50}$ similar to those indicated for native tissue (1). Activation of the receptor triggers $IP_3$ production and an increase in intracellular $Ca^{2+}$, arising from internal stores (2), as well as inhibition of cAMP accumulation in CaR-transfected human embryonic kidney cells (HEK293) (16). While a great deal of information is available concerning signal transduction pathways downstream of receptor activation, little is known about cellular targets of the receptor and stimulus-secretion coupling of the receptor in the parathyroid glands.

As illustrated in Figure 1, the CaR amino acid sequence predicts three domains: (1) a large, extracellular amino-terminus domain featuring nine potential N-linked glycosylation sites shown to be essential for normal expression of the receptor at the cell surface (17); (2) a central domain featuring seven membrane-spanning $\alpha$ helices, characteristic of the G protein-coupled receptor superfamily; and (3) a large cytosolic carboxy-terminus domain containing several putative consensus sequences for protein kinase A-(PKA) and protein kinase C-(PKC)-dependent phosphorylation processes (1).

When compared to other known proteins, there is little-to-no homology between CaR and other members of the superfamily of G protein-coupled receptors, with the sole

**Table 2.** CaR in cells outside the $Ca^{2+}$ homeostatic system

| Tissue/cell | Putative function | Reference |
|---|---|---|
| Brain | Secretion/learning | (9) |
| Microglia | Ionic homeostasis | (81) |
| Pituitary cell line | Hormone secretion | (82) |
| Eye | Ionic homeostasis | (10) |
| Stomach | Acid secretion | (11) |
| Intestine | Cell proliferation | (8) |
| Pancreas | Cell proliferation | (13) |
| Insulinoma cells | Hormone secretion | (83) |
| Breast cells | Secretion/cell proliferation | (12) |
| Ovarian cells | Cell proliferation | (33) |
| Prostate carcinoma cells | Cell proliferation | (76) |
| Leydig cells | Ionic homeostasis | (84) |
| Skin fibroblasts | Cell proliferation/ differentiation | (33) |
| Keratinocytes | Cell proliferation/ differentiation | (75) |
| Cytotrophoblast | Ionic homeostasis/ differentiation | (85) |
| Bone marrow | Cell proliferation/ differentiation | (86) |

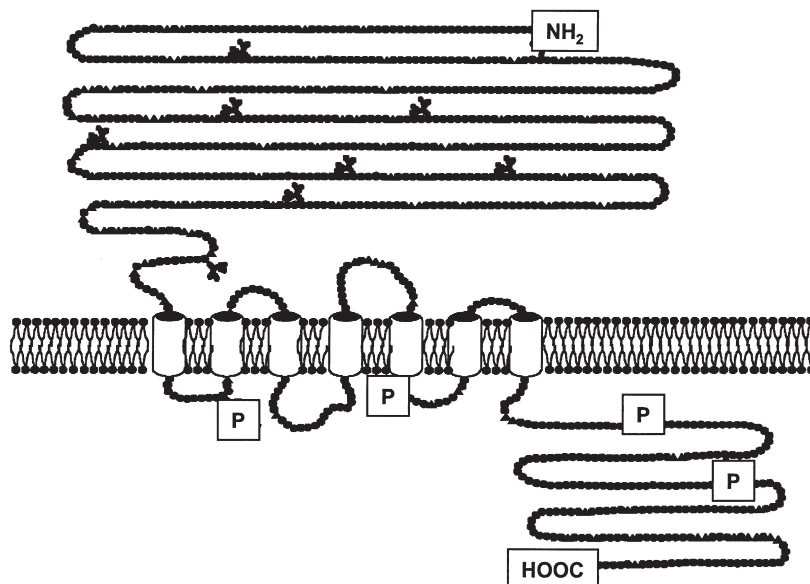


**Figure 1.** The extracellular $Ca^{2+}$-sensing receptor.

exception of metabotropic glutamate receptors (mGluR) (identity of about 23–27%) (2,18). Even though the entire protein is taken into consideration, this degree of identity does not constitute a striking similarity between CaR and mGluRs. The regions of homology are confined to precise functional domains suggested as forming part of ligand binding and/or initiating signal transduction (2). Studies using chimeric receptors for extracellular domains of CaR and mGluRs have demonstrated that the amino terminus, together with the extracellular loops, are likely to form the binding pocket, or the ion-sensing apparatus, and confer specificity for the ligand (19). However, in general, agonists for CaR do not activate mGluRs and vice versa. This indicates two different functional domains for the two classes of receptors. In this regard, CaR exhibits in the amino-terminus domain several clusters of acidic amino-acid residues not present in mGluRs. It has been suggested that these clusters bind $Ca^{2+}$ at low affinity (2). The absence of high affinity $Ca^{2+}$-binding motifs is consistent with activation of the receptor by a $Ca^{2+}$ concentration in the millimolar range. Recently, members of a large family of receptors related with CaR have been found in the genome of the puffer fish, *Fugu rubripes* (20). *Fugu* genes are expressed in the nose and, based on predicted amino acid homology, these proteins are putative pheromone receptors (21,22).

Overall, the predicted tertiary structures of CaRs, mGluRs, and *Fugu* genes are reminiscent of another class of proteins known as bacterial nutrient periplasmic binding proteins, which sense extracellular ligands as part of the chemotaxis process or cellular uptake of extracellular nutrients (e.g., amino acids, sugars, ions) (23). Several members of this protein class have been crystallized and their quaternary structure resolved. All share a two-lobe structure with an open configuration in the absence of the ligand. They also share a closed conformation, in which the two lobes join together when the ligand is bound. Conklyn and Bourne (24) have compared this structure to a Venus flytrap. If this model is correct, CaR can be seen, from an evolutionary perspective, as an ancient molecule that can be the result of the fusion of a bacterial periplasmic binding protein and a serpentine receptor, which evolved before the development of calciotropic peptide hormone calcitonin and PTH (25). Even more intriguingly, this would additionally suggest additional role(s) of CaR in sensing other ions and/or the existence of other G-coupled sensors for other ions and small molecules. In this regard, recent investigations indicate that polycationic compounds such as the polyamines, spermine, and spermidine can activate CaR and evoke intracellular responses virtually identical to those induced by high $[Ca^{2+}]_o$, suggesting the relative promiscuity of the receptor in recognizing small organic molecules (26).

The receptor exhibits a cooperative response in the presence of the physiological circulating levels of $Mg^{2+}$ concentration (27), which could enhance agonist potency. Therapeutically, this could provide important insights in developing agonists and antagonists of receptor function that can lower PTH levels in states of hyperparathyroidism, as well as prevent hypercalciuria, nephrocalcinosis, and renal impairment. In fact, one of the calcimimetic compounds (NPS-R-568)—a small organic molecule—has been shown to prevent secondary hyperparathyroidism in rats with mild chronic renal failure due to partial nephrectomy. It is currently being used for clinical trials (28). Calcimimetic compounds act as positive allosteric activators (i.e., they do not work in the absence of $[Ca^{2+}]_o$), thus increasing sensitivity of CaR to $[Ca^{2+}]_o$ in a stereoselective fashion. These com-

pounds are safely used to decrease plasma levels of PTH and calcium in patients with primary hyperparathyroidism—to date, a disease refractory to pharmacological intervention. The compounds are also equally effective in lowering PTH plasma levels in dialysis patients with secondary hyperparathyroidism (28), and are likely to provide a major breakthrough in the treatment of primary and secondary hyperparathyroidism.

Recent work by Bai et al. has shown that the CaR receptor exists in the plasma membrane in the form of a dimer, and that intermolecular interactions are indispensable for full functional activity of the receptor (29).

Finally, several polyvalent cations and polycations are either endogenously produced or administered as a part of pharmacological therapy. Their ability to activate CaR could be responsible for several physiopathological conditions. For instance, the amyloid β-peptides massively produced in Alzheimer's disease can activate CaR. Ye et al. (30) have proposed that this could be one cause of the sustained elevations of intracellular $Ca^{2+}$ responsible for neuronal dysfunction and degeneration, the hallmarks of Alzheimer's disease. In another example, polycationic aminoglycoside antibiotics, frequently used in the treatment of gram-negative infections, are also agonists for the receptor (1). Because aminoglycosides are capable of activating the receptor, Brown et al. (2) and Riccardi et al. (4) have hypothesized that the toxic effect of these antibiotics in the kidney could be mediated through activation of CaR. Their effect could be even more severe if we consider that preliminary data from our laboratory indicate that CaR is more active at acidic pH (6.8–6.9), which is characteristic of the kidney proximal tubules.

