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The Calcium-Sensing Receptor: A Window into the Physiology and Pathophysiology of Mineral Ion Metabolism

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I. Introduction

CALCIUM [1](Ca^{2+}) ions are of critical importance for a variety of vital bodily functions (1–4). Intra- and extracellular Ca^{2+} act in distinct but sometimes complementary ways to regulate a multitude of biological processes. The cytosolic free calcium concentration ($[Ca^{2+}]_i$) plays a pivotal role in controlling cellular processes such as secretion, differentiation, and motility (2, 5). Calcium ions act as key intracellular second messengers and also as cofactors for a number of enzymes. Although $[Ca^{2+}]_i$ generally exists at much lower levels than the extracellular calcium concentration ($[Ca^{2+}]_o$), it can undergo large, rapid changes due to either influx through the cell membrane or release from intracellular stores (2, 5). $[Ca^{2+}]_o$, on the other hand, remains remarkably constant, varying by only a few percentage points over much of a lifetime under normal circumstances (1, 3, 4). $[Ca^{2+}]_o$ likewise plays an essential role in numerous processes, including blood clotting, neuromuscular excitability, and maintenance of the integrity of the skeletal system.

The near constancy of $[Ca^{2+}]_o$ is the result of a complex homeostatic system, which, in mammals and other tetrapods, primarily involves the parathyroid glands, calcitonin (CT)-producing C cells, kidney, bone, and intestine (1, 3, 4). It has been known for many years that calcium ions move across cell membranes via ion channels and other transport processes, but the actual mechanism(s) that enables cells to "sense" (*i.e.* recognize and respond to) $[Ca^{2+}]_o$ was for many years poorly understood. The parathyroid glands of mammals are remarkably sensitive to $[Ca^{2+}]_o$, and studies on this cell type provided the first evidence that $[Ca^{2+}]_o$ modulates $[Ca^{2+}]_i$ and other second messengers without actually crossing the cell membrane, utilizing a mechanism similar to that of ligands acting through the so-called "calcium-mobilizing," G protein-linked receptors (1, 6–8). That this was actually the case was confirmed recently with the cloning of a G protein-coupled, $[Ca^{2+}]_o$-sensing receptor (CaR) from bovine parathyroid gland (9). Subsequently CaRs from various species have been cloned from several different tissues, including not only those involved in Ca^{2+} homeostasis, such as the kidney (10) and CT-secreting C cells (11), but also tissues, such as the brain (12), not thought to be involved directly in mineral ion homeostasis.

Soon after the cloning of the CaR, it was recognized that inherited mutations in this receptor could produce clinical disorders of mineral ion metabolism, such as familial hypocalciuric hypercalcemia (FHH) (13), neonatal severe hyperparathyroidism (NSHPT) (13), and autosomal dominant hypocalcemia (ADH) (14). It also facilitated the development of "calcimimetic" agents targeted at the receptor, which

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could potentially be of substantial use in the management of disorders with abnormal $[Ca^{2+}]_o$-sensing, such as primary and secondary hyperparathyroidism (15). This review will provide an update on these recent developments, outlining the discovery, cloning, and characterization of the receptor, as well as its known functions in various tissues and the way it is thought to malfunction in hyper- and hypocalcemic states.

II. Physiology of Calcium Homeostasis

The maintenance of $[Ca^{2+}]_o$ within the normal limits of 1.1–1.3 mM found in humans and other mammals is accomplished through the complex interplay of the various hormones and effector tissues that constitute the essential elements of the calcium homeostatic system (1, 3, 4) (Fig. 1). The most important of these hormones are PTH and vitamin D in humans, while CT also exerts a physiologically relevant hypocalcemic action in some species, such as the rat. Alterations in $[Ca^{2+}]_o$ result in activation of both short term (minutes to hours) and long term (days to weeks or longer) homeostatic responses. Lowering of $[Ca^{2+}]_o$, for instance, results in rapid release of preformed PTH from the chief cells of the parathyroid glands, which is followed within 15–30 min by an increase in net PTH production due to reduced intracellular degradation of the hormone (16, 17). Within hours, the level of the mRNA for prepro-PTH increases, which is a result of an increase in gene transcription (18) as well as a result of posttranslational mechanisms (19).

The hypocalcemia-elicited increase in the circulating level of PTH normalizes $[Ca^{2+}]_o$ by virtue of its actions on kidney, bone, and (indirectly through changes in 1,25-dihydroxyvitamin D [1,25-$(OH)_2D_3$] production) intestine (1, 3, 4). PTH

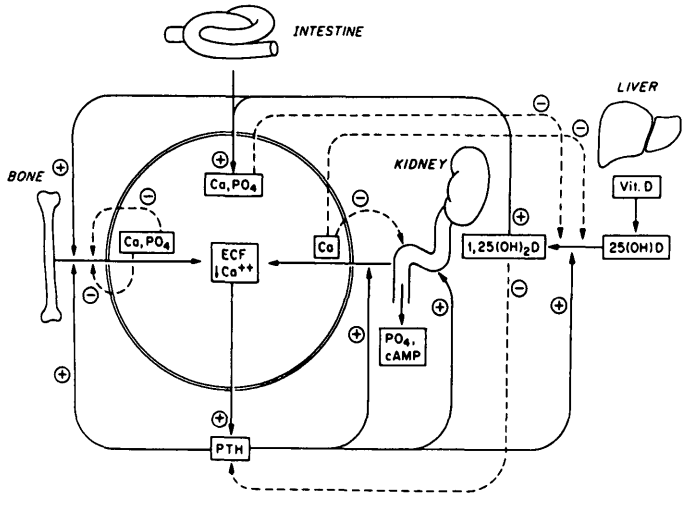

FIG. 1. Schematic diagram illustrating the regulation of $[Ca^{2+}]_o$ homeostasis. The *solid lines and arrows* indicate the actions of PTH and 1,25-$(OH)_2D_3$; the *dotted lines and arrows* demonstrate examples of direct effects of Ca^{2+} or phosphate ions. Abbreviations are as follows: Ca^{2+}, calcium; PO_4, phosphate; ECF, extracellular fluid; 1,25-$(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; + signs indicate positive actions while − signs indicate inhibitory effects. [Reproduced with permission from Brown EM, Pollak M, Hebert SC 1994 Cloning and characterization of extracellular Ca^{2+}-sensing receptors from parathyroid and kidney: molecular physiology and pathophysiology of Ca^{2+}-sensing. *The Endocrinologist* 4:419–426.]

binds to its high-affinity cell surface receptors in the kidney to increase the reabsorption of Ca^{2+} from the distal tubule and promote phosphaturia by inhibiting proximal tubular reabsorption of phosphate within a matter of minutes (4). In bone, PTH probably acts initially on the osteoblast (20), which, through mechanisms involving cellular contact and/or local humoral mediators, stimulates preformed osteoclasts to release calcium and phosphate ions into the extracellular fluid within 1–2 h by enhancing bone resorption. If hypocalcemia persists for longer periods, PTH can increase the formation of osteoclasts from their mononuclear precursors and also enhance the activity of the enzyme, 25-hydroxyvitamin D_3 [25(OH)D_3] 1-hydroxylase, in cells of the renal proximal tubule to catalyze the conversion of the prohormone, 25(OH)D_3, to the active hormone, 1,25-$(OH)_2D_3$ (1, 3, 4). The latter acts via its specific receptor in intestinal epithelial cells to promote absorption of Ca^{2+} and also to enhance release of skeletal Ca^{2+} (21). Long-term hypocalcemia, sustained for days to weeks, leads to hyperplasia of the parathyroid glands, which can result in greatly increased rates of PTH secretion (22, 23).

The homeostatic system has negative feedback elements that either reestablish the original steady state of the system or promote a new steady state if, for example, there were a persistent stress on the system, such as a change in dietary calcium availability. The restoration of $[Ca^{2+}]_o$ toward normal directly inhibits PTH synthesis and secretion, while 1,25-$(OH)_2D_3$ also exerts potent inhibitory effects of PTH gene expression (24–26), parathyroid cellular proliferation (27, 28), and PTH secretion (29) that provide an additional feedback loop.

Hypercalcemia reverses the homeostatic alterations in the $[Ca^{2+}]_o$-elevating system just described and, to a lesser extent, activates a hypocalcemic effector mechanism built around the actions of CT. High $[Ca^{2+}]_o$ acutely inhibits PTH release, an action that is opposite to the usual role of calcium in stimulus-secretion coupling. More prolonged elevations in $[Ca^{2+}]_o$ result in suppression of PTH gene expression (30). The resultant reduction in the circulating level of PTH decreases $[Ca^{2+}]_o$ by lowering its renal tubular reabsorption (4), diminishing Ca^{2+} mobilization from bone, and depressing the absorption of Ca^{2+} from the intestine due to reduced PTH-mediated synthesis of 1,25-$(OH)_2D_3$. Hypercalcemia also directly stimulates CT secretion from the C cells of the thyroid gland (31). CT is a potent inhibitor of osteoclast activity and can, therefore, reduce the flux of Ca^{2+} into the extracellular fluid from bone. In addition, CT exerts a calciuric effect, which contributes to its hypocalcemic action. However, CT has very modest hypocalcemic effects under normal circumstances in normal adult humans, although it acts more potently in this regard in states of elevated bone turnover, such as hypercalcemia or Paget's disease of bone (3). Therefore, in humans $[Ca^{2+}]_o$-mediated changes in PTH secretion and, in turn, in 1,25-$(OH)_2D_3$ production represent the dominant determinants of the activity of the tissues involved in movements of calcium (and phosphate) ions into or out of the extracellular fluid.

Discussions of calcium homeostasis have generally focused on the direct actions of $[Ca^{2+}]_o$ on parathyroid and C cells as being the major sites where $[Ca^{2+}]_o$ sensing regulates

the system. As outlined in subsequent sections, however, the capacity of cells to monitor and respond to changes in $[Ca^{2+}]_o$ is a more widespread property than generally recognized (1), and in several cases this $[Ca^{2+}]_o$ sensing is mediated by the recently cloned $[Ca^{2+}]_o$-sensing receptor. This provides an even more sophisticated mechanism for regulating mineral ion metabolism, where cells use the informational content of the local extracellular ionic environment to modify their functions in homeostatically appropriate ways. The property of $[Ca^{2+}]_o$ sensing is also used as a means of integrating homeostatic systems (*e.g.* those regulating calcium, magnesium, and water metabolism) in ways that balance the needs of free-living terrestrial organisms for divalent cations and water (32).

