Annu. Rev. Nutr. 2000. 20:507–33

THE EXTRACELLULAR Ca^{2+}-SENSING RECEPTOR: Central Mediator of Systemic Calcium Homeostasis

Edward M. Brown
Endocrine-Hypertension Division, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115; e-mail: embrown@rics.bwh.harvard.edu

Key Words parathyroid, intestine, kidney, bone, diet

■ **Abstract** The cloning of the G protein-coupled, extracellular calcium (Ca^{2+}_o)-sensing receptor (CaR) has identified a central mediator of the mechanism governing systemic Ca^{2+}_o homeostasis. This system enables organisms to adapt successfully to wide variations in dietary Ca^{2+}_o intake while maintaining near constancy of Ca^{2+}_o. Whereas discussions of Ca^{2+}_o homeostasis have generally focused on the key role of Ca^{2+}_o -elicited changes in parathyroid hormone secretion, the presence of the CaRs in effector tissues of this system enables direct regulation of processes (e.g. renal tubular Ca^{2+} reabsorption and possibly bone formation and resorption) that add additional layers of homeostatic control. As we understand more about how the CaR regulates these tissues, we may find that it participates in other processes relevant to mineral ion homeostasis, including the control of the 1-hydroxylation and activation of vitamin D_3 or reabsorption of phosphate in the renal proximal tubule. Regardless, the remarkable sensitivity of the CaR to small changes in Ca^{2+}_o allows adjustments in the response of the Ca^{2+}_o homeostatic system to increases or decreases in the intake of dietary Ca^{2+}, for instance, that cause barely detectable alterations in Ca^{2+}_o. Furthermore, the CaR likely participates in coordinating interactions among several different homeostatic control systems (including those for water, Mg^{2+}_o, Na^+, extracellular volume, and/or blood pressure), despite the fact that these systems are often considered to function largely independently of mineral ion metabolism.

CONTENTS

INTRODUCTION

Calcium (Ca^{2+}) is a key nutrient because of its diverse intra- and extracellular roles. Intracellular Ca^{2+}, particularly the cytosolic free calcium concentration (Ca^{2+}_i), is an important second messenger and cofactor for proteins and enzymes regulating key cellular processes (i.e. neurotransmission, motility, hormonal secretion, and cellular proliferation) (12). Extracellular Ca^{2+} (Ca^{2+}_o) serves as a cofactor for adhesion molecules, clotting factors, and other proteins; regulates neuronal excitability; and is an essential part of the mineral phase of bone. The skeleton provides a structural framework that protects critical bodily structures and facilitates locomotion, as well as a large reservoir of mineral ions (e.g. calcium and phosphate) that can be mobilized when needed (87). Ultimately, however, all Ca^{2+}, whether it is intra- or extracellular, originates in the diet. The increasing recognition of the importance of dietary Ca^{2+} as the ultimate source for the myriad biological roles of Ca^{2+}_o has recently led some to call it a "superstar of nutrients" (11).

It is not surprising, therefore, that the level of Ca^{2+}_o is precisely regulated (12). In the past decade, our understanding of how Ca^{2+}_o is sensed and, in turn, regulated

has advanced greatly. The Ca^{2+}_o-sensing mechanism is the body's thermostat for Ca^{2+}_o, or its "calciostat" (16). It may be surprising that this calciostat is a G-protein-coupled receptor (GPCR). Nevertheless, remarkably diverse extracellular ligands, namely proteins, prostaglandins, and photons (e.g. via rhodopsin), use GPCRs to transduce changes in their extracellular concentrations into appropriate biological responses. Now, with the cloning of the Ca^{2+}_o-sensing receptor (CaR), an inorganic ion has been shown to use a GPCR as well.

The CaR (sometimes designated CaSR or CasR) ensures the remarkable stability of Ca^{2+}_o in bodily fluids by precisely adjusting—through its direct and indirect actions on kidney, bone, and intestine—the movements of Ca^{2+} into and out of the extracellular fluid (ECF) (16). Indeed, its existence has proved definitively that Ca^{2+} is not only a key intracellular second messenger but also an important extracellular "first" messenger (12). Thus despite its near constancy, Ca^{2+}_o encodes the information that cells "decode" via the CaR to maintain Ca^{2+}_o homeostasis.

In this chapter, I describe the importance of Ca^{2+}_o sensing for maintaining Ca^{2+}_o homeostasis, briefly review the indirect evidence suggesting the existence of the CaR and leading to its isolation by expression cloning, and describe the receptor's role in coordinating the tissues that maintain Ca^{2+}_o homeostasis. I emphasize how regulation of dietary intake and absorption of Ca^{2+}, as well as its renal tubular handling and skeletal disposition, is a key mechanism through which the CaR adjusts the metabolism of this crucial nutrient to its availability. Finally, I review interactions between the CaR regulation of Ca^{2+}_o metabolism and homeostatic mechanisms for other nutrients, including magnesium (Mg^{2+}), salt, and water—some of which use the capacity of the CaR to integrate multiple homeostatic signals.

Ca^{2+}_o BALANCE AND THE IMPORTANCE OF Ca^{2+}_o SENSING FOR Ca^{2+} HOMEOSTASIS

Calcium Balance

Figure 1 illustrates the approximate magnitudes of the movements of Ca^{2+} into and out of the ECF in a normal human via kidney, bone, and intestine. These fluxes of Ca^{2+} are large compared with the total Ca^{2+} content of the ECF, particularly those fluxes that occur in the kidney, where $\sim$10 g of Ca^{2+} is filtered daily and 9.8 g (98%) is reabsorbed. That is, about every 2 h, an amount of Ca^{2+} equivalent to the total quantity in the ECF is filtered and reabsorbed. Nevertheless, this renal Ca^{2+} turnover pales in comparison with that in an egg-laying chicken (47). Here, the depositing of >1 g of Ca^{2+} over several hours in the shell of a soon-to-be-laid egg requires total turnover of plasma Ca^{2+} every few minutes. Therefore, in humans, birds, and other free-living terrestrial organisms, these fluxes of Ca^{2+}

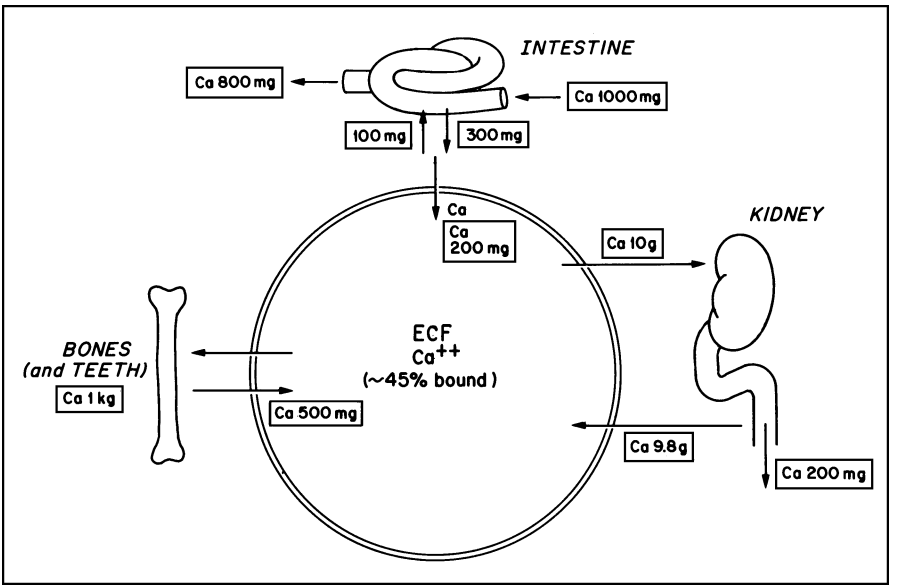


Figure 1 Overall Ca^{2+} balance in a normal individual. There is net absorption of ~200 mg of the 1 g of elemental Ca^{2+} ingested daily. Balance is achieved by renal excretion of 200 mg of Ca^{2+} because equivalent amounts of skeletal Ca^{2+} are deposited and resorbed daily. *ECF*, extracellular fluid. (Adapted from Reference 14d.)

must be precisely coordinated, lest, for instance, large reductions in Ca^{2+}_o produce life-threatening compromise of cardiac contraction.

Importance of Ca^{2+}_o Sensing in Calcium Homeostasis

Mammals and other tetrapods (e.g. mammals, birds, reptiles, and amphibians) only intermittently ingest Ca^{2+} and have evolved a complex homeostatic mechanism that ensures near constancy of Ca^{2+}_o (Figure 2). Specific cells "sense" changes in Ca^{2+}_o and respond so as to restore normocalcemia (12). Classical Ca^{2+}_o-sensing cells include the parathyroid hormone (PTH)-secreting chief cells of the parathyroid glands and the calcitonin (CT)-secreting C cells of the thyroid, which secrete less and more of these hormones, respectively, when Ca^{2+}_o rises. There is a steep inverse sigmoidal curve relating circulating PTH levels to Ca^{2+}_o in normal humans (Figure 3). Increases in circulating PTH in response to hypocalcemia stimulate tubular Ca^{2+} reabsorption, bone resorption, and, if prolonged for several hours, renal proximal tubular 1-hydroxylation of 25-hydroxyvitamin D_3 to its active form, 1,25-dihydroxy vitamin D_3 [$1,25(OH)_2D_3$] (12, 87), which increases intestinal Ca^{2+} absorption.

Raising Ca^{2+}_o reduces PTH, modulating the functions of kidney, bone, and intestine so as to dispose of the extra Ca^{2+}. The positive relationship between

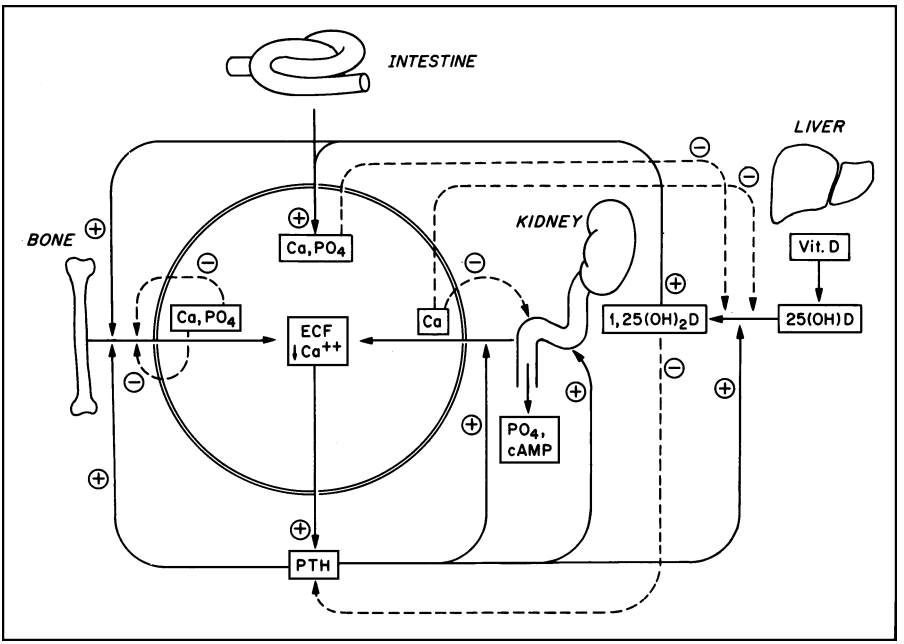


Figure 2 Schematic diagram illustrating the Ca^{2+}_o homeostatic system. *Solid arrows* and *lines* delineate actions of PTH and $1,25(OH)_2D_3$; *dotted arrows* and *lines* show direct actions of Ca^{2+}_o and phosphate ions on target tissues. Abbreviations and symbols: Ca^{2+}, calcium; PO_4, phosphate; ECF, extracellular fluid; PTH, parathyroid hormone; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; *circled minuses*, inhibitory actions; *circled pluses*, positive actions. (Reproduced with permission from Reference 14e.)