Following the cloning of the bovine parathyroid CaR and using a homology-based strategy, similar receptors from several species have been identified not only in organs known to be involved in $Ca^{2+}$ homeostasis (i.e., human parathyroid and kidney) (31,32) (Table 1), but also in many other tissues with little or no apparent link to divalent mineral ion homeostasis (Table 2).

These findings reinforce the hypothesis of an evolutionary premature development of the receptor molecule as an elementary signal system for total body fluid—and electrolyte homeostasis and/or as a regulator of cellular differentiation, as recent evidence seems to indicate. Interestingly, recent work suggests a role for the receptor in the modulation of cell proliferation by activation of c-SRC kinase, as well as through extracellular signal-regulated, kinase 1/mitogen-activated protein kinase activity (33). In addition, in the renal distal tubular-derived cell line—the Madine-Darby canine kidney (MDCK) cells—stimulation of endogenous CaR in turn stimulates cJUN N-terminal kinase (JNK) (34). This might represent a mechanism by which CaR could regulate the expression of proteins involved in calcium transport. Finally, in the human colonic cell line CaCO-2, CaR stimulation depresses, through luminal calcium, the activity of the proto-oncogene c-myc (35), thereby preventing activation of detectable levels of tumor-promoting signals present during deficient dietary calcium intake (36). Comparative studies aimed at investigating the presence and physiological relevance of the receptor in species that occupy the lower steps of the evolutionary scale will provide a major contribution to the evaluation of this fascinating hypothesis.

### CaR and the $[Ca^{2+}]_o$ Homeostatic System

At least three calciotropic factors are responsible for maintaining normocalcemia through the regulation of skeletal release, intestinal absorption, and renal excretion of $Ca^{2+}$. These factors are PTH, produced by the parathyroid glands; calcitonin, produced in the thyroid gland, and $1,25(OH)_2D_3$, produced in the skin and activated by hydroxylation in liver and kidney (1). It is not surprising that the secretion of these calciotropic hormones is strictly regulated by $[Ca^{2+}]_o$ (Table 1). It is known, for example, that the relationship between circulating PTH levels and $[Ca^{2+}]_o$ is fitted by a steep, inverse sigmoidal curve (1). As Figure 2 shows, $IC_{50}$ for inhibition of PTH secretion by $[Ca^{2+}]_o$ is about 1.1–1.3 mM in normal humans (1). Thus, small changes in $[Ca^{2+}]_o$ have a tremendous impact on PTH secretion rate. As we will discuss later, $[Ca^{2+}]_o$ affects PTH secretion through stimulation of CaR in parathyroid glands.

Kidney function is also deeply affected by changes in $[Ca^{2+}]_o$. Under physiological conditions, 60% of plasma calcium is filtered through the glomerular capillaries, but is in turn almost completely reabsorbed along the nephron. Most of the $Ca^{2+}$ is reabsorbed in the proximal tubule and thick ascending limb, is secondary to sodium transport, does not require extra energy expenditure, and has a urinary excretion that is tightly correlated linearly to plasma $[Ca^{2+}]_o$ via calciotropic hormones (37). Both PTH and vitamin D

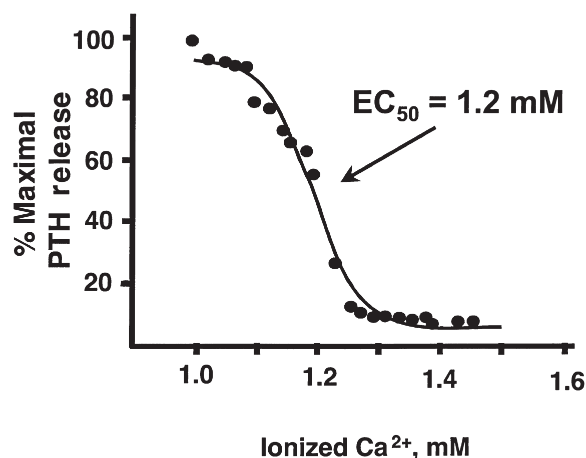


**Figure 2.** Relationship between circulating PTH levels and $[Ca^{2+}]_o$ is fitted by a steep, inverse sigmoidal curve.

control the set point for $Ca^{2+}$ reabsorption (38). However, in the absence of calciotropic mechanisms, the set point for $Ca^{2+}$ reabsorption becomes very low but surprisingly, the steep linear relationship between plasma and urinary $Ca^{2+}$ persists, suggesting the presence of a third calciotropic mechanism, i.e., extracellular $Ca^{2+}$ itself (38).

The discovery of CaR-associated genetic anomalies has triggered an intense search by pharmaceutical companies for compounds recently used successfully as calcimimetic compounds for the pharmacological treatment of hypercalcemic states and diseases, such as primary and secondary hyperparathyroidism (28). It is possible that inducible knockouts and the use of receptor antagonists not yet available will shed considerable light on CaR function in regions expressing the receptor.

## CaR in the Parathyroid

A large number of studies have clearly demonstrated the central role of CaR in the regulation of PTH secretion (1). These studies include measurements of PTH secretion in patients carrying inactivating or activating mutations in the CaR gene. As illustrated in Figure 3, an increase in $[Ca^{2+}]_o$ is sensed by CaR in plasma membranes of parathyroid gland cells, which in turn reduce PTH secretion. Hence, $[Ca^{2+}]_o$ decreases due to an increase in renal $Ca^{2+}$ excretion, a decrease in $Ca^{2+}$ absorption, and liberation from bone, which are all due to a reduction in PTH plasma levels.

The fundamental role of CaR in the $Ca^{2+}$ homeostatic system in humans has been clearly defined by analysis of the regulation of $[Ca^{2+}]_o$ in patients with the following two inherited diseases: familial (benign) hypocalciuric hypercalcemia (FBHH), and neonatal severe hyperparathyroidism (NSHPT) (39,40). These syndromes are due to loss-of-function mutations in the CaR gene. FBHH is a benign disease because it is the result of mutation in only one allele (heterozygous), whereas NSHPT is a severe disease, because of mutations in both alleles of the CaR gene, that is, the homozygous form of FBHH (40). NSHPT is a life-threatening condition characterized by severe hypercalcemia with skeletal manifestations of hyperparathyroidism, which usually requires parathyroidectomy early in onset.

FBHH is an autosomal dominant disease identified in patients initially diagnosed as having primary hyperparathyroidism, but in whom inappropriate renal tubular $Ca^{2+}$ reabsorption persisted even after parathyroidectomy (3). The observation that other relatives in the same families had asymptomatic hypercalcemia suggested the genetic nature of the disease. FBHH phenotype is characterized by moderate, usually asymptomatic, life-long elevations in serum $Ca^{2+}$ levels ($<$12 mg/dL) with PTH levels within the normal range, normal urinary concentrating ability, and (despite hypercalcemia) low urinary calcium excretion (calcium:creatinine ratio $<$0.01). FBHH is considered a benign disorder since hypercalcemia does not lead to renal stone disease or nephrogenic diabetes insipidus. Indeed, FBHH patients often exhibit hypocalciuria (41), suggesting altered $Ca^{2+}$-sensing by the kidney. It emerged from the previous study that, given the persistence of hypocalciuria after parathyroidectomy and the loss of linearity in the relationship between serum and urinary $Ca^{2+}$ concentration, FBHH must occur somewhere along the nephron (41). The authors observed that in FBHH patients, loop diuretic ethacrynic acid