III. Calcium Sensing by Various Cell Types

A. Parathyroid cells

The parathyroid cell is remarkably sensitive to alterations in $[Ca^{2+}]_o$, readily detecting changes on the order of a few percent and responding with alterations in hormonal secretion within seconds (1, 6–8). A steep inverse sigmoidal relationship between PTH secretion and $[Ca^{2+}]_o$ has been demonstrated both *in vivo* (33, 34) and *in vitro* (1, 35). A computer model can be employed to fit this relationship (Fig. 2) (36). These curves can be defined in terms of four parameters (A through D), as follows: A represents the maximal rate of PTH release and is equivalent to the secretory reserve of the parathyroid on being exposed to a maximal, acute hypocalcemic stimulus. B represents the slope of the curve at its midpoint. This curve is steepest at this point, so that large changes in PTH secretion take place in response to minor alterations in $[Ca^{2+}]_o$ around the midpoint. C refers to the set point, which is defined as the calcium concentration producing half of the maximal inhibition of PTH secretion; it has values of about 1.0 mM $[Ca^{2+}]_o$ *in vitro* (36) and 1.1–1.3 mM *in vivo* (33) in humans. Others have defined the set point as the level of $[Ca^{2+}]_o$ at which the rate of PTH secretion is one half of its maximal value (37). With the latter definition, the relative position of C on the curve will vary as a function of the suppressibility of the gland (*i.e.* if maximal suppressibility is only slightly more than 50%, the set point will be very close to the bottom of the curve, where PTH secretion is maximally suppressed). The set point of the parathyroid gland plays a key role in "setting" the level of $[Ca^{2+}]_o$, although the set point for $[Ca^{2+}]_o$ (38) (*i.e.* the level at which the serum ionized calcium concentration is set) is generally slightly higher than that for the parathyroid gland, such that the circulating level of intact PTH is usually about 20–25% of the maximal level achieved during a maximal hypocalcemic stimulus (33). Not surprisingly, changes in the set point of the parathyroid (*e.g.* a reset "calciostat") produce major changes in PTH secretion at any given level of $[Ca^{2+}]_o$ (36) and, in turn, the steady state level of the serum calcium concentration. Elevations in set point are typically seen in hyperparathyroid states (36) and in FHH (39, 40), as well as in parathyroid cells maintained in culture (28, 41), implying varying degrees of calcium "resistance" of the parathyroid $[Ca^{2+}]_o$-sensing mechanism in these conditions. The fourth parameter, D, represents the

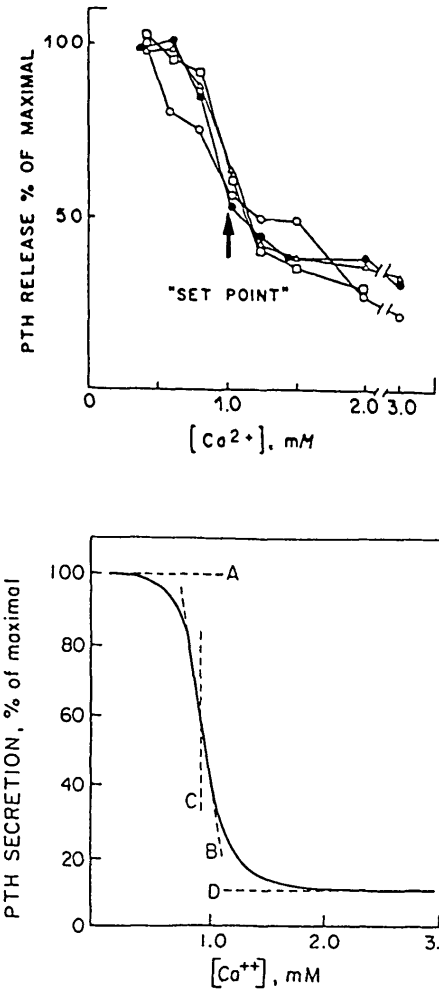

FIG. 2. Inverse sigmoidal relationship between $[Ca^{2+}]_o$ and PTH release and four-parameter model describing such curves. The data in the *upper panel* represent results obtained using dispersed normal human parathyroid cells and are expressed as the percent of the maximal rate of PTH release observed at 0.3 mM $[Ca^{2+}]_o$. The set point represents the level of $[Ca^{2+}]_o$ at which PTH release is half-maximally suppressed. The *lower panel* illustrates the four parameters that may be used to describe such curves. Details are given in the text. [Reproduced with permission from Brown EM 1982 PTH secretion *in vivo* and *in vitro*: regulation by calcium and other secretagogues. *Miner Electrolyte Metab* 8:130–150.]

minimum rate of PTH secretion that persists even at very high levels of $[Ca^{2+}]_o$, due to the existence of a basal nonsuppressible component of PTH release (42). The computer model used to fit the sigmoidal relationship between PTH and $[Ca^{2+}]_o$ was based on a similar model that had been used to describe the binding of hormones to their cell surface receptors, although at the time it had not been appreciated that $[Ca^{2+}]_o$ regulates parathyroid function through an entirely analogous, receptor-mediated mechanism.

A large body of indirect evidence supported the existence of a "receptor-like mechanism" that confers upon parathyroid cells the capacity to sense $[Ca^{2+}]_o$. Raising $[Ca^{2+}]_o$, for example, activates phospholipase C (PLC) in dispersed bovine parathyroid cells, leading to accumulation of inositol 1,4,5-trisphosphate (IP_3) due to activation of PLC (43) and consequent release of Ca^{2+} from its intracellular stores (6).

This results in an initial, transient "spike" in $[Ca^{2+}]_i$, followed by a sustained increase due to influx of Ca^{2+} through what are most likely voltage-insensitive channels (6, 44), although some studies have suggested that parathyroid cells (45), like C cells, have voltage-sensitive calcium channels (46). In addition, high levels of $[Ca^{2+}]_o$ produce a pertussis toxin-sensitive inhibition of agonist-stimulated cAMP accumulation in bovine parathyroid cells (47). In contrast, pertussis toxin has little, if any, effect on high $[Ca^{2+}]_o$-evoked changes in $[Ca^{2+}]_i$ or inositol phosphates (48). These studies also showed the capacity of this $[Ca^{2+}]_o$-sensing apparatus to recognize not only extracellular calcium ions *per se* but also a variety of other inorganic divalent cations (*e.g.* Mg^{2+}, Ba^{2+}), trivalent cations (*e.g.* La^{3+} and Gd^{3+}), and even polyvalent cations such as polylysine, protamine, and neomycin (49–51). The lectin concanavalin A, which binds to sugar moieties on the cell surface, reduces the effects of elevated $[Ca^{2+}]_o$ on $[Ca^{2+}]_i$ and PTH release, suggesting that the putative receptor was a glycoprotein (52). All these observations suggested that the apparatus for $[Ca^{2+}]_o$ sensing was probably a G protein-coupled cell surface receptor, linked to inhibition of adenylate cyclase via G_i and to activation of PLC through a pertussis toxin-insensitive G protein, presumably a member of the G_q family.

B. Kidney cells

$[Ca^{2+}]_o$ exerts direct actions on a variety of cell types in the kidney. Elevated levels of $[Ca^{2+}]_o$ directly inhibit the 1-hydroxylation of 25-hydroxyvitamin D, independent of the associated inhibition of PTH secretion (53), since this effect can be observed in experimental animals in which PTH is "clamped" by infusion of the hormone either into intact or parathyroidectomized animals. In the medullary thick ascending limb (MTAL), but not in the proximal tubule, elevated levels of $[Ca^{2+}]_o$ produce a pertussis toxin-sensitive inhibition of cAMP accumulation (54, 55), analogous to that seen in parathyroid cells (47). $[Ca^{2+}]_o$ also exerts a direct inhibitory action on NaCl reabsorption in the thick ascending limb (TAL) (56), and elevated peritubular, but not luminal, concentrations of either $[Ca^{2+}]_o$ or $[Mg^{2+}]_o$ also inhibit the reabsorption of both calcium and magnesium ions in the TAL (57). In the distal tubule, $[Ca^{2+}]_o$ acts synergistically with $1,25\text{-}(OH)_2D_3$ to increase the level of the mRNA for calbindin D_{28K} (58).

It has long been recognized that hypercalcemia produces nephrogenic diabetes insipidus (NDI) in some patients by reducing the action of vasopressin on the MTAL and/or collecting duct of the kidney (59), possibly contributing to the nephrogenic DI that can be observed in this setting. Other effects of $[Ca^{2+}]_o$ on renal function include inhibition of renin secretion from the juxtaglomerular cells (60), renal vasoconstriction (61), and reduction in glomerular filtration rate through as yet unexplained mechanisms (62). Thus, at least in the case of $[Ca^{2+}]_o$-evoked changes in vasopressin-stimulated cAMP accumulation in the TAL, some of the effects of $[Ca^{2+}]_o$ on renal function could potentially be exerted by a $[Ca^{2+}]_o$-sensing mechanism similar to that thought to be present in parathyroid cells.

C. C Cells

As discussed previously, elevations in $[Ca^{2+}]_o$ acutely elicit secretion of CT from the thyroidal C cells, which, in turn, inhibits bone resorption (although this action is of minor physiological relevance in normal adult humans) and promotes renal calcium excretion (31). C cells are highly sensitive to $[Ca^{2+}]_o$, suggesting that they too have a specific mechanism for sensing changes in extracellular calcium ions; however, in contrast to parathyroid cells, they show stimulation rather than inhibition of secretion in response to increases in $[Ca^{2+}]_o$ (63). Studies on $[Ca^{2+}]_o$ sensing in C cells suggested fundamental differences between the mechanisms used by parathyroid and C cells to detect changes in $[Ca^{2+}]_o$. Similar to parathyroid cells, C cells respond to small increases in $[Ca^{2+}]_o$ with elevations in $[Ca^{2+}]_i$, but when studied at the level of individual cells, they show slow oscillations in $[Ca^{2+}]_i$ that can be abolished by blockers of voltage-sensitive Ca^{2+} channels (46, 64). Thus release of Ca^{2+} from internal stores appears to play little, if any, role in the high $[Ca^{2+}]_o$-evoked changes in $[Ca^{2+}]_i$ dynamics in C cells, and the type(s) of Ca^{2+} channels mediating influx of extracellular calcium ions also appears to differ from those activated by elevated levels of $[Ca^{2+}]_o$ in parathyroid cells. On the basis of these results, some investigators suggested that a voltage-sensitive Ca^{2+} channel might represent the principal $[Ca^{2+}]_o$-sensing mechanism in C cells (64). The resultant high $[Ca^{2+}]_o$-activated increases in $[Ca^{2+}]_i$ would then stimulate CT secretion through the direct relationship between $[Ca^{2+}]_i$ and exocytosis of CT found in this cell type, as in most secretory cells.