Ca^{2+}_o and CT secretion also elevates CT levels during hypercalcemia (3, 12, 87). CT lowers Ca^{2+}_o by inhibiting osteoclastic bone resorption and enhancing renal Ca^{2+} excretion (3). Although CT exerts only modest hypocalcemic actions in adult humans, in whom bone turnover is slow, it can be an effective therapeutic agent in conditions in which bone turnover is high (i.e. osteoporosis, Paget's disease of bone, and hypercalcemia of malignancy caused by bony metastases).

The steepness of the curve relating PTH and Ca^{2+}_o is a major contributor to the near constancy of Ca^{2+}_o in vivo, because small perturbations in Ca^{2+}_o (of only a few percent) elicit large changes in PTH (12). An additional parameter, the midpoint or "set-point" (e.g. Figure 3), is an important determinant of the level at which Ca^{2+}_o is "set." In certain inherited and acquired diseases of Ca^{2+}_o sensing, the set-point is increased in hypercalcemic conditions and reduced in hypocalcemic disorders (13). That is, the calciostat is reset upward or downward, causing stable hypercalcemia or hypocalcemia, respectively.

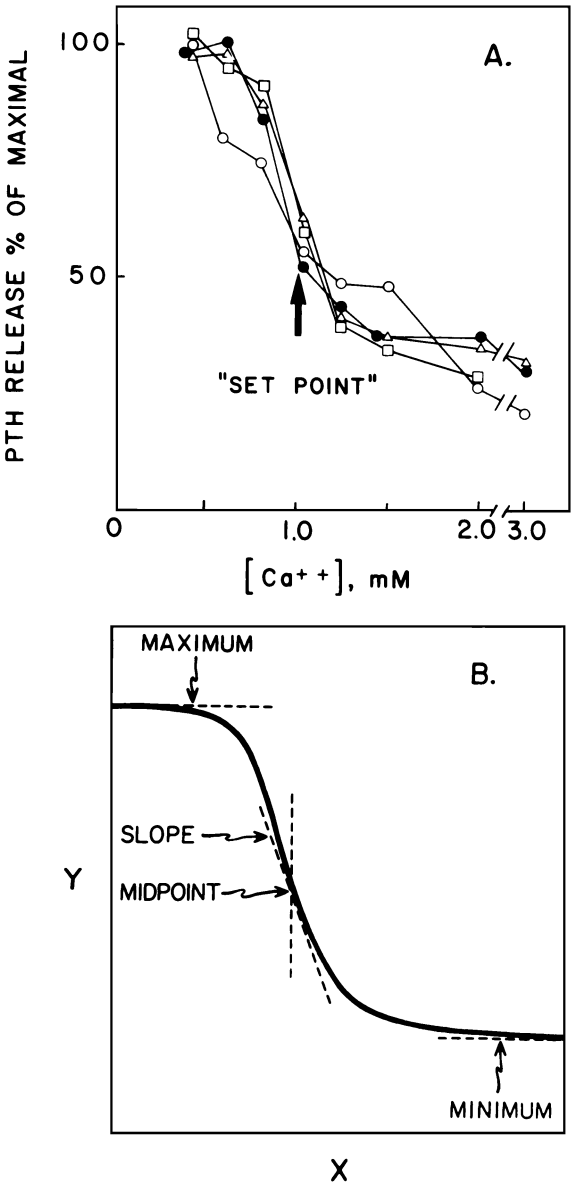


Figure 3 (*A*) Steep inverse sigmoidal relationship between PTH and $Ca^{2+}{}_o$ in vivo. Reproduced in modified form with permission from Reference 12. (*B*) Four-parameter model of the inverse sigmoidal relationship between $Ca^{2+}{}_o$ and PTH release based on the equation, $Y = \{(A - D)/[1 + (X/C)^B]\} + D.Y$, maximal secretory rate. The slope of the curve at its midpoint or set point and the minimal secretory rate are indicated. (Reproduced with permission from Reference 11a.)

Additional tissues involved in calcium homeostasis likewise sense Ca^{2+}_o. The 1-hydroxylation of 25-hydroxyvitamin D_3 to $1,25(OH)_2D_3$ is directly regulated by Ca^{2+}_o—a physiologically appropriate response to reducing Ca^{2+} absorption and availability (97). Moreover, raising peritubular but not luminal Ca^{2+}_o diminishes both Ca^{2+} and Mg^{2+} reabsorption in the thick ascending limb of Henle's loop (30, 90), thereby enhancing Ca^{2+} excretion—another homeostatically logical response. High Ca^{2+}_o inhibits bone resorption by osteoclasts (104), which are the cells that normally break down bone mineral and matrix during skeletal turnover. Because Ca^{2+}_o beneath a resorbing osteoclast can be as high as 8−40 mM (85), this resorbed Ca^{2+}, upon release into the bone microenvironment, could potentially act on nearby osteoclasts to limit further bone resorption. Raising Ca^{2+}_o also stimulates the functions of bone-forming osteoblasts (72). Thus, Ca^{2+}_o itself, like the more classical calciotropic hormones PTH, CT, and $1,25(OH)_2D_3$, can act as a local or systemic calcium-regulating "hormone" that plays a central role in maintaining Ca^{2+}_o homeostasis (12; Figure 2).

Indirect Evidence for the Existence of a Calcium-Sensing Receptor

How do cells sense Ca^{2+}_o? Indirect evidence accumulated in the 1980s strongly implicated a "receptor-like" Ca^{2+}_o-sensing mechanism that had the properties of a GPCR (12). For instance, raising Ca^{2+}_o activated phospholipases C (PLC) and A_2 (PLA_2) and elevated Ca^{2+}_i in bovine parathyroid cells (66)—responses that are characteristic of the so-called "Ca^{2+}-mobilizing" hormones acting through their GPCRs. Moreover, high Ca^{2+}_o caused a pertussis toxin-sensitive inhibition of cyclic AMP (cAMP) accumulation (12), suggesting that the putative CaR inhibited adenylate cyclase via the inhibitory G protein, G_i. These data suggested that parathyroid cells and perhaps other Ca^{2+}_o-sensing cells detected changes in Ca^{2+}_o via a GPCR that was coupled to adenylate cyclase and one or more phospholipases. The use of expression cloning in *Xenopus laevis* oocytes provided a possible avenue for cloning the putative CaR, because this approach did not require specific probes for the gene in question (e.g. DNA probes or specific antibodies) (27).

CLONING AND SELECTED STRUCTURAL AND FUNCTIONAL FEATURES OF THE CALCIUM-SENSING RECEPTOR

Cloning of the Calcium-Sensing Receptor

Indeed, the application of expression cloning yielded a single 5.3-kb cDNA encoding a CaR with pharmacological properties that were virtually identical to those of the Ca^{2+}_o-sensing mechanism in parathyroid cells (14; Figure 4). Full-length CaRs were later cloned from diverse tissues in several mammals,

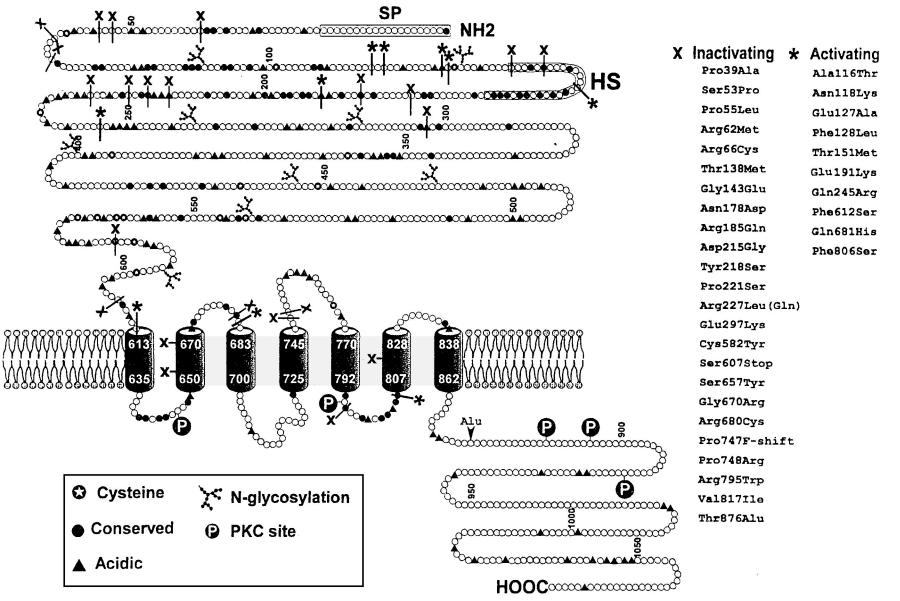


Figure 4 Predicted structure of the calcium-sensing receptor. See text for details. Abbreviations: SP, signal peptide; HS, hydrophobic segment. Also delineated are missense and nonsense mutations causing either FHH or autosomal dominant hypocalcemia, indicated with the three-letter amino acid code, with the normal amino acid preceding and the mutated amino acid after the number of the relevant codon. (Reproduced with permission from Reference 13a.)

including human parathyroid (39); rat (76), human (1), and rabbit (18) kidneys; rat C cells (36, 40); and rat brains (80). All are very similar in their amino acid sequences (>90% identical) and are tissue and species homologs of the same ancestral gene.

A full-length chicken parathyroid CaR (33) and a smaller CaR fragment from the mudpuppy, an amphibian (25), are also highly homologous to mammalian CaRs (~80% amino acid and nucleotide identities). This CaR gene's great similarity among mammals, birds, and amphibians indirectly supports its functional importance. Furthermore, humans or mice with two defective CaR genes exhibit marked hypercalcemia, firmly establishing the central, nonredundant role of the CaR in maintaining Ca^{2+}_o homeostasis. Surprisingly, however, given the diversity of related GPCRs (see next section), no additional CaR genes have been identified to date, although splice variants that are of uncertain physiological relevance have been identified.