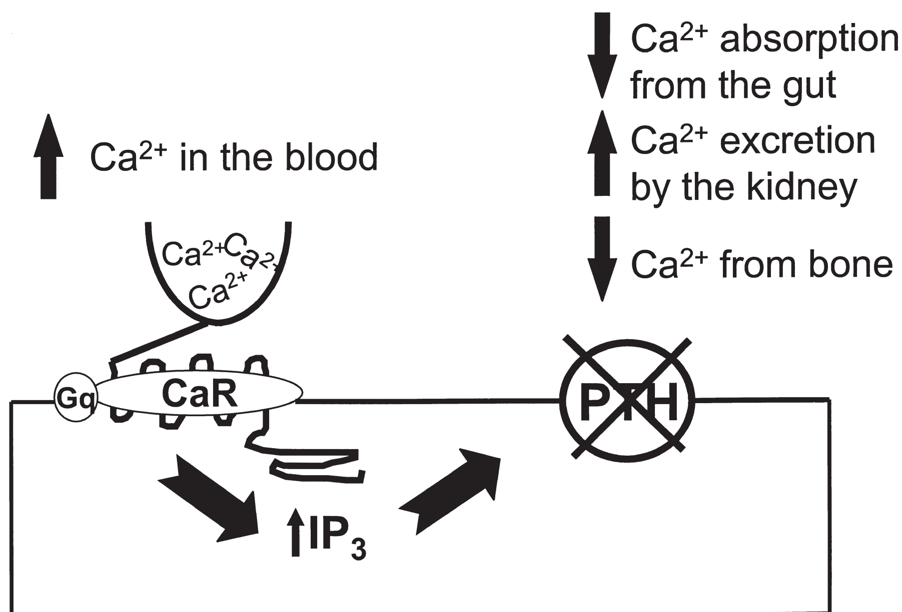


**Figure 3.** $[Ca^{2+}]_o$ regulates PTH secretion by stimulating the calcium-sensing receptor.

increased renal $Ca^{2+}$ excretion. Thus, they suggested the thick ascending limb as the possible site for reduced sensitivity to hypercalcemia and divalent cation reabsorption in FBHH patients.

Magnesium renal clearance is also reduced in FBHH patients (41), suggesting that in the kidney, CaR could also be a $Mg^{2+}$-sensor. This is interesting because it is known that both *in vivo* and *in vitro*, CaR sensitivity for $Mg^{2+}$ is threefold less than for $Ca^{2+}$. Thus, in normal conditions activation of CaR by circulating $Mg^{2+}$ concentration levels is usually improbable, in that $Ca^{2+}$ will affect CaR before $Mg^{2+}$ does. However, we cannot rule out the possibility of significant local changes that occur in the microenvironment present at the basolateral surface of thick ascending limb cells that can activate the receptor.

To date, a variety of mutations causing FBHH have been identified. So far, they fall within two general regions of the receptor. The vast majority of the mutations are localized in the first 300 amino-acid residues of the putative extracellular domain, while the remaining mutations are in proximity to the first transmembrane region, either upstream or downstream (42). Functional studies have been carried out by engineering CaR constructs for incorporating these mutations and by expressing them in *Xenopus laevis* oocytes or in HEK-transfected cells (43). In all circumstances, CaR containing FBHH-type mutants exhibited reduced responsiveness to $[Ca^{2+}]_o$ measured as second-messenger production, indicating that these mutations cause a decrease in CaR sensitivity for extracellular $Ca^{2+}$. There are, in addition, some sporadic, nonsense mutations that result in a premature stop, single-base deletion with consequent frameshift and early termination (44) and the insertion of an alu sequence in exon 7 of CaR (45). All result in loss of function of CaR. Bai et al. (46) have recently identified a novel heterozygous mutation in the CaR gene that may exert dominant negative action on the normal receptor, producing NSHPT-like syndrome with hypercalcemia more severe than usually observed in typical FBHH. It remains to be elucidated whether this occurs because mutant proteins synthesized by CaR from mutated allele prevent normal CaR of the normal allele from reaching the cell surface, or because of a direct binding of the mutated receptor with the wild-type, thereby reducing receptor-dependent activation of signal transduction pathways.

In 1995, Ho et al. (47) generated the murine equivalent for FBHH and NSHPT by targeted disruption of the CaR gene. As in humans, the heterozygous form, corresponding to FBHH, exhibits hypocalciuria with modest increases in serum $Ca^{2+}$, $Mg^{2+}$, and PTH levels. Homozygous mice show elevated $Ca^{2+}$ and PTH levels, bone abnormalities, retarded growth, and premature death, together with markedly reduced expression in the number of functional receptor molecules at the cell surface (47). The availability of this CaR knockout mice model will provide valuable insights into understanding which multiple effects of high $[Ca^{2+}]_o$ on many organs are indeed mediated by CaR and will also aid in elucidating the roles of the receptor in organs not belonging to the $Ca^{2+}$ homeostatic system.

There is evidence in the literature suggesting the possibility of at least one second gene implicated in FBHH. Linkage analysis has revealed that in the vast majority of FBHH families, the altered gene can be mapped to the long arm of chromosome 3 (39), where the CaR gene is located (32). However, two exceptions exist. In one family, the defect is located at chromosome 19 (48), and in another family no linkage was observed with either chromosome 3 or chromosome 19 (49). These studies suggest the genetic heterogeneity of FBHH and support the hypothesis that different receptor isoforms (and/or modulatory subunits) might exist.

In addition to these loss-of-function mutations, Pollak et al. (50) identified activating or gain-of-function mutations of the CaR gene that cause an interesting form of autosomal dominant hypocalcemia (ADH). Subsequently, Pearce et al. (51) studied six members of the same family diagnosed with autosomal dominant hypoparathyroidism based on hypocalcemia with hypercalciuria and normal serum PTH levels. When these patients were treated with vitamin D, hypercalciuria worsened and resulted in nephrocalcinosis and renal failure. Analysis of single-strand conformational polymorphisms identified gain-of-function mutations in the CaR gene. In five of the six patients, mutation was localized in the extracellular amino-terminal domain, indicating an enhanced affinity for extracellular $Ca^{2+}$. Pathological manifestations occurring after vitamin-D treatment can be explained in terms of increased reactivity of the homeostatic system to serum $[Ca^{2+}]_o$. Functional expression of receptor constructs carrying these activating mutations in HEK-293 cells revealed a shift toward the left of the dose-response relationship between extracellular $Ca^{2+}$ and $IP_3$ production (43), suggesting that gain-of-function mutations result in increased sensitivity of CaR for extracellular $Ca^{2+}$.

## CaR in the Kidney

A large number of indirect observations have indicated that hypercalcemia can directly alter many aspects of renal function. For instance, increases in $[Ca^{2+}]_o$ reduce glomerular filtration rate, modulate the renin-aldosterone axis (52), and induce renal vasoconstriction (53). As Figure 4 shows, there is also a step correlation between ionized $Ca^{2+}$ and urinary $Ca^{2+}$ excretion with an $IC_{50}$ of ~1.2 mM, indicating that small changes in $[Ca^{2+}]_o$ have tremendous effects on renal handling of $Ca^{2+}$.

In the proximal tubule (PT), $Ca^{2+}$ inhibits 1-hydroxylation of $25(OH)D_2$ (54) as well as PTH-induced second-messenger production (55). In thick ascending limb (TAL) and cortical collecting duct (CCD), hypercalcemia reduces hormone- (vasopressin, glucagon, calcitonin, PTH, and insulin) induced and cAMP accumulation (56). Effects of hypercal-

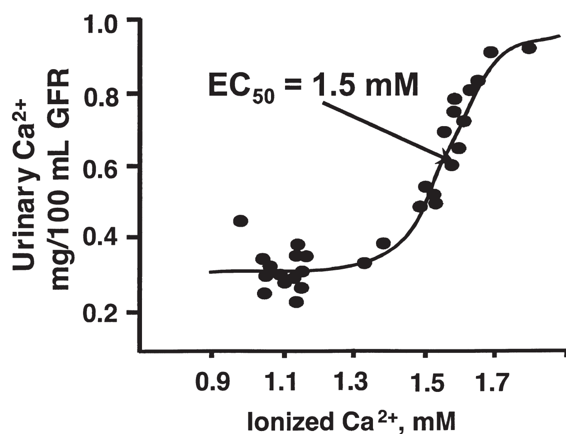


**Figure 4.** Relationship between urinary $Ca^{2+}$ excretion and $[Ca^{2+}]_o$ is fitted by a steep sigmoidal curve. The higher the $[Ca^{2+}]_o$, the higher the urinary $Ca^{2+}$ excretion.