D. $[Ca^{2+}]_o$-sensing in other cell types

$[Ca^{2+}]_o$ acts directly on additional cell types, some that are involved in mineral ion metabolism but others that are not, implying that they too have $[Ca^{2+}]_o$-sensing mechanisms potentially differing from those described to this point. For example, elevations in $[Ca^{2+}]_o$ inhibit osteoclastic bone resorption (65, 66) and produce changes in $[Ca^{2+}]_i$ in osteoclasts somewhat reminiscent of those elicited in parathyroid cells by high levels of $[Ca^{2+}]_o$. The pharmacological profile of the polycations that exert such effects differs distinctly from that for the $[Ca^{2+}]_o$-sensing mechanism in parathyroid, kidney, and C cells. In addition, $[Ca^{2+}]_o$ and other polycations stimulate the proliferation of some osteoblast-like cells and elicit changes in intracellular mediators (67) that are clearly different from those evoked by $[Ca^{2+}]_o$ in parathyroid, kidney, or C cells.

The placenta plays an important role in transporting calcium and other minerals from the mother to the developing fetus, and PTH-related protein (PTHrP) may play an important role in regulating this process (68). Not surprisingly, certain placental cells, the cytotrophoblasts, show regulation of PTHrP secretion by $[Ca^{2+}]_o$ (69). Raising $[Ca^{2+}]_o$ inhibits the proliferation of keratinocytes and promotes their differentiation (70). $[Ca^{2+}]_o$ also modulates the growth and/or differentiation of cultured mammary cells and various other epithelial cells (for review, see Ref. 1). Therefore, $[Ca^{2+}]_o$ sensing is an attribute that is not limited to cells involved in

mineral ion metabolism and might potentially be present in a wider range of cell types and regulate a larger variety of cellular functions than currently recognized.

IV. Molecular Cloning of a $[Ca^{2+}]_o$-Sensing Receptor

A. Bovine parathyroid $[Ca^{2+}]_o$-sensing receptor

Racke *et al.* (71) and Shoback and co-workers (72) independently showed that *Xenopus laevis* oocytes became responsive to $[Ca^{2+}]_o$-sensing receptor agonists, such as Ca^{2+} or Gd^{3+}, after being injected with parathyroid mRNA. Subsequently, Brown *et al.* (9) used a similar assay as a means for screening a bovine parathyroid cDNA library in an attempt to isolate full-length, functional clones of the $[Ca^{2+}]_o$-sensing receptor from this tissue source. The rationale for choosing expression cloning in *X. laevis* oocytes was that after translation of the functional receptor protein by the oocytes from mRNA synthesized *in vitro* from CaR-encoding cDNA clones of a parathyroid cell library, the receptor protein will couple to the endogenous G protein-activated PLC of the oocyte. The resultant CaR agonist-dependent increases in IP_3 and release of Ca^{2+} from intracellular stores will stimulate Ca^{2+}-activated chloride (Cl^-) currents, producing large currents that are readily measurable by standard electrophysiological means. The membrane impermeant gadolinium ion was chosen over Ca^{2+} as the CaR agonist used to screen for clones encoding functional receptor, since the latter could conceivably confound interpretation of the results by entering the oocytes through expressed channel proteins and/or transporters (9).

Use of this approach to screen a directional cDNA library prepared from size-fractionated bovine parathyroid poly(A^+) RNA exhibiting maximal Gd^{3+}-activated Cl^- currents in oocytes (of 4–6 kb in size) resulted in isolation of a single 5.3-kb clone containing the entire coding sequence of the CaR [bovine parathyroid Ca^{2+}-sensing receptor (BoPCaR)(9)]. The deduced amino acid sequence of BoPCaR predicts three major domains (Fig. 3): 1) a large hydrophilic extracellular domain at the amino terminus, consisting of 613 amino acids; 2) a hydrophobic transmembrane domain consisting of 250 amino acids with seven membrane-spanning segments characteristic of the superfamily of G protein-coupled receptors; and 3) a cytosolic carboxy-terminal tail predicted to have 222 amino acids. BoPCaR has nine predicted N-linked glycosylation sites within the extracellular amino-terminal domain, consistent with the native protein being expressed as a glycoprotein. Within the intracellular loops and carboxy-terminal tail of the protein, BoPCaR contains four predicted protein kinase C (PKC) phosphorylation sites, which may contribute to the PKC-mediated uncoupling of the receptor from activation of PLC in bovine parathyroid cells (73).

Among the G protein-coupled receptors, the $[Ca^{2+}]_o$-sensing receptor only shares amino acid sequence homology with the metabotropic glutamate receptors (mGluRs) (9), which are G protein-coupled receptors present in the central nervous system that respond to glutamate, the

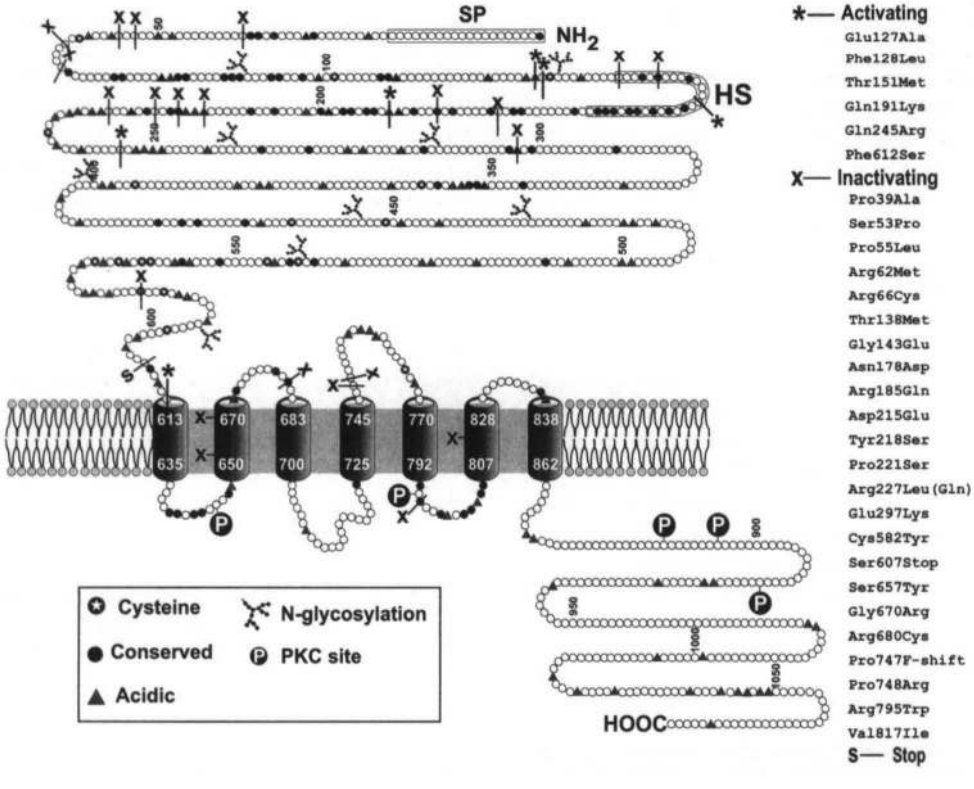

FIG. 3. Schematic diagram of the predicted structure of the $[Ca^{2+}]_o$-sensing receptor, illustrating inactivating and activating mutations. Symbols are explained in key. Additional abbreviations are: SP, predicted signal peptide; HS, hydrophobic segment. The mutations shown are from Refs. 13, 14, 98, 119, 121, 122, 151, and 152. [Modified with permission from The New England Journal of Medicine E. M. Brown *et al.*: *N Engl J Med* 333:234–240, 1995 (32). © 1995 Massachusetts Medical Society. All rights reserved].

major excitatory neurotransmitter in the brain (74). BoPCaR has only modest identity in its amino acid sequence with the mGluRs (18–24%), but it shares striking topological similarity. Both classes of receptors possess a large amino-terminal, extracellular domain as well as a total of 20 strictly conserved cysteine residues (17 within the extracellular domain and three in transmembrane segments or extracellular loops). Studies using chimeric receptors constructed from mGluRs with differing ligand specificities (75) or chimeras in which the extracellular domains are interchanged between an mGluR and the CaR (76) have established that the principal determinants of ligand binding to both classes of receptors reside within the large amino-terminal domain. The conserved cysteines may contribute to organizing this domain of the receptor into a binding pocket appropriate for interacting with these small charged ligands (*e.g.* glutamate or Ca^{2+} and other polyvalent cations for the mGluRs and CaR, respectively). O'Hara and co-workers (77) and Conklin and Bourne (78) have suggested that the mGluRs and BoPCaR bear structural homology to the bacterial periplasmic binding proteins. The latter are involved in sensing extracellular ligands as part of the processes of chemotaxis or cellular uptake of extracellular ligands in bacteria and have a bilobate structure with the binding pocket for the ligand located within the cleft between the two lobes of the protein (79). Upon binding of the ligand, the protein undergoes a conformational change in which the two lobes approximate one another, largely obliterating the remaining space between them (hence the basis for the "venus fly trap" model of ligand binding by these proteins). O'Hara *et al.* (77) have created a model of the extracellular domain of the mGluRs that successfully predicts certain features of the binding of mGluR agonists to the receptor based on an overall three-dimensional structure postulated to be similar to that of the periplasmic binding proteins. A detailed understanding of how their respective ligands interact with the mGluRs and BoPCaR, however, will require determination of the structure of their extracellular domains by x-ray crystallography. It is of interest that BoPCaR possesses several highly acidic regions within the extracellular domain that are similar to those thought to be involved in the binding of calcium ions to other low affinity calcium-binding proteins, such as calsequestrin and calreticulin, but are not present in the mGluRs (9). There are no predicted high-affinity calcium-binding motifs in this domain of the protein, such as EF hands (the high-affinity Ca^{2+}-binding domain of calmodulin and related intracellular Ca^{2+}-binding proteins), which would be inappropriate for $[Ca^{2+}]_o$ sensing as they would be persistently occupied by the ambient concentrations within the extracellular fluids.