The Principal Structural Features of the Calcium-Sensing Receptor

Figure 4 illustrates the overall topology of the CaR, including its large, amino (NH2)-terminal extracellular domain (ECD), central core with the "serpentine"

seven-membrane-spanning motif characteristic of the GPCRs, and intracellular carboxyl-terminal (C-) tail (14). Ca^{2+}_o binds to the ECD of the CaR; this ECD also contains several N-linked glycosylation sites (14), whereas its intracellular regions have several protein kinase C (PKC) and protein kinase A (PKA) phosphorylation sites. The PKC sites modulate the activity of the CaR—reducing its coupling to PLC—but the importance of the PKA sites, if any, is unknown (8, 14). The CaR resides on the cell surface largely as a dimer (6, 96), and the subunits of the dimeric CaR interact functionally (7). Determining the three-dimensional structure of the CaR by X-ray crystallography or other techniques will elucidate how the Ca^{2+}_o signal is sensed and transduced into changes in intracellular signaling.

The Homology of the Calcium-Sensing Receptor to Other G-Protein–Coupled Receptors and Related Evolutionary Issues

The CaR belongs to the recently described family C GPCRs, comprising three subfamilies with $\geq$20% amino acid identity in their seven-membrane-spanning regions. Group I includes the metabotropic glutamate receptors 1–8, which are GPCRs for the excitatory neurotransmitter glutamate (65). Group II has at least two members: the CaR and a multigene family of putative pheromone receptors in neurons of the vomeronasal organ, a small sensory organ that is prominent in rodents and regulates instinctual behavior (60). Additional receptors similar to the CaR and these vomeronasal receptors have been described in mammals (46) and fish (19) that are, respectively, taste and putative odorant receptors. Group III includes the $GABA_B$ receptors, which are GPCRs for gamma-aminobutyric acid (GABA), the key inhibitory neurotransmitter (52).

The extracellular, ligand-binding domains of the family C GPCRs are homologous to the bacterial periplasmic nutrient-binding proteins (PBPs) (26), supporting an evolutionary relationship between the ECD of these GPCRs and the PBPs, which bind diverse extracellular solutes that are destined for cellular uptake and/or that elicit chemotactic responses (84). The PBPs recognize organic nutrients (i.e. amino acids) as well as inorganic ions (e.g. phosphate and nickel) (84). After binding their respective ligands, they interact with integral membrane proteins that transmit the chemotactic signal or transport the nutrient intracellularly. Thus the family C GPCRs likely represent "fusion proteins" composing an ECD derived from an ancient family of solute-binding sensing proteins (the PBPs) and the seven-membrane-spanning motif that evolved separately to transmit extracellular signals into eukaryotic cells.

The CaR also shares functional properties with the PBPs. It recognizes a key nutrient, Ca^{2+}. The CaR usually senses Ca^{2+}_o within enclosed bodily fluids, but in the gastrointestinal tract, for example, it senses Ca^{2+}_o in a fluid arising, at least in part, from outside the organism, analogous to nutrient sensing by the PBPs. The CaR also mediates stimulation of chemotaxis by Ca^{2+}_o in monocytes (101)

and, perhaps, osteoblasts and their precursors (41, 99, 100). Thus both structural and functional attributes of the PBPs may have been conserved over a very broad evolutionary scale.

The Signal Transduction Pathways of the Calcium-Sensing Receptor

Phospholipases The CaR activates PLC, PLA_2 (81), and phospholipase D (PLD) (53). CaR-induced stimulation of phosphoinositide (PI)-specific PLC likely involves a direct, G protein-mediated process (16), probably using $G_{q/11}$, and elevates $Ca^{2+}{}_i$, owing to both mobilization of intracellular Ca^{2+} via inositol trisphosphate and activation of Ca^{2+} influx. Stimulation of PLA_2 and PLD is largely indirect, involving CaR-mediated, PLC-dependent activation of PKC (53). The CaR can activate the cytosolic form of PLA_2 through its phosphorylation by mitogen-activated protein kinase (MAPK) (O Kifor, R Diaz, I Kifor, and EM Brown, submitted for publication). The CaR is thought to stimulate phosphatidylcholine-specific PLC in sheep C cells. Inhibitors of phosphatidylcholine-specific PLC partially block CaR-evoked secretion in this model (63), but further studies are needed to understand how the CaR exerts this action.

Mitogen-Activated Protein Kinase The CaR stimulates the MAPK family member Erk-1, in association with activation of the cytoplasmic tyrosine kinase, c-Src, and stimulation of cellular proliferation, in rat-1 fibroblasts (64). Herbimycin, a tyrosine kinase inhibitor, inhibits stimulation of both Erk-1 and proliferation, suggesting that CaR-mediated activation of c-Src is upstream of these biological responses. Furthermore, inhibiting MAPK kinase, a protein kinase that activates MAPK, also blocks CaR-evoked proliferation (64), further supporting the mediatory role of MAPK. Future studies will reveal whether the CaR modulates other MAPKs, such as the c-Jun terminal kinase/stress-activated kinase or p38 MAPK.

Adenylate Cyclase The CaR inhibits adenylate cyclase in parathyroid (21) and in medullary thick ascending limb (MTAL) of mouse kidney (89) by a direct, G_i-mediated mechanism. In other cases, CaR-induced inhibition of adenylate cyclase is indirect, e.g. via the effect of a CaR-induced rise in $Ca^{2+}{}_i$ on a Ca^{2+}-inhibitable isoform of adenylate cyclase (29). In contrast, in AtT-20 cells (34) and pituitary adenomas (79), high $Ca^{2+}{}_o$ raises cAMP. Whether this involves a Ca^{2+}-stimulated adenylate cyclase or direct G_s-mediated activation requires further study. Thus the CaR regulates several signaling pathways, enabling it to exert both rapid actions (i.e. on secretion or ion channel/transporters) and longer-term effects (e.g. on cellular proliferation and gene expression) relevant to Ca^{2+} homeostasis (16).

THE TISSUE DISTRIBUTION AND FUNCTIONS OF THE CALCIUM-SENSING RECEPTOR IN Ca^{2+}_o HOMEOSTASIS

Parathyroid Gland

Parathyroid glands of humans (54), rats (4), mice (44), rabbits (18), and chickens (33) express abundant CaR. Studies of inherited diseases of Ca^{2+}_o homeostasis caused by inactivating CaR mutations and of mice with targeted disruption (e.g. "knockout") of the CaR gene strongly support the receptor's central role in Ca^{2+}_o-regulated PTH secretion (for review, see 13). Persons who are heterozygous for inactivating mutations and mice that are heterozygous for CaR knockout (44) show modest (10%–20%) increases in their parathyroid set-points. Humans that are homozygous for inactivating mutations—with a condition termed neonatal severe hyperparathyroidism—and mice that are homozygous for CaR knockout (44) exhibit severe hypercalcemia and parathyroid "resistance" to Ca^{2+}_o. In addition, persons harboring activating mutations, which render the parathyroid overly sensitive to Ca^{2+}_o, exhibit hypocalcemia and inappropriately normal or low PTH levels (i.e. a stimulus that normally increases PTH fails to do so) (13). Despite the compelling evidence that these experiments in nature provide for the central, nonredundant role of the CaR in mediating high Ca^{2+}_o-inhibited PTH release, the underlying intracellular mechanism(s) remains uncertain.

The CaR also tonically suppresses parathyroid proliferation, because, in neonatal severe hyperparathyroidism (13) and homozygous CaR knockout mice (44), there is marked parathyroid hyperplasia. Furthermore, treating rats with renal impairment caused by subtotal nephrectomy with the "calcimimetic" CaR activator R-568 (which activates the receptor through an allosteric mechanism) prevents the parathyroid hyperplasia that otherwise develops in renal insufficiency (93). These agents are currently in clinical trials for primary and secondary hyperparathyroidism and may provide the first effective medical therapy for parathyroid overactivity in hyperparathyroidism (86). The CaR likely also controls PTH gene expression, because R-568 decreased PTH mRNA levels in parathyroid cells (38).

C-Cells

Studies with sheep C-cells have suggested that the CaR stimulates CT secretion by activating phosphatidylcholine-specific PLC, which generates diacylglycerol, thereby activating PKC. The latter stimulates a nonselective cation channel, enhancing cellular uptake of $Na^+$and Ca^{2+}, producing concomitant cellular depolarization, and activating voltage-gated, principally L-type Ca^{2+} channels (63). The resultant rise in Ca^{2+}_i stimulates CT secretion.

Kidney

Localization of the Calcium-Sensing Receptor CaR mRNA is present along essentially the entire nephron: in the glomerulus, proximal convoluted tubules

(PCTs) and straight tubules, MTAL, cortical thick ascending limb (CTAL), distal convoluted tubule (DCT), cortical collecting duct, and inner medullary collecting duct (IMCD) (75). Immunohistochemistry with CaR-specific antisera has localized CaR protein to PCTs and straight tubules (74), MTAL (74), CTAL (18, 74), DCT (74), cortical collecting duct (74), and IMCD (18, 83). In PCTs, the CaR is at the base of apical brush border (74). The CaR is also localized apically in IMCD (18, 83), whereas CTAL exhibits a high basolateral level of CaR expression, enabling it to sense $Ca^{2+}{}_o$ in blood rather than tubular fluid (18, 74). The receptor likewise resides basolaterally in MTAL and DCT (74). In cortical collecting duct, the CaR is expressed in some, but not all, of the type-A intercalated cells, which participate in acid-base homeostasis (74).

Role of the Calcium-Sensing Receptor in Regulating Tubular Reabsorption of Ca^{2+} and Mg^{2+} Raising peritubular $Ca^{2+}{}_o$ or $Mg^{2+}{}_o$ decreases tubular reabsorption of both ions (71). Ca^{2+} and Mg^{2+} reabsorption in CTAL occurs by the paracellular route, driven by the lumen-positive, transepithelial potential difference (V_t) generated by the transport of Na^+, K^+, and Cl^- by the apical Na/K/2Cl cotransporter coupled with the recycling of K^+into the lumen by an apical K^+ channel (Figure 5; for reviews, see 30, 43). PTH and other hormones that elevate cAMP (e.g. glucagon) stimulate Ca^{2+} and Mg^{2+} reabsorption by increasing V_t and by stimulating overall cotransporter activity (30, 43). High $Ca^{2+}{}_o$, presumably acting through the CaR, inhibits this K^+channel, in part, through an arachidonic acid metabolite(s) generated by the P-450 pathway, probably 20-hydroxyeicosatetraenoic acid (94; Figure 4). Reduced recycling of K^+ diminishes luminal levels of K^+, overall cotransporter activity, V_t, and pari passu paracellular Ca^{2+} and Mg^{2+} transport (43).