**Table 3.** Localization and role of CaR inside the kidney

| Nephron segment | Membrane | Function |
|---|---|---|
| Proximal convoluted tubule | Apical | Pi transport* |
| | | Acidification;* Vitamin D synthesis;* $Na^+$:$K^+$:ATPase regulation* |
| Thick ascending limb of Henle | Basolateral | Reabsorption of NaCl/$Ca^{2+}$/$Mg^{2+}$; inhibition of apical $K^+$ channel; inhibition of $Na^+$:$K^+$:$2Cl^-$; reduction in cAMP |
| Macula densa cells; tubulo-glomerular feedback* | Basolateral | Renin secretion* |
| Distal convoluted tubule | Intracellular/ basolateral | $Ca^{2+}$ transport;* regulation of apical $Na^+$:$Cl^-$* |
| Collecting ducts | A-type; intercalated cells | Acidification* |
| Inner medullary ducts | Apical | Blunts cell response to vasopressin |

*Putative function.

cemia on TAL are reversed by pretreatment with pertussis toxin, reminiscent of mechanisms described for cultured bovine parathyroid cells (1), indicating an inhibitory α subunit of the G proteins at post-receptor site (57). In the same nephron segment, increases in peritubular $[Ca^{2+}]$ (and $[Mg^{2+}]$) directly inhibit reabsorption of these cations and produce a loop diuretic-like effect (57). Following molecular identification of rat renal CaR, proposal of the receptor as the best candidate for explaining some, if not all, effects of hypercalcemia on renal function was obvious. Several functional studies demonstrated the validity, at least to some extent, of this hypothesis.

To understand the role of CaR in the kidney, we examined intrarenal segmental and cellular distribution of CaR-related transcripts (58) and protein (59). Additionally, we assessed the presence of the receptor along the nephron. Interestingly, intrarenal cellular distribution is peculiar (Table 3). CaR is apical in the proximal tubules and the inner medullary-collecting duct (IMCD), but basolateral in the TAL and macula densa cells. The distal convoluted tubule (DCT) exhibits basolateral immunoreactivity and occasionally punctuate cytosolic, vesicle-like staining. Finally, in the CCD, CaR protein is confined at the basolateral membrane of A-intercalated cells (59).

Consistent with previous observations of FBHH patients (41), CaR mRNA and protein are mostly expressed in TAL. In this region, divalent mineral cation reabsorption is regulated by serum $Ca^{2+}$ levels through negative feedback on the transepithelial electrochemical potential gradient, usually positive toward the lumen, generating the driving force for passive $Ca^{2+}$ and $Mg^{2+}$ reabsorption (60) (Figure 5). Transepithelial lumen-positive electrical gradient in this nephron segment is generated by the following two systems present at the apical membrane of TAL cells simultaneously: (1) a loop diuretic-inhibitable, $Na^+$:$K^+$:$2Cl^-$ cotransporter, which removes $1Na^+$, $1K^+$, and $2Cl^-$ from the lumen without any net charge movement, and (2) apical, intermediate conductance $K^+$ channels, through which $K^+$ ions back-diffuse to the lumen, thus accumulating positive charges (25). Therefore, under physiological conditions, there is a net transcellular lumen-to-serosa transport of $1Na^+$- and $2Cl^-$ ions. Additionally, there is accumulation of $1K^+$ ion in the lumen that generates the electrical force responsible for a lumen-to-serosa transport of another cation, which could be $Na^+$, $Mg^{2+}$, or $Ca^{2+}$. In fact, inhibition of the $Na^+$:$K^+$:$2Cl^-$ cotransporter by loop diuretics constitutes the basis behind management of life-threatening hypercalcemia because inhibition of this mechanism reduces $Ca^{2+}$ reabsorption in TAL. Recent studies by Wang et al. (61) have shown that in rat TAL, increasing $[Ca^{2+}]_o$ reversibly inhibits apical $K^+$ channels, and that this effect is mediated by CaR through the arachidonic acid metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE). In addition, it is well known that 20-HETE directly inhibits activity of the $Na^+$:$K^+$:$2Cl^-$ (62). Hence, it is highly likely that CaR activation reduces divalent cation reabsorption in TAL by inhibiting both the $Na^+$:$K^+$:$2Cl^-$ cotransporter and the apical $K^+$ channels. In addition, as Figure 5 demonstrates, CaR-mediated inhibition of cAMP accumulation would also reduce stimulatory action of hormones acting through Gs-coupled receptors, such as vasopressin or PTH. The $Na^+$:$K^+$:$2Cl^-$ cotransporter can transport $NH_4^+$ instead of $K^+$, thus performing as $Na^+$: $NH_4^+$:$2Cl^-$. It is also responsible for most of the apical entry of transcellular ammonia absorption in medullary TAL (63). Therefore, it can also contribute to net urinary acid excretion by accumulating ammonia in the renal medulla, suggesting that CaR-mediated inhibition might be relevant in maintaining a correct acid-base balance (63).

The diuretic effect of hypercalcemia is enhanced by the

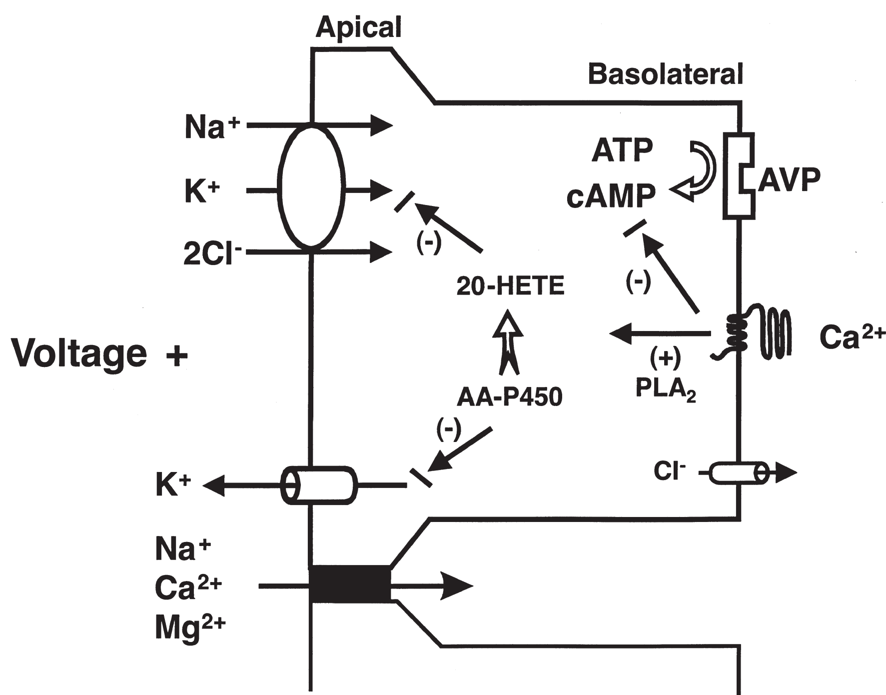


**Figure 5.** Model for action of extracellular $Ca^{2+}$ on divalent cation handling in the thick ascending limb of Henle.

presence of CaR at the apical surface of IMCD cells. As Figure 6 shows, activation of CaR in renal tubules has a dual effect that allows for an increase in $Ca^{2+}$ excretion while reducing water reabsorption as a means of preventing the formation of renal stones. In IMCD, CaR is expressed in the same vesicles as vasopressin-activated aquaporin 2 water channels. Sands et al. (64) showed that concentration of $Ca^{2+}$ within the tubular fluid regulates the function of these vesicles through CaR, resulting in a protective mechanism against stone formation. Reduction in $Ca^{2+}$ reabsorption in TAL when CaR is activated results in increased $Ca^{2+}$ concentration in the tubular fluid. Thanks to this mechanism, if luminal $Ca^{2+}$ concentration rises above a certain level, stimulation of CaR located on the aquaporin 2-containing apical vesicles will result in migration of these vesicles to a submembranal compartment, with subsequent reduction in water reabsorption. Thus, urine production will increase but $Ca^{2+}$ will not rise above certain levels to prevent stone formation. According to Hebert et al., in the case of persistent hypercalcemia, this mechanism could also explain the presence of nephrogenic diabetes insipidus frequently seen in hypercalcemic patients, thereby explaining the tradeoff of water preservation for divalent cation loss in terrestrial vertebrates (25).