B. Cloning of additional species homologs of the CaR

Recently, two cDNA clones (4.0 and 5.2 kb) have been isolated from a cDNA library prepared from a human parathyroid adenoma (80). Expression in *X. laevis* oocytes confirmed that both clones encode functional human parathyroid CaRs [human parathyroid calcium receptor (HuPCaR)]. An essentially identical $[Ca^{2+}]_o$-sensing receptor was subsequently isolated from a human kidney cDNA library (81). Highly similar, if not identical, rat $[Ca^{2+}]_o$-sensing receptors have been cloned from kidney outer medulla [rat kidney calcium-sensing receptor (RaKCaR)] (10), the striatum of the brain (12), and a C cell line derived from medullary thyroid carcinoma (rMTC 44–2) cells (11). All are almost certainly derived from a single gene. The CaRs from different species likewise all show a high degree of homology with BoPCaR (>90% amino acid identity), strongly suggesting the they are homologs of the same ancestral gene. Most of the differences between the receptors cloned from different species reside in their carboxy-terminal tails. The predicted glycosylation and PKC phosphorylation sites are more or less conserved between the bovine, rat, and human receptors, although the human and rat receptors have one and two additional PKC phosphorylation sites, respectively. In addition, the human receptor has one predicted protein kinase A (PKA) phosphorylation site and the rat receptor has two such sites, while BoPCaR has no predicted PKA sites. The functional significance of these sites and the interspecies differences between them are at present unknown. Agents stimulating PKC activity are known to uncouple the CaR from activation of PLC, as will be described in detail later (see *Section IV.E*). Activators of PKA have no obvious effect on $[Ca^{2+}]_o$ sensing in bovine parathyroid cells, consistent with the lack of predicted PKA sites in the bovine receptor. Similar studies have not yet been reported for the rat and human receptors.

There are several additional notable differences between the predicted amino acid and nucleotide sequences in the various species homologs of the $[Ca^{2+}]_o$-sensing receptor. Within the coding sequence, 10 amino acids are inserted between codons 536–545 in one fully characterized clone (HuPCaR-5.2) of two full-length human CaRs isolated from a cDNA library prepared from a parathyroid adenoma (80). None of the other forms of the receptor isolated to date have shown this insertion. Therefore, this insert presumably represents a rare splice variant in which a portion of an intron is incorporated into the coding sequence, without any apparent effect on the function of the expressed receptor (80). There is, in addition, a benign polymorphism (or possibly a cloning artifact) in another of the CaR clones isolated from this same cDNA library (HuPCaR-4.0), with replacement of the normal Arg at codon 990 (as assessed by its presence in BoPCaR) with Gly (80). Again, this change in amino acid sequence has no obvious impact on the function of the expressed receptor. There are additional differences in the noncoding sequence of the receptor. In their 5′-untranslated regions, the two HuPCaR clones diverge 242 bp upstream of the translational start site, with the two distinct sequences 5′ to this site presumably representing splice variants within noncoding sequences that are of uncertain physiological significance.

C. Receptor-effector coupling of the CaR

In bovine parathyroid cells, high levels of $[Ca^{2+}]_o$ activate PLC in a pertussis toxin-insensitive manner (48), unlike its actions on cAMP accumulation (47), suggesting that the CaR is coupled to PLC through a member of the G_q family (82).

In X. *laevis* oocytes expressing the CaR, high $[Ca^{2+}]_o$, $[Gd^{3+}]_o$, or neomycin levels also increased the accumulation of inositol 1,4,5-trisphosphate and $[Ca^{2+}]_i$ by severalfold, the latter arising primarily from intracellular Ca^{2+} stores (9). Unlike the case in bovine parathyroid cells, however, high concentrations of pertussis toxin (10 μg/ml) markedly attenuated the high $[Ca^{2+}]_o$-elicited increases in IP_3 in oocytes. Therefore, unlike parathyroid cells, the CaR activated PLC in the oocyte via a pertussis toxin-sensitive G protein. The specificity for G protein coupling of other receptors can also vary when they are expressed in oocytes compared with the cell of origin (83). Of interest, the high $[Ca^{2+}]_o$-elicited increase in inositol phosphate accumulation in AtT-20 cells, a mouse pituitary cell line expressing the CaR, is also pertussis toxin-sensitive (84), indicating that the specificity of the G protein through which the CaR couples to PLC can vary in mammalian cells as well.

The expressed CaR confers high $[Ca^{2+}]_o$-mediated inhibition of cAMP accumulation on human embryonic kidney cells (HEK 293) stably expressing the receptor (85). Recent studies in tubules from the MTAL, however, have raised the possibility that high levels of $[Ca^{2+}]_o$ inhibit agonist-stimulated cAMP accumulation through an indirect mechanism involving arachidonic acid (86). Direct addition of arachidonic acid to tubule suspensions leads to a pertussis toxin-sensitive inhibition of cAMP accumulation. It remains to be determined whether the high $[Ca^{2+}]_o$-evoked inhibition of cAMP accumulation in cells stably expressing the cloned CaR involves a similar mechanism or whether the CaR can directly couple to inhibition of adenylate cyclase via G_i.

D. Tissue distribution of the CaR

By Northern blot analysis, transcripts for the $[Ca^{2+}]_o$-sensing receptor are present in a variety of tissues. The sizes of these transcripts vary both within and between species. In the bovine species, the major transcript is 5.6 kb in length (9), with a minor transcript of 9.5 kb, while in rat the major transcript is 7.5–8.5 kb with a minor transcript of 4.1 kb (10–12). The significance of the presence of several CaR transcripts is at present unknown. Some of the tissues expressing transcripts for the CaR play an important role in maintaining calcium homeostasis and are known to sense $[Ca^{2+}]_o$, including the parathyroid and thyroid glands (where the mRNA for the receptor is present exclusively in the C cells) as well as several regions of the kidney. The use of *in situ* hybridization and immunohistochemistry, utilizing antibodies raised against synthetic peptides within the extracellular domain of the CaR, has permitted more detailed localization of the receptor within these tissues. In the parathyroid gland, CaR mRNA is present within the chief cells. The receptor protein is present on the cell surface of the chief cell but can also be visualized in some cases over the cytoplasm (87). It is not yet clear whether the latter represents receptor protein within the biosynthetic pathway or that resident within intracellular organelles, such as the endoplasmic reticulum and secretory vesicles. The total calcium concentration within such organelles is generally maintained within the millimolar range (2) and $[Ca^{2+}]_o$ sensing via the CaR could potentially be involved in regulating the state of filling of the organelles or other aspects of their calcium homeostasis. C cells likewise contain abundant CaR protein on the plasma membrane (11).

The location of CaR mRNA and protein within the kidney has recently been clarified not only by the use of *in situ* hybridization and immunohistochemistry but also by performing PCR on reverse-transcribed RNA isolated from individually microdissected and identified segments of the nephron (88). In this way it has been possible to identify which of the segments of the nephron contain transcripts for the CaR. mRNA for the receptor is present within nearly all of them, including the glomerulus, proximal convoluted and straight tubules, MTAL and cortical thick ascending limb, distal convoluted tubule, and cortical, outer medullary, and inner medullary collecting ducts. In the TAL, the CaR has a similar, if not identical, distribution to that of the PTH/PTHrP receptor and is present predominantly on the basolateral surface of the cells. Since it is known that high $[Ca^{2+}]_o$ inhibits both PTH-induced cAMP production and Ca^{2+} reabsorption by this segment of the nephron, the presence of both the CaR and PTH receptors in TAL and also in distal convoluted tubule is strong supporting evidence for a crucial role of the $[Ca^{2+}]_o$-sensing receptor in calciotropic hormone-regulated mineral ion homeostasis (32). Moreover, it implies that the CaR carries out this function not only by regulating PTH secretion but also by modulating its action(s) on the kidney.

In brain, immunolocalization of the CaR protein revealed the presence of the receptor throughout the central nervous system with particular abundance in the cerebellum and hippocampus, olfactory bulbs, ependymal zones of the cerebral ventricles, and cerebral arteries (12). The high level of expression of the CaR in several types of cerebellar cells (*e.g.* Purkinje and granule cells) may suggest that it has a role in motor coordination, while its presence in the CA1 to CA3 layers of the hippocampus raises the possibility that the CaR may also have some role(s) in cognitive functions, such as learning and memory, in this region of the central nervous system (12, 89). It is significant that the structurally and functionally homologous mGluRs are also present abundantly in the hippocampus, where they are also thought to play an important role in the generation of long-term potentiation and other processes related to cognition (74). The site of CaR immunostaining included nerve fibers and terminals in addition to a lesser degree of staining of neuronal cell bodies (*e.g.* in the hippocampal pyramidal cells). Cerebral arteries also displayed prominent CaR staining within a network of branching nerve fibers, a pattern resembling the distribution of neuronal nitric oxide synthase (12). The authors suggested a role for the CaR in detecting local changes in $[Ca^{2+}]_o$ concentration in the vicinity of neurons, including within synaptic clefts, thereby potentially regulating release of neurotransmitters or other aspects of neuronal function.

Alterations in serum calcium concentration are reflected in changes in the level of calcium in the cerebrospinal fluid. Could the CaR in the ventricular ependymal cells play a role in sensing changes in $[Ca^{2+}]_o$ or alterations in the gradient for $[Ca^{2+}]_o$ between its systemic level and that within the cerebrospinal fluid and/or brain, thereby contributing to regulation of the calcium concentration in the cerebrospinal fluid?

Finally, it is possible that the CaR in brain responds to ligands other than calcium (*e.g.* polycations, such as spermine) and may play a more general role as an ion sensor than in the parathyroid.