The CaR may modulate the function of the thick ascending limb through additional or alternative mechanisms. In rat CTAL, high $Ca^{2+}{}_o$ -evoked inhibition of NaCl reabsorption involves lowering of cAMP (28). In rabbit CTAL, high $Ca^{2+}{}_o$-induced increases in $Ca^{2+}{}_i$ result from Ca^{2+} influx via basolateral Ca^{2+} channels rather than activation of PI-PLC (31). Finally, in mouse CTAL, high $Ca^{2+}{}_o$ inhibits Ca^{2+} and, to a lesser extent, Mg^{2+} but not NaCl transport; this is accomplished by lowering cAMP (32). Clearly, further studies are needed to firmly establish the roles of the CaR in these processes and the relative importance of various signaling pathways.

Very little is presently known about the role of the CaR in regulating Ca^{2+} reabsorption in DCT. The CaR is expressed in a murine DCT cell line in which elevating $Ca^{2+}{}_o$ or $Mg^{2+}{}_o$ raises $Ca^{2+}{}_i$ and inhibits adenylate cyclase (9). The second of these actions could inhibit PTH-stimulated Ca^{2+} reabsorption (37). Thus, as in CTAL, the CaR and PTH receptor in DCT could potentially exert mutually antagonistic actions on Ca^{2+} reabsorption. Ca^{2+}-permeable channels have recently been cloned that are expressed in rat intestine (70) and DCT of rabbit kidney (45), respectively, and likely represent the apical Ca^{2+} uptake mechanisms for

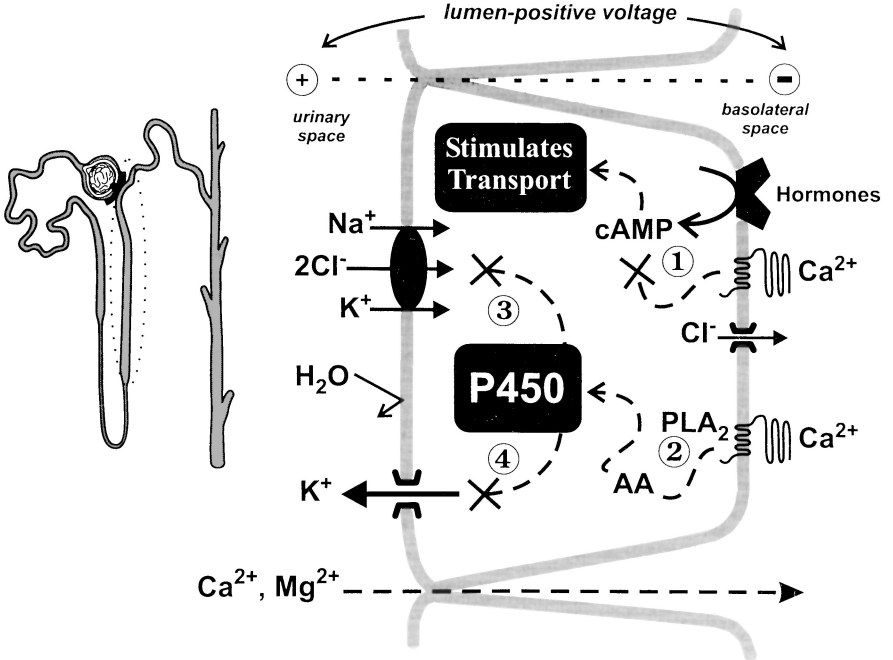


Figure 5 Possible mechanism by which the CaR regulates intracellular second messengers and ionic transport in the thick ascending limb. Hormones elevating cAMP (e.g. PTH) activate paracellular Ca^{2+} and Mg^{2+} reabsorption by stimulating the Na-K-2Cl cotransporter and an apical K^+ channel and increasing V_t. The CaR, also on the basolateral membrane, stimulates PLA_2 (2), increasing free arachidonic acid, which is metabolized via the P-450 pathway to an inhibitor of the apical K^+ channel (4) and, perhaps, the cotransporter (3). Both actions reduce overall cotransporter activity, reduce V_t, and, therefore, inhibit paracellular divalent cation transport. The CaR also inhibits adenylate cyclase (1) and, therefore, hormone-stimulated divalent cation transport. (Reproduced with permission from Reference 14b.)

transcellular Ca^{2+} absorption and reabsorption in intestine and distal nephron, respectively. These channels have been termed CaT1 (calcium transport proteins, subtype 1) and ECaC (epithelial calcium channel) (45, 70), respectively. Both proteins have PKC sites that may inhibit their functions, as well as PKA sites. Thus the PTH receptor and the CaR could potentially exert opposite actions on distal tubular Ca^{2+} reabsorption by phosphorylating these channels on different sites.

Regardless of exactly how the CaR modulates renal tubular handling of divalent cations, the reduced capacity of persons with inactivating CaR mutations to increase urinary Ca^{2+} excretion in response to hypercalcemia indirectly supports the role of the CaR in this process (2, 43). Conversely, individuals with activating CaR mutations have excessively high urinary Ca^{2+} excretion, presumably caused by activated CaRs in CTAL (13).

Bone and Cartilage

Osteoclasts and Their Precursors The CaR is expressed in cells of the monocyte/macrophage lineage (102) that can serve as osteoclast precursors by differentiating and fusing to form mature, multinucleated osteoclasts (56, 87). Moreover, Ca^{2+}_o and the calcimimetic CaR activator, NPS R-467 (68), inhibit the formation of osteoclasts from their precursors in vitro (51), which suggests a role for the CaR in regulating osteoclastogenesis. Other studies, however, have suggested that the osteoclast Ca^{2+}_o-sensing mechanism differs pharmacologically from the CaR (103). Further studies are needed, therefore, to understand at a molecular level how the osteoclast senses Ca^{2+}_o. If the CaR is expressed in osteoclasts and/or their precursors and mediates Ca^{2+}_o-induced inhibition of osteoclastogenesis, it could clearly contribute to Ca^{2+}_o homeostasis by its direct actions on these bone cells and/or their precursors in addition to doing so indirectly through CaR-mediated alterations in PTH and $1,25(OH)_2D_3$ levels.

Osteoblasts, Their Precursors, and Osteocytes The CaR is expressed in osteoblastlike cell lines (100), as well as in osteoblasts in bone sections (23). This makes the CaR a candidate for mediating known actions of high Ca^{2+}_o on osteoblast function, such as the stimulation of preosteoblast proliferation and chemotaxis, which may contribute to their appearance at sites of recent bone resorption in preparation for eventual osteoblastic replacement of the missing bone. The murine ST-2 stromal cell line also expresses the CaR, and high Ca^{2+}_o stimulates the proliferation and chemotaxis of these cells (99). Stromal cells can serve as osteoblast precursors and also produce mediators that modulate osteoblast and osteoclast function (56). Therefore, the CaR in stromal cells could participate in bone turnover either directly (if these cell differentiate to osteoblasts) or indirectly (by influencing production of these mediators).

Osteocytes are osteoblasts that have completed their role as bone-forming cells and have become encased within the bone substance, in which they extend processes into narrow bony canaliculi and may sense mechanical forces and/or directly regulate Ca^{2+} fluxes into and out of bone (87). Raising Ca^{2+}_o increases Ca^{2+}_i in chick osteocytes by mobilizing intracellular Ca^{2+}—presumably by a Ca^{2+}_o-sensing mechanism (50). The pharmacology for the effects of various divalent cations on Ca^{2+}_i in osteocytes suggests that the Ca^{2+}_o-sensing mechanism of the osteocyte is more similar to that of the osteoclast than to the CaR (103). Thus, additional work is needed to clarify the Ca^{2+}_o-sensing mechanism(s) in bone cells. Genetic approaches [e.g. bone cells from CaR knockout mice (44) and dominant negative CaRs (5, 64)] and/or pharmacological tools [i.e. specific CaR agonists (68) and antagonists (67)] should clarify the role of the CaR in various bone cells.

Cartilage Ca^{2+} is important for the transformation of the skeleton's cartilaginous model to bone during growth. Moreover, Ca^{2+}_o modulates the differentiation

and other functions of cartilage cells (98), which arise from the same mesenchymal stem cell that gives rise to osteoblasts, adipocytes, smooth muscle cells, and fibroblasts (20). It is interesting that the RCJ3.1C5.18 chondrocytic cell line expresses the CaR and that raising Ca^{2+}_o dose dependently reduces the mRNAs for aggrecan (a major proteoglycan in cartilage), the α_1 chains of types II and X collagen (components of the cartilaginous matrix), and alkaline phosphatase (which contributes to cartilage mineralization) (22). Thus the CaR, by its actions on both cartilage and bone cells as well as other elements of the Ca^{2+}_o homeostatic system, may coordinate the need for mineral ions in the growing skeleton with their availability.

Intestine

The duodenum and proximal colon are the major sites of Ca^{2+} absorption in the intestine. Ca^{2+} taken up by the apical (e.g. luminal) plasma membrane diffuses down its intracellular concentration gradient to the basolateral cell membrane—likely using the Ca^{2+}-binding protein, calbindin, as a "shuttle"—and is extruded by the Ca^{2+}-ATPase and Na^+-Ca^{2+} exchanger (87). Researchers have cloned and characterized Ca^{2+} channels that likely represent the major apical Ca^{2+} uptake mechanisms in intestine and distal renal tubule (45, 70; see also above). The CaR is expressed in small intestinal villus and crypt cells, as well as colonic surface and crypt cells (24). The villus and surface cells both absorb Ca^{2+}. Thus, the CaR could directly or indirectly participate in Ca^{2+} homeostasis through its actions on these small and large intestinal epithelial cells, but no such actions have been investigated. The CaR on the apical surface of colonic crypt cells could potentially mediate the known inhibitory action of Ca^{2+}_o on crypt cell proliferation that has been implicated in the protective effect of dietary Ca^{2+}_o against colon cancer (49). The CaR is also expressed in the enteric nervous system (24), in which it may contribute to clinically significant actions of Ca^{2+}_o on intestinal motility, which is reduced in hypercalcemia and increased in hypocalcemia (87). The physiological importance of any such role in the enteric nervous system, however, is unknown.