It is now clear that $[Ca^{2+}]_o$ is a calciotropic hormone that therefore can modulate the effects of other hormones acting on the kidney. Why would this occur? One possible explanation is a fine-tuning of local perturbations, bypassing the total body feedback system. For example, it is known that $[Ca^{2+}]_o$ inhibits PTH-induced cAMP accumulation in PT and cortical TAL (55,57). In a previous study, we demonstrated that transcripts for CaR are present in the regions where PTH/PTHrP mRNA is expressed (e.g., glomerulus, PCT, PST, cortical TAL, DCT, and cortical CD) (58). This suggests a peripheral role of $[Ca^{2+}]_o$ on modulation of renal PTH action in addition to the central, inhibitory role of PTH secretion by the parathyroid glands.

More obscure and hypothetical is the role of the receptor in other nephron segments not directly involved in renal tubular divalent mineral cation handling (Table 3). In the macula densa, for example, in which the receptor is highly present basolaterally (59), CaR could sense changes in $[Ca^{2+}]_o$ and accordingly regulate tubuloglomerular feedback responses. In proximal regions, the receptor is present in the subapical compartment of both convoluted and straight tubules, in which it could mediate some reported effects of high $Ca^{2+}$ on 1-hydroxylation of 25-(OH)vitD (54) or PTH-induced cAMP production (55). In addition, we have recently hypothesized a role for the phosphate transport receptor in the regulation of local ionic homeostasis, bypassing systemic levels of PTH (59). Alternatively, the receptor could also be involved in bicarbonate transport. A possible involvement of the receptor in urinary pH regulation is suggested by the presence of CaR at the basolateral surface of A-type intercalated cells in the cortical CD. Because endogenous acid production enhances urinary $Ca^{2+}$ excretion and, in turn, urinary pH regulates divalent cation solubility, it is plausible to hypothesize a feedback mechanism exerted by CaR on $H^+$ excretion to prevent nephrolethiasis or nephrocalcinosis.

CaR is also expressed at the basolateral/intracellular as-

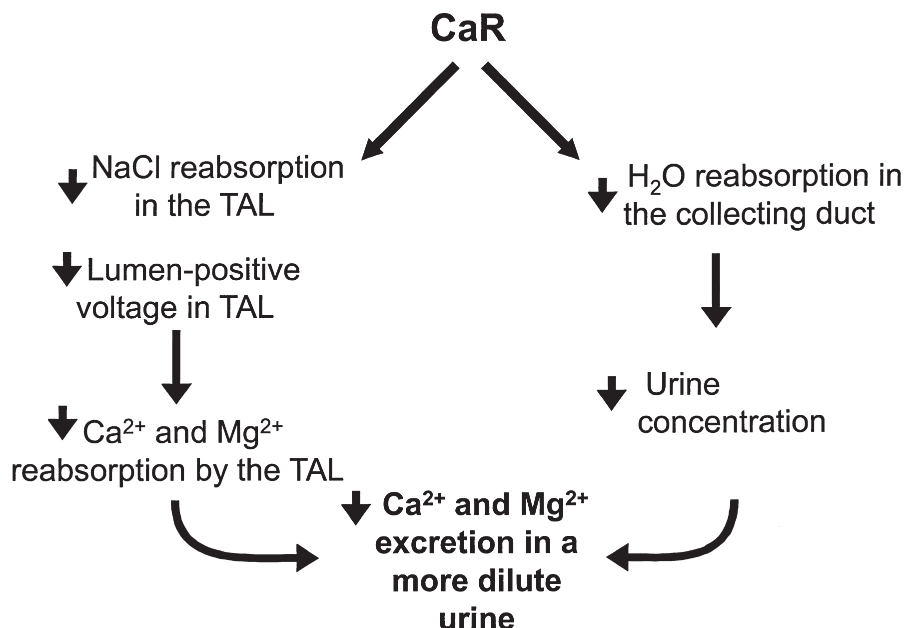


**Figure 6.** Dual effect of CaR activation on kidney function.

pect of DCT cells and occasionally, apically in a punctuate, in a vesicle-like pattern (59). In this nephron segment, $Ca^{2+}$ is reabsorbed transcellularly against a transepithelial potential difference (65). In DCT, $Ca^{2+}$ reabsorption is not accompanied by $Mg^{2+}$. It is strongly regulated by calciotropic factors and inversely related to $Na^+$ reabsorption (66). In addition, the calcium-binding protein calbindin $D_{28K}$ selectively increases $Ca^{2+}$ uptake into luminal membrane vesicles of rabbit distal nephrons (67), suggesting possible entry via the apical $Ca^{2+}$ channels (68). It is known that changes in $[Ca^{2+}]_o$ alter the expression of calbindin $D_{28K}$, but little is known concerning the effects of $Ca^{2+}$ on the transport system. Several putative effects could include modulation of the expression of calbindin $D_{28K}$ and, due to a similar immunoreactive distribution pattern, of the thiazide-sensitive $Na^+$:$Cl^-$ cotransporter via vesicular trafficking (69), following a mechanism analogous to that identified in the inner-medullary collecting duct for CaR-mediated regulation of aquaporin 2 (64). In addition, in early DCT calcitonin stimulates $Ca^{2+}$ reabsorption, natriuresis, and an $Na^+$-independent, $HCO_3^-$-dependent mechanism via an increase in cAMP content (70). Recent work has shown that Madine-Darby canine kidney (MDCK) cells, a model for DCT, express CaR and that stimulation of the receptor by $Ca^{2+}$ or $Gd^{3+}$ selectively elicits the α-inhibitory subunits of G proteins (i.e., αi-2 and -3) (71), thereby inhibiting intracellular cAMP content. These findings appear to validate the hypothesis of a role for CaR in urinary acid balance regulation by inhibiting calcitonin-induced cAMP accumulation during hypercalcemia.

Finally, little is known about the role and relevance of the receptor in the PT, macula densa, DCT, CCD, or the outer-medullary collecting duct. Current work is in progress to assess the role of the receptor in these regions in which the link with calcium metabolism is not apparent (Table 3).

### CaR in Bone and Intestine

Bone and intestine are an integral component of the $Ca^{2+}$ homeostatic system. Functional and molecular data have demonstrated the existence of CaR-like molecules, but our understanding of receptor roles in these two regions is still somewhat limited.

In osteoclasts, changes in $[Ca^{2+}]_o$ alter cell morphology and inhibit bone reabsorption, while in osteoblasts, raising $[Ca^{2+}]_o$ induces a proliferative response (1). Interestingly, recent work from Brown's laboratory indicates that the osteoblast-derived cell lines MC3T3-E1, UMR-106, and SaOS-2 also express CaR mRNA and protein, and that CaR agonists stimulate chemotaxis and proliferation (6,7). However, in bone, $[Ca^{2+}]_o$ can be as high as 26 mM (1), well above the range of CaR activation in other organs. Thus, it is difficult to explain the physiological role of such a receptor in regions where $[Ca^{2+}]_o$ is one order of magnitude higher than the physiological range of activation for the known CaR. This issue, together with a pharmacological profile differing from that reported for the previously cloned receptor (72), indicates that there might be additional CaRs and/or modulatory subunits in bone that regulate CaR sensitivity.

In the intestine, the receptor is present mainly at the base of the epithelial cells of small intestinal villi and crypts, basolaterally and apically in colonic crypts, and in the regions of Auerbach's myenteric and Meissner's plexi (8). Although we lack direct evidence to pin down the function of

the receptor in the intestine, the overall distribution pattern is consistent with a role for CaR in the modulation of absorptive and/or secretomotory functions (8).

## Distribution of CaR Outside the Calcium Homeostatic System

Recently, an increasing number of observations has demonstrated the presence of CaR in many organs not belonging to the $Ca^{2+}$ homeostatic system (Table 2). The receptor is heavily expressed in several regions of the central nervous system (9), such as hippocampus, cerebellum, olfactory bulbs, and ependymal areas of the cerebral ventricles. Many hypotheses have been formulated to explain the role of CaR in these areas, such as an involvement in long-term potentiation, learning, and other cognitive functions, and regulation of neurotransmitter release and non-selective cation channels causing membrane depolarization and neuronal excitability (73). Demonstration that polyamines such as spermine and spermidine stimulate CaR suggests that the brain may utilize these compounds as physiological agonists of the receptor (26).