E. Regulation of the CaR

To date there have been relatively few studies on the regulation of the function and/or expression of the CaR. Activators of PKC blunt substantially the high $[Ca^{2+}]_o$-elicited increases in inositol phosphate accumulation and $[Ca^{2+}]_i$ in bovine parathyroid cells (1, 73, 90). The presence of predicted sites for PKC-mediated phosphorylation on intracellular domains of the CaR suggest that these effects of altering PKC activity in parathyroid cells may be the result of PKC-mediated phosphorylation of one or more of these sites (9–12, 81). PKC could also, of course, exert additional effects on the PLC/IP_3 pathway by phosphorylating G protein(s), PLC, or other elements within the signal transduction pathway. Direct documentation of PKC-mediated phosphorylation of the CaR as well as determination of the functional consequences of removal of its PKC sites will enable more detailed dissection of the mechanisms involved and the importance of these sites in regulating the receptor's function. Activators of cAMP-dependent PKA, in contrast, have no apparent effect on high $[Ca^{2+}]_o$-elicited elevations in the levels of inositol phosphates and $[Ca^{2+}]_i$ in bovine parathyroid cells (91).

Studies on the regulation of the level of expression of the CaR are likewise at an early stage. Treatment of rats with 1,25-$(OH)_2$D *in vivo* produced a modest increase in the level of CaR mRNA in the parathyroid in one study (92) but not in another (93), while chronic decreases or increases in serum calcium concentration had no effect on CaR mRNA levels in the parathyroid in either study. In contrast, elevated levels of $[Ca^{2+}]_o$ produced ~2-fold increases in CaR mRNA levels in AtT-20 cells (84). The physiological relevance of the latter effect is unclear, but it raises the possibility that the regulation of the receptor in tissues involved in calcium homeostasis, such as parathyroid cells, may differ from that in tissues such as the brain that are not directly involved in mineral ion metabolism. Of interest, calcium infusion does elevate ACTH levels *in vivo* in normal human volunteers (94, 95), suggesting that the presence of the CaR in AtT-20 cells is probably not simply due to ectopic expression of the receptor in a tumor cell line.

Bovine parathyroid cells placed into culture undergo a rapid and marked decrease in CaR transcript and protein expression, with 75–80% reductions in both parameters within 18 h and 36–48 h, respectively (87). This is accompanied by a progressive increase in the set point for $[Ca^{2+}]_o$-regulated PTH secretion followed by a loss of the high $[Ca^{2+}]_o$-mediated suppression of PTH release. Therefore, the level of expression of the CaR may play a key role in determining both parameters C and D in the four-parameter model of $[Ca^{2+}]_o$-regulated PTH release (see *Section III.A*). In contrast, there is a dramatic up-regulation of CaR mRNA and protein expression in rat kidney during the first postnatal week of life (96). This correlates temporally with development of the TAL and reflects, therefore, the tissue-specific expression of the receptor in the kidney. Thus large changes in the expression of the CaR can take place both *in vivo* and *in vitro*; however, the mechanisms underlying these changes as well as the determinants of the tissue-specific expression of the CaR remain to be clarified.

F. The CaR gene

The gene encoding the $[Ca^{2+}]_o$-sensing receptor resides on chromosome 3 in humans (97). It is known to contain six exons comprising the coding region of the gene (13) as well as at least one additional upstream, noncoding exon (98). Five of these exons encode the amino-terminal, extracellular domain of the CaR, while the sixth codes for the entire transmembrane domain and the carboxy-terminal tail. This exonic structure provides indirect support for the concept (78) that the CaR represents the fusion of a seven membrane-spanning, signal-transducing motif with additional exons involved in determining the $[Ca^{2+}]_o$-sensing properties of the receptor. Virtually nothing is presently known about the regions upstream of the translational start site of the receptor gene that are involved in regulating the level of expression of the CaR and the tissue specificity of its expression.

V. Are There Additional Forms of $[Ca^{2+}]_o$-Sensors/Receptors?

It is probable that there are $[Ca^{2+}]_o$-sensors or receptors in addition to the CaR. cDNAs encoding a putative $[Ca^{2+}]_o$-sensor, which is related to the low density lipoprotein receptor superfamily and expressed on parathyroid and placental cells as well as on the cells of the kidney proximal tubules, have recently been isolated but have not yet been fully characterized in terms of their $[Ca^{2+}]_o$-sensing properties (99, 100). The $[Ca^{2+}]_o$-sensing mechanism mediating the effects of calcium on osteoclasts also differs from the CaR in its properties, generally only responding to levels of $[Ca^{2+}]_o$ higher than those needed to modulate the activity of the CaR in parathyroid and kidney cells and also being responsive to divalent cations (*e.g.* Cd^{2+}) that have no effect on the cloned and expressed CaR (65, 66). In addition, much more work will be required to define fully the nature of the $[Ca^{2+}]_o$-receptors/sensors that mediate the actions of $[Ca^{2+}]_o$ on the proliferation and differentiation of epithelial cells and other cell types (for review, see Ref. 1). It is entirely possible that there are diseases of $[Ca^{2+}]_o$ sensing that result from dysfunction of such additional $[Ca^{2+}]_o$-receptors/sensors.

VI. Syndromes of Extracellular Calcium Resistance — Experiments of Nature Elucidating the Functions of the CaR *in Vivo*

A. Familial hypocalciuric hypercalcemia (FHH)

FHH (first named familial benign hypercalcemia or FBH) is a rare, heritable disorder of mineral metabolism, first described in 1972 (101). FHH is characterized by lifelong, moderate (<12 mg/dl) but generally asymptomatic hypercalcemia (102, 103). The degree of hypercalcemia is similar to that seen in primary hyperparathyroidism, but in FHH it is characteristically accompanied by inappropriately low urinary

calcium excretion (a calcium to creatinine clearance ratio of <0.01) (102, 103), along with inappropriately "normal" circulating levels of PTH in the face of hypercalcemia, which are often midnormal and may, in some cases, be in the lower part of the normal range. It is sometimes difficult to differentiate patients with FHH from those with mild primary hyperparathyroidism, particularly those individuals with hyperparathyroidism who have levels of PTH in the upper part of the normal range (104). Hypophosphatemia is variable, and mild elevations in serum magnesium level may also be present (102, 105, 106). Serum levels of 25(OH)D and 1,25-$(OH)_2$D are normal (107–109). The disorder is generally considered to be benign, as patients do not develop complications of hypercalcemia such as nephrolithiasis and NDI. Older patients with FHH, however, show a seemingly higher prevalence of chondrocalcinosis than would be expected in the normal population (102). Moreover, Marx and co-workers (102) described three cases of pancreatitis in their FHH kindreds. Two of these had known predisposing factors (102), however, and Law and Heath (103) have described an apparently increased incidence of gallstones in FHH. Thus it is probably unlikely that pancreatitis is a direct consequence of FHH (103, 110). Other nonspecific symptoms reported in earlier series (102) could not be attributed to FHH in later studies (103, 111), perhaps because ascertainment bias attributed symptoms to the disease process in probands that were not confirmed in more detailed analyses of entire kindreds. An unusual variant of the disease has been reported in a family from Oklahoma, which showed an age-related, progressive elevation in PTH levels along with hypophosphatemia and osteomalacia (112).

Individuals with FHH show abnormal parathyroid and renal responsiveness to extracellular calcium (113). It has been shown using intravenous calcium infusion that patients with FHH have an elevated set point for calcium-regulated PTH release (39, 40). That is, for any given level of serum calcium, FHH patients have a higher concentration of PTH than normal subjects. To suppress the circulating PTH level to 50% of its maximal value, these individuals require a serum calcium level slightly higher than that necessary to achieve a comparable degree of suppression in normals. These findings suggest that the parathyroid glands of patients with FHH show a defect in $[Ca^{2+}]_o$ sensing, exhibiting mild to moderate resistance to the inhibitory effects of extracellular calcium on PTH secretion.

Calcium handling by the kidney is also abnormal in individuals with FHH, with these patients failing to show a hypercalciuric response to hypercalcemia (102, 105). Indeed, they are often overtly hypocalciuric (102, 106). This phenomenon is PTH-independent, as these individuals remain hypocalciuric even after total parathyroidectomy (107, 114). Interestingly, some persons with FHH reported by Heath and co-workers (108) displayed normal gastrointestinal absorption of calcium and normal levels of 1,25-$(OH)_2$D despite being on a low calcium diet, suggesting a blunted homeostatic response to low calcium intake. By the late 1980s, convincing evidence had accumulated that FHH is a generalized disorder of calcium ion sensing with wide variability in its phenotypic presentation (106, 108).

1. Genetics of FHH. FHH is inherited as an autosomal dominant disorder, with greater than 90% penetrance (113). A severe variety of hyperparathyroidism that was found in some neonates from FHH families has been shown to represent most commonly the homozygous form of FHH and will be discussed in detail later. Over the past few years, several studies have confirmed that, in the majority of FHH families, the disease gene could be linked to a locus on the long arm of chromosome 3 (97). Genetic analysis of four families with FHH mapped the gene to band 3q21–24 (115), and subsequent studies have confirmed that the great majority of families with FHH exhibit this same genetic linkage. In one family, however, the disorder maps to chromosome 19 (band 19p13.3) (115). Furthermore, the disorder in the Oklahoma kindred does not show linkage to either chromosomes 3 or 19, confirming the genetic heterogeneity of FHH (116).