Placenta

The placenta is crucial for fetal Ca^{2+} metabolism because it is the source of all fetal mineral ions. The fetal skeleton forms in the third trimester, during which ~30 g of skeletal calcium is laid down (77). Ca^{2+}_o-sensing placental cytotrophoblasts could potentially regulate Ca^{2+} transport between mother and fetus. These cells express both full-length CaR transcript(s) and an additional, alternatively spliced transcript (10) that lacks exon 3 and encodes a truncated, presumably inactive receptor protein. The presence of the full-length CaR, however, makes it a candidate for mediating the actions of Ca^{2+}_o on placental function. Recent studies with CaR knockout mice have suggested that the CaR contributes to placental calcium transport, although the relative contributions of maternal and fetal CaRs require

further study (55). Thus, the CaR directly or indirectly influences placental Ca^{2+} transfer, a key parameter for providing Ca^{2+} for the mineralizing fetal skeleton. The CaR is also essential for normal Ca^{2+}_o -regulated fetal PTH release and, indirectly, for normal fetal bone turnover and renal Ca^{2+} excretion (by ensuring normal fetal levels of PTH and Ca^{2+}_o) (55).

Possible Central Mechanisms Regulating Ca^{2+}_o Homeostasis

It is generally thought that Ca^{2+}_o homeostasis does not involve a central endocrine control mechanism(s) (e.g. analogous to pituitary adrenocorticotropic hormone secretion). It is interesting that the CaR is expressed in numerous regions of brain (78, 80), including those involved in the central regulation of other endocrine systems, such as hypothalamus, pituitary, and circumventricular organs of the third ventricle (78). Some of these CaRs could potentially participate in "central" control of Ca^{2+}_o homeostasis, as described below, whereas others may coordinate Ca^{2+}_o homeostasis with homeostatic systems for other key constituents in the ECF, as described in the next section. For instance, rats exhibit a specific "calcium appetite" (92) that could promote intake of calcium-containing food during calcium deficiency, although the role of the CaR in this process has not been investigated. The CaR is expressed in the area postrema of the hypothalamus, an important center for generating sensations of nausea or anorexia (78). If the CaR contributed to the anorexia occurring during hypercalcemia (87), it could reduce ingestion of food and, therefore, calcium intake. Studies in CaR knockout mice or the local application of specific CaR agonists or antagonists in the brain should permit direct testing of these hypotheses.

The CaR and the Integrated Control of Systemic Mineral Ion Homeostasis

An important consequence of the cloning of the CaR was the recognition that it is expressed not only in Ca^{2+}_o-sensing cells that secrete calciotropic hormones (e.g. parathyroid and C cells) but also in effector tissues that are acted on by these hormones (e.g. kidney and bone) (16). Thus, Ca^{2+}_o serves as a local and systemic Ca^{2+}_o-regulating "hormone" (Figure 2) controlling both calciotropic hormone secretion and the functions of these effector tissues.

For example, the CaR directly regulates renal tubular Ca^{2+} reabsorption (Figure 5) and may mediate local feedback inhibition of bone resorption by its actions on mature osteoclasts and/or the process of osteoclastogenesis, as well as the "coupling" of bone resorption to formation through actions of Ca^{2+}_o on chemotaxis and proliferation of preosteoblasts (72). Although the CaR may be an important mediator of these actions of Ca^{2+}_o, however, additional Ca^{2+}_o sensors/receptors may also contribute (72, 103). The dashed lines in Figure 2 illustrate direct actions of Ca^{2+}_o on tissues involved in mineral ion metabolism. This figure also points out that phosphate directly modulates cellular function. There may well be some

type of phosphate-sensing mechanism (12), but its structure and properties remain obscure.

POSSIBLE ROLES OF THE CALCIUM-SENSING RECEPTOR IN Mg^{2+}_o, WATER, AND SALT METABOLISM AND THEIR INTERRELATIONSHIPS

Role of the CaR in Sensing and Regulating Mg^{2+}_o

Mg^{2+}_o has long been known to mimic the actions of Ca^{2+}_o on certain cells. For instance, raising Mg^{2+}_o inhibits PTH secretion (42), reduces cAMP accumulation (59), and diminishes renal tubular Mg^{2+} reabsorption (71). Both Mg^{2+}_o and Ca^{2+}_o are agonists of the cloned CaR (14, 18, 21, 33, 81), although Mg^{2+}_o is two- to threefold less potent than Ca^{2+}_o (18, 21, 81). Because Mg^{2+}_o in the ECF is, if anything, slightly lower than Ca^{2+}_o, can Mg^{2+}_o serve as a physiologically relevant CaR agonist in vivo? Genetic diseases of the CaR suggest that it does, in fact, contribute to "setting" Mg^{2+}_o (13). Individuals with inactivating CaR mutations can have mildly elevated levels of Mg^{2+}_o (57), whereas those with activating mutations may have mild hypomagnesemia (13). Mg^{2+}_o could act in several ways to control its own homeostasis. All CaR agonists potentiate one another's actions (15, 81). Therefore, raising Mg^{2+}_o may activate the CaR by sensitizing it to Ca^{2+}_o. Furthermore, Mg^{2+}_o in specific microenvironments differs from its blood level. The proximal tubule reabsorbs less Mg^{2+} than Na^+, Cl^-, Ca^{2+}, and water. Therefore, Mg^{2+}_o rises progressively along the nephron and is 1.6- to 1.8-fold higher in the thick ascending limb than in PCTs (30). This level of Mg^{2+}_o may activate CaRs in CTAL that regulate both Ca^{2+} and Mg^{2+} reabsorption. An elevated Mg^{2+}_o would promote urinary Mg^{2+} loss, whereas low Mg^{2+}_o would increase Mg^{2+} retention in CTAL, thereby promoting Mg^{2+}_o homeostasis.

Possible Roles of the Calcium-Sensing Receptor in Sodium Chloride, Volume, and Blood Pressure Regulation

Roles of the CaR in Volume and Blood Pressure Regulation Figure 5 shows that activating the CaR reduces transcellular NaCl transport in CTAL by inhibiting the Na/K/2Cl cotransporter and that *pari passu* reduces paracellular NaCl reabsorption by diminishing V_t. Therefore, high Ca^{2+}_o exerts a "loop diuretic-like" action that likely contributes to the volume depletion of severely hypercalcemic persons (e.g. via urinary loss of NaCl) (43). The action of Ca^{2+}_o could also potentially contribute to the salutary action of dietary calcium supplementation in certain forms of genetic hypertension in experimental animals (e.g. the spontaneously hypertensive rat) (62, 69) and, perhaps, in treating pregnancy-induced hypertension (91) or preventing preeclampsia (61). Because of its exquisite sensitivity to changes in Ca^{2+}_o resulting from alterations in dietary calcium intake, the CaR in

the kidney could potentially modulate NaCl reabsorption and sensitize the kidney to other agents that promote diuresis.

There are additional actions through which the CaR could modulate blood pressure. It is expressed in perivascular sensory nerve endings in rat mesenteric artery (17) and other vascular beds (e.g. mesenteric branch artery > basilar artery = renal interlobar artery > main renal trunk artery > left anterior descending coronary artery) (95). Moreover, stimulating the CaR in nerve endings releases a vasodilatory substance—likely an endogenous cannabinoid [e.g. N-arachidoylethanolamine (anandamide)]—that then acts on a cannabinoid receptor in the vascular wall (48). It is possible that the inhibition of renin release from the juxtaglomerular apparatus by high $Ca^{2+}{}_o$ involves the CaR (35). Further studies, therefore, may reveal that the CaR regulates renal fluid transport, electrolyte metabolism, vascular tone, and, perhaps, central vasomotor control by multiple mechanisms. These effects of the CaR could contribute to overall blood pressure regulation and therefore provide therapeutic targets for novel CaR-based, dietary and/or pharmacological treatments of hypertension and, perhaps, other vascular disorders.

Sensing of Ionic Strength by the CaR Increases in ionic strength reduce the sensitivity of the CaR to $Ca^{2+}{}_o$ and vice versa (73). These effects occur independently of the monovalent cation (e.g. sodium or choline) or anion (chloride or iodide) used to alter ionic strength. Changes in osmolality per se (e.g. achieved via substitution of sodium chloride with sucrose), in contrast, do not modify $Ca^{2+}{}_o$ sensing by the CaR (73). Substantial alterations in ionic strength occur in specific microenvironments even under normal circumstances. For example, the urinary sodium concentration (and, therefore, ionic strength) in the distal collecting system varies from ~50 to 300 mM. The CaR on the IMCD apical membrane, therefore, may experience changes in ionic strength that are sufficient to alter substantially the 50% effective concentration for its activation by $Ca^{2+}{}_o$. Moreover, in CTAL, NaCl is reabsorbed largely without water because of the low permeability of this nephron segment to water (43). Therefore, the basolateral CaR in CTAL epithelial cells may experience elevated levels of ionic strength that could change its $Ca^{2+}{}_o$-sensing capacity in physiologically relevant ways.

Role of the Calcium-Sensing Receptor in Renal Integration of $Ca^{2+}{}_o$ and Water Homeostasis

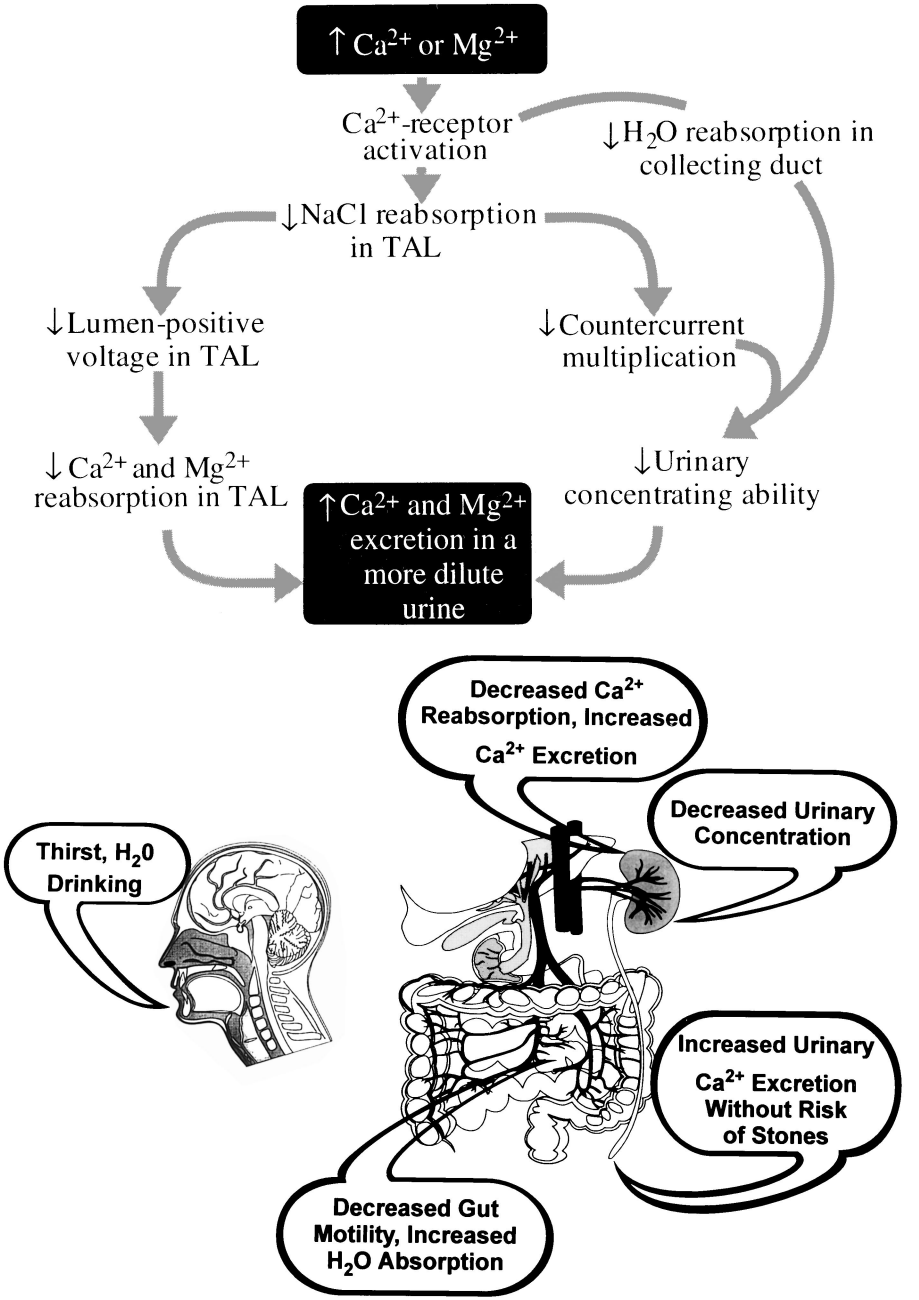
Severely hypercalcemic patients can exhibit defective urinary concentrating ability and, occasionally, frank nephrogenic diabetes insipidus (88). The CaR in nephron segments involved in urinary concentration (74, 83) may provide a mechanism that underlies this action of high $Ca^{2+}{}_o$. Perfusing rat IMCD with high $Ca^{2+}{}_o$ reversibly decreases vasopressin-stimulated water flow by 35%–40% (83). Vasopressin, whose hypothalamic secretion is activated by dehydration and resultant increases in serum osmolality, increases water reabsorption in the collecting duct by stimulating insertion of water channel-containing endosomes into the apical