CaR immunoreactivity has recently been found in the eye, where hypocalcemia has been associated with initiation of cataract formation (10). In the human lens, epithelial-cell activation of CaR directly modulates the activity of $K^+$ channels present in the epithelium. This has been indicated to play a role in ionic homeostasis of the lens (10).

The receptor is also heavily expressed in the gastrointestinal tract (8). In the human stomach, it is localized in antral gastrin cells (74), where it is proposed to modulate the acid-rebound phenomenon associated with calcium-containing antacid preparations (74). Recently, Bruce et al. showed that a CaR is expressed in both exocrine (acinar cells and pancreatic interlobular ducts) and endocrine (islet of Langerhans) components of rat pancreas (13). In the acini, CaR agonists induce an increase in intracellular $Ca^{2+}$ similar to that induced by the secretagogue, cholecystokinin, while in the pancreatic ducts, CaR regulates bicarbonate (and hence fluid) secretion (13). In the islet of Langerhans, CaR seems to be involved in the regulation of secretion of hormones regulating glycemia, because antibody studies show immunoreactivity confined to insulin and glucagon-secreting cells (13).

A recent study by Chattopadhyay et al. (8) identified the receptor along the entire intestine. This is very interesting, in that several studies appear to indicate a novel role for CaR in cell differentiation. In human colonic cell line CaCo2, for example, decrease in luminal $Ca^{2+}$ concentration is associated with up-regulation of the proto-oncogene c-myc. This effect could be mediated by a CaR expressed on the luminal membrane (35). Thus, this information suggests a new role for CaR in mediating the tumor-promoting effects of low luminal $Ca^{2+}$. This observation, together with preliminary epidemiological indications that dietary $Ca^{2+}$ load inhibits cell proliferation (36), suggests the intriguing possibility that CaR could be involved in cell cycle regulation. This hypothesis is supported by an analogous role of the receptor in differentiating keratinocytes, in which CaR is present as full-length, wild-type CaR and also as a splice variant lacking exon 5 that is unable to produce a $Ca^{2+}$-dependent increase in $IP_3$ production when transfected in keratinocytes (75). Interestingly, the balance between the two CaR isoforms is associated with the differentiation state, the splice variant absent at the beginning of the differentiation process and maximally expressed at the end.

Finally, a role for the receptor in programmed cell death has been proposed by Lin et al. (76). These authors showed that in prostate carcinoma cells, CaR can prevent apoptosis and provide a novel mechanism by which $Ca^{2+}$ ions can modulate cell survival.

## Conclusions

Molecular identification of novel cell-surface CaR from many tissues of different species has shown, for the first time, that changes in $[Ca^{2+}]_o$ act as first messengers and can evoke a variety of biological responses, some beyond extracellular calcium homeostasis. The ability of endogenous organic polyvalent cations and polycations to activate CaR indicates that the receptor can have different physiological agonists in different regions. On the one hand, this proposes CaR as a primitive ion-sensing mechanism. On the other hand, it proposes the intriguing perspective of the existence of similar mechanisms for other ions and/or small molecules. Demonstrating that mutations of the CaR gene are linked to disturbances in $Ca^{2+}$ metabolism has had considerable impact in understanding the urinary concentrating mechanism and mineral ion homeostasis under physiological and pathological conditions, such as hypercalcemic states of various natures. Clinical relevance of the assessment of these genetic diseases can eliminate unnecessary surgical procedures in FBHH individuals or irreversible renal damage when vitamin-D treatment is undertaken to normalize serum $Ca^{2+}$ in patients with autosomal-dominant hypercalcemia. Availability of CaR mutants has additionally allowed the development of organic calcium-like compounds that can either mimic the effects of $Ca^{2+}$ or act as positive allosteric modulators to enhance CaR sensitivity. Currently, these calcimimetic compounds are used to treat secondary hyperparathyroidism.

In the kidney, CaR actively participates in divalent mineral cation and water handling, minimizing the risk of nephrolithiasis and nephrocalcinosis during dehydration states. Broad distribution and different polarity of receptor expression throughout the body indicate additional roles for the receptor, possibly in ion transport regulation and in other phenomena such as cell proliferation and differentiation.

Our understanding of CaR receptor function in physiologic (and therefore pathologic) conditions is strongly limited

due to the lack of known pharmacological compounds that can act as CaR antagonists. Their availability will shed considerable light on CaR function in regions expressing the receptor, either inside or outside the $Ca^{2+}$ homeostatic system.