Since FHH seemed to be a disorder of calcium ion sensing, the need to look for abnormalities in the newly cloned CaR was obvious. Pollak *et al.* (13) mapped the human homolog of the bovine CaR gene to chromosome 3. Using ribonuclease A protection assays, they screened the human calcium-sensing receptor gene in affected individuals from three unrelated FHH families and found unique missense mutations in each family (*i.e.* point mutations in which a change in a single nucleotide base substitutes a new amino acid for the one originally coded for). None of the observed mutations were found in the genomic DNA of 50 normocalcemic individuals. All three mutations occurred in amino acid residues conserved between the human and bovine receptor genes. Two of the three families exhibited a mutation in the amino-terminal extracellular domain [R185Q (inadvertently described as R185E in the original paper) and E297K] of the CaR, which is thought to be involved in the binding of extracellular calcium. Based on homology with the mGluRs, the authors inferred that one of the extracellular mutations (R185Q) could potentially disrupt a salt bridge that contributes to the general structure of the binding pocket. The third family showed a mutation (R795W) in the third intracellular loop, which could be important for signal transduction, as seen in other G protein-linked receptors. This intracellular residue is also part of a consensus PKC phosphorylation site, and disruption of the latter could potentially also contribute to malfunction of the mutated receptor. Furthermore, *X. laevis* oocytes, injected with synthetic mRNA encoding a CaR engineered to contain the R795W missense mutation, exhibited a markedly blunted response to $[Ca^{2+}]_o$, $[Gd^{3+}]_o$, and neomycin, providing convincing evidence that this mutation impaired $[Ca^{2+}]_o$ sensing (13). Studies on other G protein-linked receptors have suggested the possibility that mutant receptors may interfere with the function of the wild type receptor on the cell surface or that mutant receptors could decrease the number of the receptors produced from the normal allele that are able to reach the cell surface (*i.e.* exert a dominant negative effect) (117). For example, a mutation in the human thromboxane A2 receptor appears to cause a familial bleeding disorder through a dominant negative mechanism (118). It is possible that this mechanism also contributes to abnormal $[Ca^{2+}]_o$ sensing, particularly in families with more severe hypercalcemia in heterozygotes.

The same group later studied eight families with FHH and reported missense mutations in the extracellular domain in five of them, each mutation producing a nonconservative amino acid change (119). However, three of the families studied did not show a detectable mutation within the coding sequence of the CaR protein, despite showing linkage to the FHH locus on chromosome 3. The methods that were employed to detect mutations are generally able to detect more than 95% of all point mutations, and the riboprobes used contained all intron and exon boundaries except those flanking exons 5 and 6. Therefore, failure to identify mutations that interrupt consensus splice sites, thereby disrupting processing of mRNA precursors, is highly unlikely. From these results, it appears that the defect in these three families could lie in noncoding sequences of the CaR gene (*i.e.* promoter or enhancer sequences). These defects may result in alterations in CaR gene transcription or expression that reduce the number of otherwise normal receptors reaching the cell surface and thereby produce functional "resistance" to $[Ca^{2+}]_o$. The authors' attempts to predict phenotype based on the different mutations observed were not successful, and one family member, who was homozygous for a mutation that in heterozygotes exhibited very mild hypercalcemia, suffered from NSHPT (119).

The list of CaR mutations reported in FHH is growing rapidly (13, 98, 119–122). So far, with few exceptions (98, 121), each family has been found to have its own unique mutation. Most of these mutations have been detected in the large extracellular domain (exons 2 and 3), and it is likely that they interfere with ligand binding. A recent report from Japan describes a mutation at codon 39 (P39A) in a hypercalcemic proband from a family with FHH (122). The proband was homozygous for this mutation, while the consanguineous parents, who had borderline elevated serum calcium concentrations, were heterozygous for the defect. Normocalcemic family members did not show any CaR mutation. Although the authors did not perform expression studies, this mutation appeared to result in a very mild defect in calcium sensing in heterozygotes and manifests overt hypercalcemia only in the homozygous state (122). Several clinical features of the proband are noteworthy in this regard. First, she had serum magnesium concentration ~50% above the upper limit of normal, which was similar to the degree of elevation of the serum calcium concentration (15–17 mg/dl). Moreover, the presence of hypermagnesemia supports the role of the CaR in contributing to "setting" $[Mg^{2+}]_o$. Finally, this report serves to highlight once again the genetic and phenotypic heterogeneity of the condition. The aforementioned reports also emphasize the importance of the extracellular domain in determining the responsiveness of the CaR to $[Ca^{2+}]_o$, a point that has been further established with the recognition of activating mutations in the same region causing hypocalcemia (14), as detailed later.

The remainder of the missense mutations described to date fall within exon 7 of the CaR gene, which encodes the transmembrane domains and cytoplasmic, carboxy-terminal tail of the receptor (13, 98, 119–122). A novel type of mutation has been described recently in two presumptively related families from Nova Scotia whose family members express either FHH in heterozygotes or NSHPT in homozygotes (120, 123). The authors found an unusually large exon 7 in all affected individuals. This is due to the insertion of a 383-bp Alu repetitive sequence at codon 877. This sequence is in opposite orientation to the CaR gene and contains an exceptionally long poly A tract. Stop signals are present in all three reading frames within the Alu sequence, leading to a predicted truncation of the CaR protein after a long stretch of repeated phenylalanines (encoded by the triplet AAA), which would likely result in production of a nonfunctional protein that might well fail to reach the cell surface (120). Interestingly, this insertion seems to have expanded in a subsequent generation of this family (123). In addition to the functionally significant mutations described to this point, three benign polymorphisms that are not associated with any abnormalities in mineral ion homeostasis have been described in the carboxy-terminal tail of the CaR (121).

Based on these studies pertaining to FHH, we conclude the following: 1) FHH is characterized by genotypic and phenotypic heterogeneity. 2) Approximately one half to two thirds of all individuals with FHH, independent of their genetic locus, show inactivating mutations within the coding region of the recently cloned CaR (*e.g.* because of the rarity of the disorder arising from the other loci). However, each family generally has its own unique mutation. The majority of mutations occur within the extracellular domain and likely reduce the affinity of the receptor for extracellular Ca^{2+}. Some occur in transmembrane or cytoplasmic domains and might conceivably disrupt signal transduction. 3) Of the remaining one third to one half of individuals with FHH, the defect maps to the CaR gene in most, although there are no detectable mutations in the coding region. These individuals could have defects in promoter or enhancer sequences of the CaR gene, which have not yet been characterized in detail. 4) In some families the defect maps to other, as yet undefined, genes either on chromosome 19p or elsewhere. Because of the generally benign clinical course of patients with FHH, parathyroidectomy should not be performed except in very unusual clinical circumstances.

2. The CaR in FHH. Implications for regulation of parathyroid and renal function by $[Ca^{2+}]_o$

As discussed in preceding sections, several lines of evidence suggest that $[Ca^{2+}]_o$ exerts direct actions on parathyroid function as well as renal mineral and water handling, potentially through cellular mechanisms mediated by the CaR (32). The recognition of an abnormal CaR in FHH provides useful experiments in nature that have elucidated the role of the CaR in regulating the normal function of parathyroid and kidney.

CaR and $[Ca^{2+}]_o$-regulated parathyroid function. As noted above, individuals with FHH have a modest increase in their set point for $[Ca^{2+}]_o$-regulated PTH release (39, 40), while *in vitro* studies on parathyroid glands from two individuals with NSHPT exhibited much more severe resistance to $[Ca^{2+}]_o$ (2-fold or greater increases in the value of the set point) (124, 125). In addition, similar results have recently been reported in mice heterozygous or homozygous for targeted deletion of the murine homolog of the CaR gene (126). Thus, the alterations in $[Ca^{2+}]_o$-regulated parathyroid function in FHH and NSHPT, as well as in mice homozygous or

heterozygous for "knockout" of the CaR, provide strong evidence that this receptor plays a key role in mediating the inhibitory effects of $[Ca^{2+}]_o$ on PTH secretion. Recent data also suggest that the CaR mediates the high $[Ca^{2+}]_o$-induced reduction in expression of the PTH gene (127). It remains to be determined whether the $[Ca^{2+}]_o$-sensing receptor is also responsible for additional effects of $[Ca^{2+}]_o$ on parathyroid function (for review, see Ref. 1), including inhibition of parathyroid cellular proliferation (the parathyroid hyperplasia in NSHPT provides indirect support for a role for the receptor in regulating this aspect of parathyroid function), alterations in the intracellular degradation of PTH as well as changes in cellular respiration, and the activity of the hexose monophosphate shunt.

The CaR and $[Ca^{2+}]_o$ regulation of the function of the TAL. As discussed above, FHH is characterized by excessive, inappropriate reabsorption of calcium ions by the kidney, which persists after parathyroidectomy (107, 114). Studies by Attie *et al.* (114) suggested that this abnormality in renal calcium handling takes place in the TAL, since a loop diuretic, ethacrynic acid, was able to increase urinary calcium excretion in individuals with FHH. The CaR is present in cells of the TAL on the basis of studies employing *in situ* hybridization, immunohistochemistry, and RT-PCR (88). Moreover, increases in the peritubular, but not luminal, levels of $[Ca^{2+}]_o$ to which the TAL is exposed inhibit reabsorption of calcium ions (57). Therefore, the alteration in tubular handling of calcium in FHH provides additional evidence that the CaR is responsible for high $[Ca^{2+}]_o$-mediated modulation of Ca^{2+} reabsorption by this segment of the nephron. In effect, the TAL, like the parathyroid cell, is "resistant" to $[Ca^{2+}]_o$ in persons with FHH, limiting their capacity to up-regulate urinary calcium excretion in the face of an increase in $[Ca^{2+}]_o$, which, in turn, contributes to maintenance of their hypercalcemia.

The CaR and the control of $[Mg^{2+}]_o$ homeostasis. Individuals with FHH show mild hypermagnesemia due, in large part, to excessive magnesium reabsorption in the distal nephron (102). It has been shown that elevated peritubular levels of not only $[Ca^{2+}]_o$ but also $[Mg^{2+}]_o$ inhibit reabsorption of both ions in the TAL (57). Moreover, both the cloned parathyroid (BoPCaR) (9) and renal (RaKCaR) (10) CaRs respond to Mg^{2+} when expressed in *X. laevis* oocytes, albeit at somewhat higher concentrations than for Ca^{2+}. It is likely that the concentration of Mg^{2+} in the MTAL is higher than in the initial glomerular filtrate, since relatively little Mg^{2+} is reabsorbed before this region of the nephron (57). The concentrations achieved may be sufficient to be sensed by the CaR, which could thereby play an important, although as yet unproven, role in the regulation of renal Mg^{2+} reabsorption by the TAL. In FHH, the presence of abnormal CaRs with reduced activity leads to excessive Mg^{2+} reabsorption and consequent hypermagnesemia (102, 105). However, unlike Ca^{2+} reabsorption, the presence of PTH may be necessary for this inappropriate Mg^{2+} reabsorption in FHH, as this abnormality does not persist after parathyroidectomy (114). Of interest, mice that are heterozygous or homozygous for deletion of the CaR gene also show statistically significant increases in $[Mg^{2+}]_o$ relative to wild type mice, providing additional evidence for a role of the CaR in Mg^{2+} homeostasis (126).