plasma membrane, thereby enhancing their water permeability. The CaR may inhibit water flow by stimulating the removal and/or inhibiting the insertion of water channels into the apical plasma membrane (83). Moreover, chronic vitamin D-induced hypercalcemia in rats reduces water channel levels in IMCD (82), thereby diminishing further vasopressin-stimulated water flow. CaR-induced inhibition of vasopressin-evoked water flow in IMCD during hypercalcemia may set an upper limit for $Ca^{2+}{}_o$ in this nephron segment, thereby mitigating the risk of forming calcium-containing stones. Of interest, the CaR modifies $Ca^{2+}{}_o$ in IMCD by altering water flow rather than Ca^{2+} transport, as it does in systemic $Ca^{2+}{}_o$ homeostasis.

In addition to CaR-mediated actions in IMCD, the receptor regulates urinary concentrating ability and water intake in additional ways. For instance, CaR-induced inhibition of NaCl reabsorption in MTAL (94) diminishes the medullary countercurrent gradient, thereby reducing urinary concentrating power (Figure 6).

Moreover, abundant CaRs in the subfornical organ (78), an important hypothalamic thirst center, may promote a CaR-mediated increase in thirst that could minimize dehydration from accompanying renal water loss caused by reduced urinary concentrating ability (see Figure 6B). It is interesting that individuals with inactivating CaR mutations concentrate their urine normally and do not manifest excessive thirst despite their hypercalcemia (58), presumably because they are resistant to the effects of $Ca^{2+}{}_o$ on the renal concentrating mechanism and hypothalamic thirst centers. Conversely, persons with activating CaR mutations can develop diminished urinary concentrating capacity and excessive thirst at normal or even low levels of $Ca^{2+}{}_o$ when treated with vitamin D and calcium supplementation, probably because their CaRs are overly sensitive to $Ca^{2+}{}_o$ (13). Thus, the CaR may provide a mechanism for integrating the renal handling of Ca^{2+} and water, permitting appropriate "tradeoffs" in how these parameters of renal function are regulated under specific physiological conditions (43). For instance, when disposing of a systemic Ca^{2+} load, a CaR-mediated increase in luminal $Ca^{2+}{}_o$ in IMCD, particularly in a dehydrated individual, could predispose one to Ca^{2+}-containing renal stones, were it not for the concomitant CaR-mediated inhibition of maximal urinary concentrating capacity. Thus, there may be multiple layers of CaR-mediated integration and coordination of water and calcium metabolism that optimize the ability of terrestrial organisms to adapt to intermittent dietary access to Ca^{2+} and water (43). Therefore, the CaR likely participates in coordinating several of the body's homeostatic systems (e.g. for Na^+, $Ca^{2+}{}_o$, $Mg^{2+}{}_o$, and water) via $Ca^{2+}{}_o$-induced actions on their central control centers as well as their effector tissues.

Figure 6 Possible mechanisms interrelating systemic $Ca^{2+}{}_o$ and water homeostasis. See text for details. *Upper panel* illustrates renal mechanisms by which the CaR inhibits maximal urinary concentrating capacity. (Reproduced with permission from Reference 14c.) *Lower panel* shows that activating CaRs in the subfornical organ could increase water intake and mitigate loss of free water resulting from diminished urinary concentration. (Reproduced with permission from Reference 14a.)



SUMMARY AND CONCLUSIONS—A NUTRITIONAL PERSPECTIVE ON THE CALCIUM-SENSING RECEPTOR

The cloning of the CaR has identified a key player in systemic Ca^{2+}_o homeostasis, enabling maintenance of near constancy of Ca^{2+}_o via its coordinated actions on the tissues involved in mineral ion homeostasis. This system enables organisms to adapt successfully to wide variations in dietary Ca^{2+}_o intake. Whereas descriptions of the Ca^{2+}_o homeostatic system have traditionally focused on the crucial role of Ca^{2+}_o -induced changes in PTH and, to a lesser extent, CT secretion, the presence of the CaR in effector elements of this system likely enables direct regulation of processes (such as renal tubular Ca^{2+} reabsorption, osteoblastic bone formation, and osteoclastic bone resorption) that add further layers of homeostatic control. As more is learned about the role of the CaR in these tissues, researchers may find that it participates in other processes that are also relevant to mineral ion homeostasis, such as controlling 1-hydroxylation of vitamin D or phosphate reabsorption in the proximal tubule. In any event, the exquisite sensitivity of the CaR to even minute changes in Ca^{2+}_o permits adjustments in the Ca^{2+}_o homeostatic system's responses, for example, to increases or decreases in dietary Ca^{2+} intake that produce barely detectable changes in Ca^{2+}_o. Moreover, it appears increasingly likely that the CaR participates in additional, previously unappreciated complexities in the regulation of mineral ion metabolism. Finally, it may participate in coordinating interactions among several different homeostatic systems, such as those for water, Mg^{2+}_o, Na^+, extracellular volume, and/or blood pressure, which are usually thought of as functioning largely independently of mineral ion metabolism.

ACKNOWLEDGMENTS

This work was supported by generous grants from the National Institutes of Health (DK41415, DK48330, and DK52005), NPS Pharmaceuticals, Inc., the St. Giles Foundation, the National Dairy Council, and the National Space Bioscience Research Institute (NSBRI).

LITERATURE CITED

1. Aida K, Koishi S, Tawata M, Onaya T. 1995. Molecular cloning of a putative $Ca^{(2+)}$ sensing receptor cDNA from human kidney. *Biochem. Biophys. Res. Commun.* 214:524–29
2. Attie MF, Gill J Jr, Stock JL, Spiegel AM, Downs RW Jr, et al. 1983. Urinary calcium excretion in familial hypocalciuric hypercalcemia: persistence of relative hypocalciuria after induction of hypoparathyroidism. *J. Clin. Invest.* 72:667–76
3. Austin LA, Heath H III 1981. Calcitonin: physiology and pathophysiology. *N. Engl. J. Med.* 304:269–78
4. Autry CP, Brown EM, Fuller FH, Rogers KV, Halloran BP 1997. Ca^{2+} receptor

mRNA and protein increase in the rat parathyroid gland with age. *J. Endocrinol.* 153:437–44

5. Bai M, Quinn S, Trivedi S, Kifor O, Pearce SHS, et al. 1996. Expression and characterization of inactivating and activating mutations in the human $Ca^{2+}{}_o$-sensing receptor. *J. Biol. Chem.* 271:19537–45
6. Bai M, Trivedi S, Brown EM. 1998. Dimerization of the extracellular calcium-sensing receptor (CaR) on the cell surface of CaR-transfected HEK293 cells. *J. Biol. Chem.* 273:23605–10
7. Bai M, Trivedi S, Kifor O, Quinn SJ, Brown EM. 1999. Intermolecular interactions between dimeric calcium-sensing receptor monomers are important for its normal function. *Proc. Natl. Acad. Sci. USA* 96:2834–39
8. Bai M, Trivedi S, Lane CR, Yang Y, Quinn SJ, Brown EM. 1998. Protein kinase C phosphorylation of threonine at position 888 in $Ca^{2+}{}_o$-sensing receptor (CaR) inhibits coupling to Ca2+ store release. *J. Biol. Chem.* 273:21267–75
9. Bapty BW, Dai LJ, Ritchie G, Jirik F, Canaff L, et al. 1998. Extracellular $Mg^{(2+)}$-and $Ca^{(2+)}$ -sensing in mouse distal convoluted tubule cells. *Kidney Int.* 53:583–92
10. Bradbury RA, Sunn KL, Crossley M, Bai M, Brown EM, et al. 1998. Expression of the parathyroid $Ca^{(2+)}$ -sensing receptor in cytotrophoblasts from human term placenta. *J. Endocrinol.* 156:425–30
11. Brody JE. 1998. Calcium takes its place as a superstar of nutrients. *NY Times Oct. 13*

11a. Brown EM. 1983. Four parameter model of the sigmoidal relationship between PTH release and the extracellular calcium concentration in normal and abnormal parathyroid tissue. *J. Clin. Endocrinol. Metab.* 56:572–81

12. Brown EM. 1991. Extracellular Ca^{2+} sensing, regulation of parathyroid cell function, and role of Ca^{2+} and other ions as extracellular (first) messengers. *Physiol. Rev.* 71:371–411
13. Brown EM. 1999. Physiology and pathophysiology of the extracellular calcium-sensing receptor. *Am. J. Med.* 106:238–53

13a. Brown EM, Bai M, Pollak MM. 1997. Familial benign hypocalciuric hypercalcemia and other syndromes of altered responsiveness to extracellular calcium. In *Metabolic Bone Disease*, ed. SM Karne, LV Avioli, pp. 479–99. San Diego, CA: Academic. 3rd ed.

14. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, et al. 1993. Cloning and characterization of an extracellular Ca^{2+}-sensing receptor from bovine parathyroid. *Nature* 366:575–80

14a. Brown EM, Harris WH Jr, Vassilev P, Hebert SC. 1996. The biology of the extracellular Ca^{2+}-sensing receptor. In *Principles of Bone Biology*, ed. JP Bilezikian, LG Raisz, GA Rodan, pp. 243–62. San Diego, CA: Academic

14b. Brown EM, Hebert SC. 1997. Calcium-receptor regulated parathyroid and renal function. *Bone* 20:303–9

14c. Brown EM, Hebert SC. 1997. Novel insights into the physiology and pathophysiology of Ca^{2+} homeostasis from the cloning of an extracellular Ca^{2+}-sensing receptor. *Regul. Pept. Lett.* 7:43–47

14d. Brown EM, LeBoff MS. 1986. Pathophysiology of hyperparathyroidism. In *Progress in Surgery: Parathyroid Surgery*, ed. M Rothmund, SA Wells Jr, pp. 13–22. Basel: Karger

14e. Brown EM, Pollak M, Hebert C. 1994. Cloning and characterization of extracellular Ca^{2+}-sensing receptors from parathyroid and kidney: molecular physiology and pathophysiology of Ca^{2+} sensing. *Endocrinologist* 4:419–26

15. Brown EM, Thatcher JG, Watson EJ, Leombruno R. 1984. Extracellular calcium potentiates the inhibitory effects of magnesium on parathyroid function in

dispersed bovine parathyroid cells. *Metabolism* 33:171–76

16. Brown EM, Vassilev PM, Quinn S, Hebert SC. 1999. G-protein-coupled, extracellular $Ca^{(2+)}$ -sensing receptor: a versatile regulator of diverse cellular functions. *Vitam. Horm.* 55:1–71
17. Bukoski RD, Bian K, Wang Y, Mupanomunda M. 1997. Perivascular sensory nerve Ca^{2+} receptor and Ca^{2+}-induced relaxation of isolated arteries. *Hypertension* 30:1431–39
18. Butters RR Jr, Chattopadhyay N, Nielsen P, Smith CP, Mithal A, et al. 1997. Cloning and characterization of a calcium-sensing receptor from the hypercalcemic New Zealand white rabbit reveals unaltered responsiveness to extracellular calcium. *J. Bone Miner. Res.* 12:568–79
19. Cao Y, Oh BC, Stryer L. 1998. Cloning and localization of two multigene receptor families in goldfish olfactory epithelium. *Proc. Natl. Acad. Sci. USA* 95:11987–92
20. Caplan AI, Dennis JE. 1996. Mesenchymal stem cells: progenitors, progeny, and pathways. *J. Bone Miner. Metab.* 14:193–201
21. Chang W, Pratt S, Chen TH, Nemeth E, Huang Z, Shoback D. 1998. Coupling of calcium receptors to inositol phosphate and cyclic AMP generation in mammalian cells and Xenopus laevis oocytes and immunodetection of receptor protein by region-specific antipeptide antisera. *J. Bone Miner. Res.* 13:570–80
22. Chang W, Tu C, Bajra R, Komuves L, Miller S, et al. 1999. Calcium sensing in cultured chondrogenic RCJ3.1C5.18 cells. *Endocrinology* 140:1911–19
23. Chang W, Tu C, Chen T-H, Komuves L, Oda Y, et al. 1999. Expression and signal transduction of calcium-sensing receptors in cartilage and bone. *Endocrinology* 140:5883–93
24. Chattopadhyay N, Cheng I, Rogers K, Riccardi D, Hall A, et al. 1998. Identification and localization of extracellular $Ca^{(2+)}$ -sensing receptor in rat intestine. *Am. J. Physiol.* 274:G122–30
25. Cima RR, Cheng I, Klingensmith ME, Chattopadhyay N, Kifor O, et al. 1997. Identification and functional assay of an extracellular calcium-sensing receptor in Necturus gastric mucosa. *Am. J. Physiol.* 273:G1051–60
26. Conklin BR, Bourne HR. 1994. Homeostatic signals: marriage of the flytrap and the serpent. *Nature* 367:22
27. Dascal N. 1987. The use of Xenopus oocytes for the study of ion channels. *CRC Crit. Rev. Biochem.* 22:317–87
28. De Jesus Ferreira MC, Bailly C. 1998. Extracellular Ca^{2+} decreases chloride reabsorption in rat CTAL by inhibiting cAMP pathway. *Am. J. Physiol.* 275:F198–203
29. de Jesus Ferreira MC, Helies-Toussaint C, Imbert-Teboul M, Bailly C, Verbavatz JM, et al. 1998. Co-expression of a Ca^{2+}-inhibitable adenylyl cyclase and of a Ca^{2+}-sensing receptor in the cortical thick ascending limb cell of the rat kidney: inhibition of hormone-dependent cAMP accumulation by extracellular Ca^{2+}. *J. Biol. Chem.* 273:15192–202
30. De Rouffignac C, Quamme GA. 1994. Renal magnesium handling and its hormonal control. *Physiol. Rev.* 74:305–22
31. Desfleurs E, Wittner M, Pajaud S, Nitschke R, Rajerison RM, Di Stefano A. 1999. The Ca^{2+}-sensing receptor in the rabbit cortical thick ascending limb (CTAL) is functionally not coupled to phospholipase C. *Pfluegers Arch.* 437:716–23
32. Desfleurs E, Wittner M, Simeone S, Pajaud S, Moine G, et al. 1998. Calcium-sensing receptor: regulation of electrolyte transport in the thick ascending limb of Henle's loop. *Kidney Blood Press. Res* 21:401–12
33. Diaz R, Hurwitz S, Chattopadhyay N, Pines M, Yang Y, et al. 1997. Cloning, expression, and tissue localization of the calcium-sensing receptor in chicken (*Gallus domesticus*). *Am. J. Physiol.* 273:R1008–16
34. Emanuel RL, Adler GK, Kifor O, Quinn

SJ, Fuller F, et al. 1996. Calcium-sensing receptor expression and regulation by extracellular calcium in the AtT-20 pituitary cell line. *Mol. Endocrinol.* 10:555–65
35. Fray J, Park C, Valentine N. 1987. Calcium and the control of renin secretion. *Endocr. Rev.* 8:53–93
36. Freichel M, Zink-Lorenz A, Holloschi A, Hafner M, Flockerzi V, Raue F. 1996. Expression of a calcium-sensing receptor in a human medullary thyroid carcinoma cell line and its contribution to calcitonin secretion. *Endocrinology* 137:3842–48
37. Friedman PA, Gesek FA. 1995. Cellular calcium transport in renal epithelia: measurement, mechanisms, and regulation. *Physiol. Rev.* 75:429–71
38. Garrett J, Steffey M, Nemeth E. 1995. The calcium receptor agonist R-568 suppresses PTH mRNA levels in cultured bovine parathyroid cells. *J. Bone Miner. Res.* 10(Suppl. 1):S387 (Abstr. M539)
39. Garrett JE, Capuano IV, Hammerland LG, Hung BC, Brown EM, et al. 1995. Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. *J. Biol. Chem.* 270:12919–25
40. Garrett JE, Tamir H, Kifor O, Simin RT, Rogers KV, et al. 1995. Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. *Endocrinology* 136:5202–11
41. Godwin SL, Soltoff SP. 1997. Extracellular calcium and platelet-derived growth factor promote receptor-mediated chemotaxis in osteoblasts through different signaling pathways. *J. Biol. Chem.* 272:11307–12
42. Habener JF, Potts JT Jr. 1976. Relative effectiveness of calcium and magnesium in the secretion and biosynthesis of parathyroid hormone in vitro. *Endocrinology* 98:197–202
43. Hebert SC. 1996. Extracellular calcium-sensing receptor: implications for calcium and magnesium handling in the kidney. *Kidney Int.* 50:2129–39
44. Ho C, Conner DA, Pollak MR, Ladd DJ, Kifor O, et al. 1995. A mouse model of human familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. *Nat. Genet.* 11:389–94
45. Hoenderop JG, van der Kemp AW, Hartog A, van de Graaf SF, van Os CH, et al. 1999. Molecular identification of the apical Ca^{2+} channel in 1, 25-dihydroxyvitamin D_3-responsive epithelia. *J. Biol. Chem.* 274:8375–78
46. Hoon MA, Adler E, Lindemeier J, Battey JF, Ryba NJ, Zuker CS. 1999. Putative mammalian taste receptors: a class of taste-specific GPCRs with distinct topographic selectivity. *Cell* 96:541–51
47. Hurwitz S. 1996. Homeostatic control of plasma calcium concentration. *Crit. Rev. Biochem. Mol. Biol.* 31:41–100
48. Ishioka N, Bukoski RD. 1999. A role for N-arachidonylethanolamine (anandamide) as the mediator of sensory nerve-dependent Ca^{2+}-induced relaxation. *J. Pharmacol. Exp. Ther.* 289:245–50
49. Kallay E, Kifor O, Chattopadhyay N, Brown EM, Bischof MG, et al. 1997. Calcium-dependent c-myc proto-oncogene expression and proliferation of Caco-2 cells: a role for a luminal extracellular calcium-sensing receptor. *Biochem. Biophys. Res. Commun.* 232:80–83
50. Kamioka H, Sumitani K, Tagami K, Miki Y, Terai K, et al. 1994. Divalent cations elevate cytosolic calcium of chick osteocytes. *Biochem. Biophys. Res. Commun.* 204:519–24
51. Kanatani M, Sugimoto T, Kanzawa M, Yano S, Chihara K. 1999. High extracellular calcium inhibits osteoclast-like cell formation by directly acting on the calcium-sensing receptor existing in osteoclast precursor cells. *Biochem. Biophys. Res. Commun.* 261:144–48
52. Kaupmann K, Huggel K, Heid J, Flor PJ, Bischoff S, et al. 1997. Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptor. *Nature* 386:239–46