## References


1. Brown EM. Extracellular $Ca^{2+}$-sensing, regulation of parathyroid cell function and role of $Ca^{2+}$ and other ions as extracellular (first) messengers. Physiol Rev 1991;71:371.
2. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Hebert SC. Cloning and characterization of an extracellular $Ca(^{2+})$-sensing receptor from bovine parathyroid. Nature 1993;366:575.
3. Pollak MR, Seidman CE, Brown EM. Three inherited disorders of calcium sensing. Medicine 1996;75:115.
4. Riccardi D, Park J, Lee W-S, Gamba G, Brown EM, Hebert SC. Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. Proc Natl Acad Sci USA 1995;92:131.
5. Garrett JE, Tamir H, Kifor O, Simin RT, Rogers KV, Mithal A, Gagel RF, Brown EM. Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. Endocrinology 1995;136:5202.
6. Yamaguchi T, Chattopadhyay N, Kifor O, Butters RR, Sugimoto T, Brown EM. Mouse osteoblastic cell line (MC3T3-E1) expresses extracellular calcium $(Ca^{2+})_o$-sensing receptor and its agonists stimulate chemotaxis and proliferation of MC3T3-E1 cells. J Bone Miner Res 1998;13:1530.
7. Yamaguchi T, Kifor O, Chattopadhyay N, Brown EM. Expression of extracellular calcium $(Ca^{2+}{}_o)$-sensing receptor in the clonal osteoblast-like cell lines, UMR-106 and SAOS-2. Biochem Biophys Res Commun 1998;243:753.
8. Chattopadhyay N, Cheng I, Rogers K, Riccardi D, Hall A, Diaz R, Hebert SC, Soybel D, Brown EM. Identification and localization of extracellular $Ca^{2+}$-sensing receptor in rat intestine. Am J Physiol 1998;274:G122.
9. Ruat M, Molliver ME, Snowman AM, Snyder SH. Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. Proc Natl Acad Sci USA 1995;92:3161.
10. Chattopadhyay N, Ye C, Singh DP, Kifor O, Vassilev PM, Shinohara T, Chylack LT, Brown EM. Expression of extracellular calcium-sensing receptor by human lens epithelial cells. Biochem Biophys Res Commun 1997;233:801.
11. Cheng I, Qureshi I, Chattopadhyay N, Qureshi A, Butters RR, Hall AE, Cima RR, Rogers KV, Hebert SC, Geibel JP, Brown EM, Soybel DI. Expression of an extracellular calcium-sensing receptor in rat stomach. Gastroenterology 1999;116:118.
12. Cheng I, Klingensmith ME, Chattopadhyay N, Kifor O, Butters RR, Soybel DI, Brown EM. Identification and localization of the extracellular calcium-sensing receptor in human breast. J Clin Endocrinol Metab 1998;83:703.
13. Bruce JIE, Yang X, Ferguson CJ, Elliott AC, Steward MC, Case RM, Riccardi D. Molecular and functional identification of a $Ca^{2+}$(polyvalent cation)-sensing receptor in rat pancreas. J Biol Chem. In press 1999.
14. Dascal N. The use of *Xenopus* oocytes for the study of ion channels. Crit Rev Biochem Mol Biol 1987;22:317.
15. Nemeth EF, Scarpa A. Rapid mobilization of cellular $Ca^{2+}$ in bovine parathyroid cells evoked by extracellular divalent cations. Evidence for a cell surface calcium receptor. J Biol Chem 1987;26:5188.
16. Kifor O, Diaz R, Butters R, Brown EM. The $Ca^{2+}$-sensing receptor (CaR) activates phospholipases C, A2, and D in bovine parathyroid and CaR-transfected, human embryonic kidney (HEK293) cells. J Bone Miner Res 1997;12:715.
17. Fan G, Goldsmith PK, Collins R, Dunn CK, Krapcho KJ, Rogers KV, Spiegel AM. N-linked glycosylation of the human $Ca^{2+}$ receptor is essential for its expression at the cell surface. Endocrinology 1997;138:1916.
18. Masu M, Tanabe Y, Tsuchida K, Shigemoto R, Nakanishi S. Sequence and expression of a metabotropic glutamate receptor. Nature 1991; 349:760.
19. Hammerland LG, Krapcho KJ, Garrett JE, Alasti N, Hung BC, Simin RT, Levinthal C, Nemeth EF, Fuller FH. Domains determining ligand specificity for $Ca^{2+}$ receptors. Mol Pharmacol 1999;55:642.
20. Naito T, Saito Y, Yamamoto J, Nozaki Y, Tomura K, Hazama M, Nakanishi S, Brenner S. Putative pheromone receptors related to the $Ca^{2+}$-sensing receptor in *Fugu*. Proc Natl Acad Sci USA 1998; 95:5178.
21. Herrada G, Dulac C. A novel family of putative pheromone receptors in mammals with a topographically organized and sexually dimorphic distribution. Cell 1997;90:763.
22. Ryba NJP, Tirindelli R. A new multigene family of putative pheromone receptors. Neuron 1997;19:371.
23. O'Hara PJ, Sheppard PO, Thogersen H, Venezia D, Haldeman BA, McGrane V, Houamed KM, Thomsen C, Gilbert TL, Mulvihill ER. The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. Neuron 1993;11:41.
24. Conklyn BR, Bourne HR. The marriage of the flytrap and the serpent. Nature 1993;367:22.
25. Hebert SC, Brown EM, Harris HW. Role of the $Ca^{2+}$-sensing receptor in divalent mineral ion homeostasis. J Exp Biol 1997;200:295.
26. Quinn SJ, Ye C, Diaz R, Kifor O, Bai M, Vassilev P, Brown EM. The $Ca^{2+}$ receptor: a target for polyamines. Am J Physiol 1997;273:C1315.
27. Ruat M, Snowman AM, Hester LD, Snyder SH. Cloned and expressed rat $Ca^{2+}$-sensing receptor. Differential cooperative responses to calcium and magnesium. J Biol Chem 1996;271:5972.
28. Nemeth EF. Calcium receptors as novel drug targets. In: Bilezikian JP, Raisz LG, Rodan GA, editors. Principles of bone biology. New York: Academic Press;1996. p. 1019.
29. Bai M, Trivedi S, Kifor O, Quinn SJ, Brown EM. Intermolecular interactions between dimeric calcium-sensing receptor monomers are important for its normal function. Proc Natl Acad Sci USA 1999;96:2834.
30. Ye C, Ho-Pao CL, Kanazirska M, Quinn S, Rogers K, Seidman CE, Seidman JG, Brown EM, Vassilev PM. Amyloid-B proteins activate $Ca^{2+}$-permeable channels through calcium-sensing receptors. J Neurosci Res 1997;47:547.
31. Garrett JE, Capuano IV, Hammerland LG, Hung BC, Brown EM, Hebert SC, Nemeth EF, Fuller F. Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. J Biol Chem 1995 26;270:12919.
32. Aida K, Koishi S, Tawata M, Onaya T. Molecular cloning of a putative $Ca(^{2+})$-sensing receptor cDNA from human kidney. Biochem Biophys Res Commun 1995;214:524.
33. McNeil SE, Hobson SA, Nipper V, Rodland KD. Functional calcium-sensing receptors in rat fibroblasts are required for activation of SRC kinase and mitogen-activated protein kinase in response to extracellular calcium. J Biol Chem 1998;273:1114.
34. Arthur JM, Lawrence MS, Rane MJ, McLeish KR. Calcium-sensing receptors (CaR) on the basolateral surface of MDCK cells stimulate c-JUN N-terminal kinase (JNK) through pertussis toxin-sensitive G proteins. J Am Soc Nephrol 1998;9:418A.
35. Kallay E, Kifor O, Chattopadhyay N, Brown EM, Bischof MG, Peterlik M, Cross HS. Calcium-dependent c-myc proto-oncogene expression and proliferation of CACO-2 cells: a role for luminal extracellular calcium-sensing receptor. Biochem Biophys Res Commun 1997;232:80.
36. Garland CF, Garland FC, Gorham ED. Can colon cancer incidence and death rates be reduced with calcium and vitamin D? Am J Clin Nutr 1991;54:193S.
37. Friedman PA, Gesek FA. Cellular calcium transport in renal epithelia: measurement, mechanisms, and regulation. Physiol Rev 1995;75:429.
38. Kurokawa K. The kidney and calcium homeostasis. Kidney Int Suppl 1994;44:S97.
39. Thakker RV. Disorders of the calcium-sensing receptor. Biochim Biophys Acta 1998;1448:166.