The CaR and urinary concentrating ability. One of the well described effects of hypercalcemia on the kidney is to decrease maximal urinary concentrating ability, leading to hyposthenuria and, in some cases, overt NDI (59). This could occur in two ways: 1) elevations in extracellular calcium are known to inhibit NaCl absorption in the TAL (56), where the CaR is located (88). NaCl reabsorption in the TAL is crucial for generation of the countercurrent gradient that is needed for subsequent hydrosmotic water flow in the collecting duct under the influence of vasopressin. 2) Hypercalcemia likewise exerts an inhibitory effect on the action of vasopressin on water transport in the collecting duct (128). Recent data indicate that the CaR is present on the apical surface of cells of the collecting ducts, and, therefore, this effect on vasopressin action might also be CaR-mediated (96).

Individuals with FHH show an alteration in their maximal urinary concentrating ability in response to hypercalcemia, which provides additional indirect support for a role for the CaR in regulating water metabolism. In contrast to most hypercalcemic patients, persons with FHH concentrate their urine normally despite being hypercalcemic (129). This could occur because of deranged $[Ca^{2+}]_o$ sensing in either MTAL or collecting duct resulting from reduced activity of mutant CaRs. Impaired $[Ca^{2+}]_o$ sensing in the MTAL could lead to a reduced or absent inhibitory effect of hypercalcemia on NaCl reabsorption and consequent generation of the medullary countercurrent gradient. Alternatively or in addition, the presence of CaRs with reduced activity in the collecting duct could block the inhibitory action of hypercalcemia on vasopressin action in this nephron segment, likewise mitigating the development of NDI. In normal humans, the excretion of a dilute urine in the setting of hypercalciuria may be a protective mechanism that reduces the risk of developing nephrolithiasis and/or nephrocalcinosis when concentrated urine is being elaborated in states of dehydration (Fig. 4).

B. Neonatal severe hyperparathyroidism (NSHPT)

Neonatal primary hyperparathyroidism can be defined as symptomatic hypercalcemia with hyperparathyroid bone disease occurring in children under the age of 6 months (113). A recent analysis of 49 cases of neonatal severe primary hyperparathyroidism showed that most cases present at birth or within the first week of life (113). Failure to thrive, anorexia, constipation, and respiratory distress are common presenting features. Chest deformity, craniotabes, dysmorphic facies, and anovaginal or rectovaginal fistulas have also been described (130–140). Respiratory complications due to thoracic deformity are a major cause of morbidity (113, 137).

Skeletal radiographs show marked demineralization, with fractures of the ribs and long bones, subperiosteal erosions, metaphyseal widening, and, occasionally, rickets (137). The hypercalcemia is severe, typically ranging from 14–20 mg/dl, although values as high as 30.8 mg/dl have been described (138). Interestingly, relative hypocalciuria has been documented in some cases, even in the absence of a family history of FHH (141). Serum PTH levels were high in the cases in which they were measured, although the degree of elevation can be mild (124, 141–144). Histological examina-

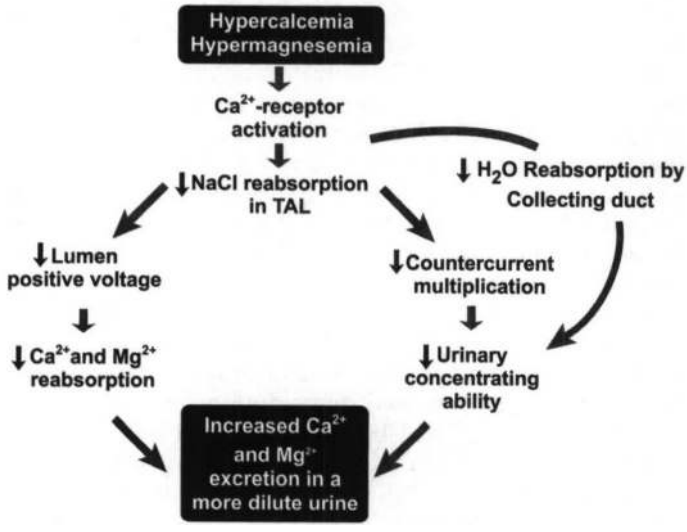

FIG. 4. Role of the CaR in mediating interactions between the renal handling of divalent cations and water. With a systemic calcium load, reduced Ca^{2+} reabsorption in the TAL increases the urinary Ca^{2+} concentration. The high $[Ca^{2+}]_o$-mediated reduction in maximal urinary concentrating ability, however, mitigates the risk of deposition of calcium salts in the final urine. [Reproduced with permission from Brown EM, Hebert SC 1996 Novel insights into the physiology and pathophysiology of Ca^{2+}-sensing receptor. *Regul Pept Lett*, in press.]

tion of bone may show typical osteitis fibrosa cystica (136). The disorder is often fatal if aggressive medical and surgical management is delayed, although occasionally the disease runs a self-limited course, reverting to milder hypercalcemia by the age of 6 to 7 months with conservative treatment (137, 145). Initial management includes hydration and aggressive respiratory support. If the clinical condition is very severe or deteriorates during treatment, however, total parathyroidectomy with autotransplantation of part of one of the glands is recommended during the first month of life (113). Some authors recommend total parathyroidectomy with lifelong management of the resultant hypoparathyroidism (124, 136). There is a dramatic improvement after parathyroidectomy, and rapid healing of the skeletal changes is usually observed, even though the hypercalcemia recurs with less than total parathyroidectomy or after autotransplantation (113, 136). In all cases where parathyroid glands have been removed, they have been enlarged, sometimes up to 10 times the mass of the normal parathyroid glands in infants of this age. Histological examination has shown chief cell or water-clear cell hyperplasia (113, 136, 146).

1. Genetics of NSHPT: association with FHH. Since the initial descriptions of FHH and NSHPT, the occurrence of NSHPT in FHH families has been noted by several investigators (102, 135, 136, 147). In 1981 Marx *et al.* (102) reported their analysis of 15 kindreds with FHH, where they found that three patients from two families had severe primary hyperparathyroidism in the neonatal period. One of the explanations they offered was that NSHPT may be the homozygous form of FHH. The same group later studied a family with two children affected by NSHPT: their consanguineous parents had mild elevations in serum ionized calcium levels (total calcium levels were normal) as well as hypocalciuria, while multiple family members had borderline, sometimes intermittent, hypercalcemia (147). These clinical findings lent support to the belief that NSHPT is actually the homozygous form of FHH and that some of the apparently sporadic occurrences of NSHPT could be due to failure to recognize very mild hypercalcemia in family members heterozygous for the defect. (It is also entirely possible that in some cases the alteration in $[Ca^{2+}]_o$ sensing by the mutant CaR could be so mild that rather than hypercalcemia *per se*, the serum calcium concentration is increased but remains within the normal range). After the localization of the gene for FHH to chromosome 3q in four families (97) and to chromosome 19p (115) in a single family, Pollak *et al.* (148) studied 11 families with FHH and demonstrated that the disease gene mapped to chromosome 3q2 in all of them (148). Four of these families had consanguineous marriages with affected NSHPT offspring. Analysis of genetic markers closely linked to the FHH gene showed that the pattern of inheritance of these markers was consistent with NSHPT representing the homozygous form of the same disease.

2. Mutations in the CaR in NSHPT. After the confirmation that NSHPT is the homozygous form of FHH, analysis of the CaR gene in families where both of these disorders coexisted confirmed that NSHPT patients inherit mutated CaR genes from both parents (13, 119, 120). Thus these patients do not possess a copy of the normal CaR, and as a result show much more severe hypercalcemia, elevated PTH, and parathyroid cell hyperplasia, as well as a markedly altered set point for calcium-regulated PTH secretion. All of these findings indicate a state of severe, albeit variable, parathyroid resistance to $[Ca^{2+}]_o$.

3. Spectrum of NSHPT. It is unlikely, however, that all cases of NSHPT represent the homozygous form of FHH. Cases of NSHPT have been reported to occur sporadically or in FHH families with only one affected parent (132, 143, 145). Indeed, recent studies have documented the occurrence of heterozygous, *de novo* CaR mutations in two sporadic cases (98) (*i.e.* caused by a single *de novo* CaR mutation in the child of normal parents), both of whom had evidence of hyperparathyroid bone disease, but with less severe hypercalcemia than is seen in typical NSHPT. This may provide an explanation for the observed clinical spectrum in NSHPT, which ranges from the typically severe, life-threatening manifestations (in patients homozygous for mutated CaR) to a more benign, possibly even self-limited form of the disorder (*i.e.* in patients with *de novo* heterozygous CaR mutations).

NSHPT that occurs in families where one parent has FHH could sometimes reflect unrecognized FHH in the other parent due to intermittent hypercalcemia. Another possible explanation for this observation could be the level of the maternal serum calcium level to which the developing fetus is exposed *in utero*. Calcium is actively transported across the placenta from the mother to the fetus, resulting in a higher concentration of serum calcium in the fetus (68). A mother with a normal serum calcium concentration would expose the fetus with FHH to a relatively hypocalcemic environment, which could conceivably "overstimulate" the fetal parathyroid glands and lead to the development of more

severe, "secondary" fetal/neonatal hyperparathyroidism superimposed upon the abnormal FHH-associated $[Ca^{2+}]_o$ sensing already present. This hypothesis is supported by the finding that cases of NSHPT with autosomal dominant inheritance have been reported in which the father had FHH while the mother was normocalcemic (106, 113, 136). Some of these cases have had a relatively benign, self-limited course, lending further credence to this theory. The possibility that an unidentified factor/protein may interact with the mutated FHH gene has also been suggested. Incomplete penetrance or uniparental disomy are other possible mechanisms.

These studies on NSHPT, which can, in effect, represent a "knockout" of the human CaR, provide the following perspective on the function of the receptor in humans: 1) They highlight the importance of the CaR in fetal and neonatal calcium homeostasis. 2) They suggest a possible role for the CaR in the regulation of parathyroid cellular proliferation, since there is marked parathyroid hyperplasia in NSHPT.