53. Kifor O, Diaz R, Butters R, Brown EM. 1997. The Ca^{2+}-sensing receptor (CaR) activates phospholipases C, A_2, and D in bovine parathyroid and CaR-transfected, human embryonic kidney (HEK293) cells. *J. Bone Miner. Res.* 12:715–25
54. Kifor O, Moore FD Jr, Wang P, Goldstein M, Vassilev P, et al. 1996. Reduced immunostaining for the extracellular Ca^{2+}-sensing receptor in primary and uremic secondary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 81:1598–606
55. Kovacs CS, Ho-Pao CL, Hunzelman JL, Lanske B, Fox J, et al. 1998. Regulation of murine fetal-placental calcium metabolism by the calcium-sensing receptor. *J. Clin. Invest.* 101:2812–20
56. Manolagas SC, Jilka RL. 1995. Bone marrow, cytokines, and bone remodeling: emerging insights into the pathophysiology of osteoporosis. *N. Engl. J. Med.* 332:305–11
57. Marx SJ, Attie MF, Levine MA, Spiegel AM, Downs RW Jr, Lasker RD. 1981. The hypocalciuric or benign variant of familial hypercalcemia: clinical and biochemical features in fifteen kindreds. *Medicine* 60:397–412
58. Marx SJ, Attie MF, Stock JL, Spiegel AM, Levine MA. 1981. Maximal urine-concentrating ability: familial hypocalciuric hypercalcemia versus typical primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* 52:736–40
59. Mathias RS, Brown EM. 1991. Divalent cations modulate PTH-dependent 3′,5′-cyclic adenosine monophosphate production in renal proximal tubular cells. *Endocrinology* 128:3005–12
60. Matsunami H, Buck LB. 1997. A multigene family encoding a diverse array of putative pheromone receptors in mammals. *Cell* 90:775–84
61. Mattar F, Sibai BM. 1999. Prevention of preeclampsia. *Semin. Perinatol.* 23:58–64
62. McCarron DA, Hatton D, Roullet JB, Roullet C. 1994. Dietary calcium, defective cellular Ca2+ handling, and arterial pressure control. *Can. J. Physiol. Pharmacol.* 72:937–44
63. McGehee DS, Aldersberg M, Liu KP, Hsuing S, Heath MJ, Tamir H. 1997. Mechanism of extracellular Ca^{2+} receptor-stimulated hormone release from sheep thyroid parafollicular cells. *J. Physiol.* 502:31–44
64. McNeil SE, Hobson SA, Nipper V, Rodland KD. 1998. 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.* 273:1114–20
65. Nakanishi S. 1994. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. *Neuron* 13: 1031–37
66. Nemeth E, Scarpa A. 1987. Rapid mobilization of cellular Ca^{2+} in bovine parathyroid cells by external divalent cations. *J. Biol. Chem.* 202:5188–96
67. Nemeth EF, Fox J, Delmar EG, Steffey ME, Lambert LD, et al. 1998. Stimulation of parathyroid hormone secretion by a small molecule antagonist of the calcium receptor. *J. Bone Miner. Res.* 23:S156 (Abstr. 1030)
68. Nemeth EF, Steffey ME, Hammerland LG, Hung BC, Van Wagenen BC, et al. 1998. Calcimimetics with potent and selective activity on the parathyroid calcium receptor. *Proc. Natl. Acad. Sci. USA* 95:4040–45
69. Oparil S, Chen YF, Jin HK, Yang RH, Wyss JM. 1991. Dietary Ca^{2+} prevents $NaCl^-$sensitive hypertension in spontaneously hypertensive rats via sympatholytic and renal effects. *Am. J. Clin. Nutr.* 54:227–36S
70. Peng J-B, Chen XZ, Berger UV, Vassilev PM, Tsukaguchi H, et al. 1999. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. *J. Biol. Chem.* 274:22739–46

71. Quamme GA. 1982. Effect of hypercalcemia on renal tubular handling of calcium and magnesium. *Can. J. Physiol. Pharmacol.* 60:1275–80
72. Quarles LD. 1997. Cation-sensing receptors in bone: a novel paradigm for regulating bone remodeling? J. *Bone Miner. Res.* 12:1971–74
73. Quinn SJ, Kifor O, Trivedi S, Diaz R, Vassilev P, Brown E. 1998. Sodium and ionic strength sensing by the calcium receptor. *J. Biol. Chem.* 273:19579–86
74. Riccardi D, Hall AE, Chattopadhyay N, Xu JZ, Brown EM, Hebert SC. 1998. Localization of the extracellular Ca^{2+}/polyvalent cation-sensing protein in rat kidney. *Am. J. Physiol.* 274:F611–22
75. Riccardi D, Lee WS, Lee K, Segre GV, Brown EM, Hebert SC. 1996. Localization of the extracellular $Ca^{(+)}$-sensing receptor and PTH/PTHrP receptor in rat kidney. *Am. J. Physiol.* 271:F951–56
76. Riccardi D, Park J, Lee WS, Gamba G, Brown EM, Hebert SC. 1995. Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. *Proc. Natl. Acad. Sci. USA* 92: 131–35
77. Rodda CP, Caple IW, Martin TJ. 1992. Role of PTHrP in fetal and neonatal physiology. In *Parathyroid Hormone Related Protein: Normal Physiology and its Role in Cancer*, ed. BP Halloran, RA Nissenson, pp. 169–96. Boca Raton, FL: CRC Press
78. Rogers KV, Dunn CK, Hebert SC, Brown EM. 1997. Localization of calcium receptor mRNA in the adult rat central nervous system by in situ hybridization. *Brain Res.* 744:47–56
79. Romoli R, Lania A, Mantovani G, Corbetta S, Persani L, Spada A. 1999. Expression of calcium-sensing receptor and characterization of intracellular signaling in human pituitary adenomas. *J. Clin. Endocrinol. Metab.* 84:2848–53
80. Ruat M, Molliver ME, Snowman AM, Snyder SH. 1995. Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. *Proc. Natl. Acad. Sci. USA* 92:3161–65
81. Ruat M, Snowman AM, Hester LD, Snyder SH. 1996. Cloned and expressed rat Ca^{2+}-sensing receptor. *J. Biol. Chem.* 271:5972–75
82. Sands JM, Flores FX, Kato A, Baum MA, Brown EM, et al. 1998. Vasopressin-elicited water and urea permeabilities are altered in IMCD in hypercalcemic rats. *Am. J. Physiol.* 274:F978–85
83. Sands JM, Naruse M, Baum M, Jo I, Hebert SC, et al. 1997. Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited water permeability in rat kidney inner medullary collecting duct. *J. Clin. Invest.* 99:1399–405
84. Sharff AJ, Rodseth LE, Spurlino JC, Quiocho FA. 1992. Crystallographic evidence for a large ligand-induced hinge-twist motion between the two domains of the maltodextrin binding protein involved in active transport and chemotaxis. *Biochemistry* 31:10657–63
85. Silver IA, Murrils RJ, Etherington DJ. 1988. Microlectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. *Exp. Cell. Res.* 175:266–76
86. Silverberg SJ, Bone III HG, Marriott TB, Locker FG, Thys-Jacobs S, et al. 1997. Short-term inhibition of parathyroid hormone secretion by a calcium-receptor agonist in patients with primary hyperparathyroidism. *N. Engl. J. Med.* 337:1506–10
87. Stewart AF, Broadus AE. 1987. Mineral metabolism. In *Endocrinology and Metabolism*, ed. P Felig, JD Baxter, AE Broadus, LA Frohman, pp. 1317–453. New York: McGraw-Hill. 2nd ed.
88. Suki WN, Eknoyan G, Rector FC Jr, Seldin DW. 1969. The renal diluting and concentrating mechanism in hypercalcemia. *Nephron* 6:50–61
89. Takaichi K, Kurokawa K. 1988. Inhibitory

guanosine triphosphate-binding protein-mediated regulation of vasopressin action in isolated single medullary tubules of mouse kidney. *J. Clin. Invest.* 82:1437–44

90. Tal M, Ammar DA, Karpuj M, Krizhanovsky V, Naim M, Thompson DA. 1995. A novel putative neuropeptide receptor expressed in neural tissue, including sensory epithelia. *Biochem. Biophys. Res. Commun.* 209:752–59
91. Tomoda S, Kitanaka T, Ogita S, Hidaka A. 1995. Prevention of pregnancy-induced hypertension by calcium dietary supplement: a preliminary report. *J. Obstet. Gynaecol.* 21:281–88
92. Tordoff MG. 1994. Voluntary intake of calcium and other minerals by rats. *Am. J. Physiol.* 167:R470–75
93. Wada M, Furuya Y, Sakiyama J-I, Kobayashi N, Miyata S, et al. 1997. The calcimimetic compound NPS R-568 suppresses parathyroid cell proliferation in rats with renal insufficiency. *J. Clin. Invest.* 100:2977–83
94. Wang WH, Lu M, Hebert SC. 1996. Cytochrome P-450 metabolites mediate extracellular $Ca^{(2+)}$ -induced inhibition of apical K^+ channels in the TAL. *Am. J. Physiol.* 271:C103–11
95. Wang Y, Bukoski RD. 1998. Distribution of the perivascular nerve Ca^{2+} receptor in rat arteries. *Br. J. Pharmacol.* 125:1397–404
96. Ward DT, Brown EM, Harris HW. 1998. Disulfide bonds in the extracellular calcium-polyvalent cation-sensing receptor correlate with dimer formation and its response to divalent cations in vitro. *J. Biol. Chem.* 273:14476–83
97. Weisinger JR, Favus MJ, Langman CB, Bushinsky D. 1989. Regulation of 1,25-dihydroxyvitamin D_3 by calcium in the parathyroidectomized, parathyroid hormone-replete rat. *J. Bone Miner. Res.* 4:929–35
98. Wong M, Tuan RS. 1995. Interactive cellular modulation of chondrogenic differentiation in vitro by subpopulations of chick embryonic calvarial cells. *Dev. Biol.* 167:130–47
99. Yamaguchi T, Chattopadhyay N, Kifor O, Brown EM. 1998. Extracellular calcium Ca^{2+}_o-sensing receptor in a murine bone marrow-derived stromal cell line (ST2): potential mediator of the actions of Ca^{2+}_o on the function of ST2 cells. *Endocrinology* 139:3561–68
100. Yamaguchi T, Chattopadhyay N, Kifor O, Butters RR Jr, Sugimoto T, Brown EM. 1998. 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.* 13:1530–38
101. Yamaguchi T, Kifor O, Chattopadhyay N, Bai M, Brown EM. 1998. Extracellular calcium (Ca^{2+}_o)-sensing receptor in a mouse monocyte-macrophage cell line (J774): potential mediator of the actions of Ca^{2+}_o on the function of J774 cells. *J. Bone Miner. Res.* 13:1390–97
102. Yamaguchi T, Olozak I, Chattopadhyay N, Butters RR, Kifor O, et al. 1998. Expression of extracellular calcium (Ca^{2+}_o)-sensing receptor in human peripheral blood monocytes. *Biochem. Biophys. Res. Commun.* 246:501–6
103. Zaidi M, Adebanjo OA, Moonga BS, Sun L, Huang CL. 1999. Emerging insights into the role of calcium ions in osteoclast regulation. *J. Bone Miner. Res.* 14:669–74
104. Zaidi M, Datta HK, Patchell A, Moonga B, MacIntyre I. 1989. "Calcium-activated" intracellular calcium elevation: a novel mechanism of osteoclast regulation. *Biochem. Biophys. Res. Commun.* 183:1461–65