40. Polak MR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinmann B, Levi T, Seidman CE, Seidman JG. Mutations in the human $Ca(^{2+})$-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell 1993;75:1297.
41. Attie MF, Gill JR, Stock JL, Spiegel AM, Downs RW Jr, Levine MA, Marx SJ. Urinary calcium excretion in familial hypocalciuric hypercalcemia. Persistence of relative hypocalciuria after induction of hypoparathyroidism. J Clin Invest 1983;72:667.
42. Heath H III, Odelberg S, Jackson CE, The BT, Hayward N, Larsson C, Buist NRM, Krapcho KJ, Hung B, Capuano IV, Garrett JE, Leppert MF. Clustered inactivating mutations and benign polymorphism of the calcium receptor gene in familial benign hypocalciuric hypercalcemia suggest receptor functional domains. J Clin Endocrinol Metab 1996;81:1312.
43. Bai M, Quinn S, Trivedi S, Kifor O, Pearce SHS, Pollak MR, Krapcho K, Hebert SC, Brown EM. Expression and characterization of inactivating and activating mutations in the human $Ca^{2+}{}_o$-sensing receptor. J Biol Chem 1996;271:19537.
44. Pearce SH, Bai M, Quinn SJ, Kifor O, Brown EM, Thakker RV. Functional characterization of calcium-sensing receptor mutations expressed in human embryonic kidney cells. J Clin Invest 1996;98:1860.
45. Bai M, Janicic N, Trivedi S, Quinn SJ, Cole DE, Brown EM, Hendy GN. Markedly reduced activity of mutant calcium-sensing receptor with an inserted Alu element from a kindred with familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. J Clin Invest 1997;99:1917.
46. Bai M, Pearce SH, Kifor O, Trivedi S, Stauffer UG, Thakker RV, Brown EM, Steinmann B. *In vivo* and *in vitro* characterization of neonatal hyperparathyroidism resulting from a de novo, heterozygous mutation in the $Ca^{2+}$-sensing receptor gene: normal maternal calcium homeostasis as a cause of secondary hyperparathyroidism in familial benign hypocalciuric hypercalcemia. J Clin Invest 1997;99:88.
47. Ho C, Conner DA, Pollak MR, Ladd DJ, Kifor O, Warren HB, Brown EM, Seidman JG, Seidman CE. A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Nat Genet 1995;11:389.
48. Lloyd SE, Pannett AA, Dixon PH, Whyte MP, Thakker RV. Localization of familial benign hypercalcemia, Oklahoma variant (FBHOk), to chromosome 19q13. Am J Hum Genet 1999;61:1899.
49. Heath H III. Familial benign hypercalcemia—from clinical description to molecular genetics. West J Med 1994;160:554.
50. Polak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, Hebert SC, Seidman CE, Seidman JG. Autosomal dominant hypocalcaemia caused by a $Ca^{2+}$-sensing receptor gene mutation. Nat Genet 1994;8:303.
51. Pearce SH, Williamson C, Kifor O, Bai M, Coulthard MG, Davies M, Lewis-Barned N, McCredie D, Powell H, Kendall-Taylor P, Brown EM, Thakker RV. A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor. N Engl J Med 1996;335:1115.
52. Porter L, Conlin PR, Scott J, Brown EM, El-Hajj Fuleihan G. Calcium modulation of the rennin-aldosterone axis. J Endocrinol Invest 1999;22:115.
53. Fynn M, Onomakpome N, Peart WS. The effects of ionophores (A23187 and RO2-2985) on renin secretion and renal vasoconstriction. Proc R Soc Lond B Biol Sci 1977;199:199.
54. Bland R, Walker EA, Hughes SV, Stewart PM, Hewison M. Constitutive expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in a transformed human proximal tubule cell line: evidence for direct regulation of vitamin D metabolism by calcium. Endocrinology 1999; 140:2027.
55. Mathias RS, Brown EM. Divalent cations modulate PTH-dependent 3′,5′-cyclic adenosine monophosphate production in renal proximal tubular cells. Endocrinology 1991;128:3005.
56. De Rouffignac C. Multihormonal regulation of nephron epithelia: achieved through combinational mode? Am J Physiol 1995;269:R739.
57. Takaichi K, Kurokawa K. Inhibitory guanosine triphosphate-binding protein-mediated regulation of vasopressin action in isolated single medullary tubules of mouse kidney. J Clin Invest 1988;82:1437.
58. Riccardi D, Lee WS, Lee K, Segre GV, Brown EM, Hebert SC. Localization of the extracellular $Ca(^{2+})$-sensing receptor and PTH/PTHrP receptor in rat kidney. Am J Physiol 1996;271:F951.
59. Riccardi D, Hall AE, Chattopadhyay N, Xu JZ, Brown EM, Hebert SC. Localization of the extracellular $Ca^{2+}$/polyvalent cation-sensing protein in rat kidney. Am J Physiol 1998;274:F611.
60. Di Stefano A, Roinel N, de Rouffignac C, Wittner M. Transepithelial $Ca^{2+}$ and $Mg^{2+}$ transport in the cortical thick ascending limb of Henle's loop of the mouse is a voltage-dependent process. Ren Physiol Biochem 1993;16:157.
61. Wang WH, Lu M, Hebert SC. Cytochrome P-450 metabolites mediate extracellular $Ca(^{2+})$-induced inhibition of apical $K^+$ channels in the TAL. Am J Physiol 1996;271:C103.
62. Escalante B, Erlij D, Falck JR, McGiff JC. Effect of cytochrome P450 arachidonate metabolites on ion transport in rabbit kidney loop of Henle. Science 1991;251:799.
63. Amlal H, Legoff C, Vernimmen C, Paillard M, Bichara M. $Na(^+)$-$K^+(NH_4{}^+)$-$2Cl^-$ cotransport in medullary thick ascending limb: control by PKA, PKC, and 20-HETE. Am J Physiol 1996;271:C455.
64. Sands JM, Naruse M, Baum M, Jo I, Hebert SC, Brown EM, Harris HW. Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited water permeability in rat kidney inner medullary collecting duct. J Clin Invest 1997;99:1399.
65. Bourdeau JE. Mechanisms and regulation of calcium transport in the nephron. Semin Nephrol 1993;13:191.
66. Costanzo LS. Localization of diuretic action in microperfused rat distal tubules: Ca and Na transport. Am J Physiol 1985;248:F527.
67. Bouhtiauy I, Lajeunesse D, Christakos S, Brunette MG. Two vitamin D3-dependent calcium binding proteins increase calcium reabsorption by different mechanisms. I. Effect of CaBP 28K. Kidney Int 1994;45:461.
68. Bacskai BJ, Friedman PA. Activation of latent $Ca^{2+}$ channels in renal epithelial cells by parathyroid hormone. Nature 1990;27:347.
69. Plotkin MD, Kaplan MR, Verlander JW, Lee WS, Brown D, Poch E, Gullans SR, Hebert SC. Localization of the thiazide sensitive Na-Cl cotransporter, rTSC1 in the rat kidney. Kidney Int 1996;50:174.
70. Dagher G, Thomas SR, Griffiths N, Siaume-Perez S, Sauterey C. Calcitonin activates an $Na(+)$-independent $HCO_3(^-)$-dependent pathway in the rabbit distal convoluted tubule. Am J Physiol 1997;273:F97.
71. Arthur JM, Collinsworth GP, Gettys TW, Quarles LD, Raymond JR. Specific coupling of a cation-sensing receptor to G protein alpha-subunits in MDCK cells. Am J Physiol 1997;273:F129.
72. Quarles LD, Hartle JE II, Siddhanti SR, Guo R, Hinson TK. A distinct cation-sensing mechanism in MC3T3-E1 osteoblasts functionally related to the calcium receptor. J Bone Miner Res 1997;12:393.
73. Ye C, Rogers K, Bai M, Quinn SJ, Brown EM, Vassilev PM. Agonists of the $Ca(^{2+})$-sensing receptor (CaR) activate nonselective cation channels in HEK293 cells stably transfected with the human CaR. Biochem Biophys Res Commun 1996;13:226.
74. Ray JM, Squires PE, Curtis SB, Meloche MR, Buchan AM. Expression of the calcium-sensing receptor on human antral gastrin cells in culture. J Clin Invest 1997;99:2328.
75. Oda Y, Tu C-L, Pillai S, Bikle DD. The calcium sensing receptor and its alternatively spliced form in keratinocyte differentiation. J Biol Chem 1998;273:23344.
76. Lin KI, Chattopadhyay N, Bai M, Alvarez R, Dang CV, Baraban JM, Brown EM, Ratan RR. Elevated extracellular calcium can prevent apoptosis via the calcium-sensing receptor. Biochem Biophys Res Commun 1998;19:249.
77. Gama L, Baxendale-Cox LM, Breitwieser GE. $Ca^{2+}$-sensing receptors in intestinal epithelium. Am J Physiol 1997;273:C1168.
78. Kovacs CS, Ho-Pao CL, Hunzelman JL, Lanske B, Fox J, Seidman JG, Seidman CE, Kronenberg HM. Regulation of murine fetal-placental calcium metabolism by calcium-sensing receptor. J Clin Invest 1998;101:2812.

79. Chang W, Tu C, Bajra R, Komuves L, Miller S, Strewler G, Shoback D. Calcium sensing in cultured chondrogenic RCJ3.1C5.18 cells. Endocrinology 1999;140:1911.
80. Yoshida N, Sato T, Kobayashi K, Okada Y. High exracellular $Ca^{2+}$ and $Ca^{2+}$-sensing receptor agonists activate nonselective cation conductance in freshly isolated rat osteoclast. Bone 1998;22:495.
81. Chattopadhyay N, Ye C, Yamaguchi T, Nakai M, Kifor O, Vassilev PM, Nishimura RN, Brown EM. The extracellular calcium-sensing receptor is expressed in rat microglia and modulates an outward $K^+$ channel. J Neurochem 1999;72:1915.
82. Emanuel RL, Adler GK, Kifor I, Quinn SJ, Fuller F, Krapcho K, Brown EM. Calcium-sensing receptor expression and regulation by extracellular calcium in AtT-20 pituitary cell line. Mol Endocrinol 1996;10:555.
83. Kato M, Doi R, Imamura M, Furutani M, Hosotani R, Shimada Y. Calcium-evoked insulin release from insulinoma cells is mediated via calcium-sensing receptor. Surgery 1997;122:1203.
84. Adebanjo OA, Igietseme J, Huang CL, Zaidi M. The effect of extracellularly applied divalent cations on cytosolic $Ca^{2+}$ in murine Leydig cells: evidence for a $Ca^{2+}$-sensing receptor. J Physiol 1998;513:399.
85. Bradbury RA, Sunn KL, Crossley M, Bai M, Brown EM, Delbridge L, Conigrave AD. Expression of the parathyroid $Ca(^{2+})$-sensing receptor in cytotrophoblasts from human term placenta. J Endocrinol 1998;156:425.
86. House MG, Kohlmeier L, Chattopadhyay N, Kifor O, Yamaguchi T, Leboff MS, Glowacki J, Brown EM. Expression of an extracellular calcium-sensing receptor in human and mouse bone marrow cells. J Bone Miner Res 1997;12:1959.