VII. Activating Mutations of the CaR: Autosomal Dominant Hypocalcemia

ADH refers to a familial syndrome characterized by mild hypocalcemia with few or no symptoms and detectable PTH levels (14). Since these patients appear to tolerate their hypocalcemia very well, it was hypothesized that they could have an altered set point for recognizing extracellular calcium, *i.e.* their parathyroid glands could be "hyperresponsive" to extracellular calcium (14, 149). Thus their calcium homeostatic system might be "reset" to maintain extracellular calcium at subnormal concentrations, the converse of FHH, where the system has been reset upward due to loss-of-function mutations in the CaR gene. Interestingly, in one such family, EDTA infusion was able to provoke PTH secretion, suggesting that the parathyroid glands were able to secrete PTH, but there was indeed a downward shift in the set point for $[Ca^{2+}]_o$-regulated PTH secretion (149).

After the cloning of BoPCaR and the recognition of inactivating CaR mutations in families with FHH and NSHPT, and in light of the fact that activating mutations in other G protein-coupled receptors had been demonstrated in several disorders (32), it was thought that an "overactive" CaR in families with ADH could provide an explanation for this syndrome. Therefore, Pollak *et al.* (14) searched for activating mutations in the CaR in two families with ADH. In one of the probands, they demonstrated a missense mutation (E127A) in the CaR gene (14). This mutation was found in all hypocalcemic members of the family. Moreover, *X. laevis* oocytes expressing the mutant receptor exhibited severalfold higher levels of IP_3 at both low and high $[Ca^{2+}]_o$ compared with oocytes expressing the wild type receptor. They concluded that this mutation in the extracellular domain increased CaR activity "inappropriately" at low extracellular calcium concentrations, causing hypocalcemia in individuals heterozygous for such a mutation. No mutation in the CaR could be detected in the second family.

Since this report, another family with "autosomal dominant hypoparathyroidism" has been reported to have a missense mutation in the extracellular domain of the CaR (150, 151). Pearce *et al.* (152) recently studied six families with ADH on the basis of clinical criteria. In addition to having asymptomatic hypocalcemia with detectable PTH levels, affected family members also had hypomagnesemia and hyperphosphatemia. The reason for classifying these families as ADH rather than familial isolated hypoparathyroidism was their unusual response to vitamin D treatment. Attempts to normalize their serum calcium levels with calcitriol resulted in marked hypercalciuria, which led to nephrocalcinosis and renal impairment in several cases (153). Some of them also complained of thirst and polyuria, possibly due to the development of NDI, with normalization of their serum calcium concentrations. It thus appears that the calcium homeostatic system in these individuals is adjusted to a lower than normal serum calcium concentration, and attempts to make them normocalcemic result in "hypercalcemic" manifestations (*i.e.* hypercalciuria, NDI). Four of these six families demonstrated mutations in the extracellular domain of the CaR that presumably produce inappropriate activation of the receptor at normal or even low levels of $[Ca^{2+}]_o$. Each of the six families with mutations so far reported have had unique mutations in the extracellular domain (two families in the latter study did not exhibit any mutations in the CaR gene), although several additional families have been reported in preliminary studies with autosomal dominant hypocalcemia associated with the presence of activating mutations within transmembrane domains of the receptor. Interestingly, the homozygous form of ADH, which would presumably present with more severe hypocalcemia, has not been identified.

The mechanism(s) by which these mutations could activate the CaR is presently unclear. Activating point mutations in other G protein-coupled receptors, like the TSH and LH receptors, are present in transmembrane domains and presumably enhance the processes of signal transduction or mimic the active state of the receptor after ligand binding if the activating mutation is truly ligand-independent (154, 155). In the CaR, it is likely that mutations within the extracellular domain enhance the affinity of the CaR for extracellular calcium or mimic the ligand-bound state of the extracellular domain, thereby imitating subsequent events in signal transduction at inappropriately low levels of extracellular calcium or even in the total absence of calcium.

These studies provide additional evidence for the importance of the CaR in setting the responsiveness of parathyroid and kidney to changes in $[Ca^{2+}]_o$. The parathyroid glands of individuals with ADH are hyperresponsive to extracellular calcium. In addition, normalization of serum calcium in these individuals leads to hypercalciuria that may be in excess of that expected in hypoparathyroid patients as individuals with ADH seem excessively prone to the complications of hypercalciuria and hypercalcemia even at low-to-normal levels of $[Ca^{2+}]_o$ (153). These observations complement the earlier ones in individuals with inactivating CaR mutations (FHH), in which there is inappropriate hypocalciuria despite hypercalcemia, and confirms a major role for the CaR in regulating tubular reabsorption of calcium. In addition, the apparently reciprocal abnormalities in water metabolism in

ADH and FHH provide additional indirect evidence that the CaR is intimately involved in coordinating renal handling of divalent cations and water.

Furthermore, these studies suggest the existence of ADH as an entity distinct from typical hypoparathyroidism and could lead to recognition of a larger number of cases with ADH, many of which may previously have been classified as mild cases of familial isolated hypoparathyroidism. This distinction is of great clinical importance, since treating ADH patients with calcitriol to normalize their serum calcium concentration may result in irreversible renal damage.

VIII. Alterations in CaR Function in Other Disease States

After the detection of mutations in the CaR in inherited disorders of calcium metabolism, it was of interest to determine whether there were alterations in CaR expression and/or function in acquired states of parathyroid dysfunction. One study has shown loss of heterozygosity encompassing the CaR locus in 10% of parathyroid adenomas (156). A detailed study of the CaR gene in various forms of primary and secondary hyperparathyroidism, including cases of adenoma, carcinoma, and hyperplastic glands from patients with sporadic or familial hyperparathyroidism or those with uremic hyperparathyroidism (157), however, failed to find any evidence for mutations in the CaR similar to those found in FHH. Furthermore, we found a substantial reduction in the immunoreactivity of the CaR protein in parathyroid adenomas as well as in uremic hyperparathyroidism as assessed by immunohistochemistry using a polyclonal antiserum raised against a peptide derived from the extracellular domain of the CaR (158). These alterations in CaR expression could possibly contribute to the altered set point for $[Ca^{2+}]_o$-regulated PTH secretion seen in various forms of primary hyperparathyroidism as well as in severe uremic hyperparathyroidism (36).

IX. Diagnostic Implications

Detection of mutations in the CaR in patients with sporadic asymptomatic hypercalcemia could clearly be helpful in diagnosing FHH, although the size of the CaR-coding sequence makes this a substantial undertaking if direct sequencing were performed. More rapid screening procedures, such as denaturing gradient gel electrophoresis or the use of ribonuclease protection, could facilitate the process of screening for point mutations (98). A negative screen for mutations is not of much value, however, as not all FHH patients show CaR mutations, even when the disorder is linked to the chromosome 3 locus. Therefore, the diagnosis of FHH will likely continue to be established by the traditional approach of documenting an autosomal dominant inheritance of asymptomatic hypercalcemia in family members other than the proband that is accompanied by relative hypocalciuria (calcium/creatinine clearance ratio of <0.01). In our experience, it is not uncommon for patients with mild hyperparathyroidism to restrict their calcium intake voluntarily to the point where separation of their clearance ratios from those encountered in FHH patients can be problematic. In this situation, particularly when first-degree relatives are not readily available for family screening, dietary supplementation to a total of 1000 mg elemental calcium/day can be very helpful, as it will usually increase urinary calcium excretion in patients with true primary hyperparathyroidism well above the levels seen with FHH. Other causes of apparent hypocalciuria in otherwise typical primary hyperparathyroidism are vitamin D deficiency, the use of hypocalciuric agents such as lithium or thiazides, and, occasionally, hypothyroidism. Hypercalcemia screening of as many family members as possible of patients with a provisional diagnosis of FHH is important since even borderline hypercalcemic patients may harbor mutations. Mutational screening of the spouse of a hypercalcemic patient could potentially be of value in predicting risk for NSHPT in the offspring. In conjunction with family screening for hypercalcemia, mutational analysis could also be useful for the diagnosis of cases presenting with hyperparathyroidism in the neonatal period, especially in the absence of a family history of hypercalcemia.

The recognition of cases with autosomal dominant hypocalcemia due to activating mutations of the CaR is important, since many of these individuals could be "over" treated with calcium/vitamin D with deleterious, sometimes irreversible, renal consequences if the disorder is not identified. The documentation of this condition requires careful clinical and genetic characterization. The clinician should carefully consider this diagnosis in individuals with the presumed diagnosis of familial hypoparathyroidism, particularly those who develop marked hypercalciuria and/or renal impairment when treated with vitamin D, so that complications such as nephrocalcinosis and renal failure can be prevented.

X. Therapeutic Implications

The recognition that $[Ca^{2+}]_o$ sensing by parathyroid cells might involve a cell surface, G protein-coupled receptor led to attempts directed at the development of drugs targeted at the receptor. Drugs that mimic the actions of high levels of $[Ca^{2+}]_o$ ("calcimimetics") on the receptor, for example, could conceivably be employed to lower PTH levels in states of hyperparathyroidism. Indeed, drugs of the latter type were developed more or less simultaneously with the cloning of the CaR. This made it possible to employ transfection of cells normally lacking the receptor to document that calcimimetics actually acted at the level of the CaR to enhance the degree of receptor activation at any given level of $[Ca^{2+}]_o$ (15). One such calcimimetic, NPS R-568, is the most promising drug developed to date and is currently undergoing clinical trials in hyperparathyroid states (159). NPS R-568 is a small organic molecule that inhibits PTH secretion, resulting in sustained hypocalcemia in normal rats. This hypocalcemia is thought to be due to a reduced Ca^{2+} efflux from bone resulting from decreased PTH secretion. In rats with mild chronic renal failure due to partial nephrectomy, NPS R-568 treatment for 4 weeks prevented the development of secondary hyperparathyroidism and an increase in bone formation rate, indicating its potential use in this condition in

humans (160). Preliminary data indicate that the drug also lowers both PTH and serum Ca^{2+} levels acutely in normal human subjects (159). Short-term use of the drug caused no discernible side effects. NPS R-568, therefore, could prove to be a major advance in the modalities available for treatment of both primary and secondary hyperparathyroidism.

Recent data suggest that NPS R-568 may inhibit not only PTH secretion but also hormonal synthesis. Cultured bovine parathyroid cells show a substantial reduction in PTH mRNA level when incubated overnight in the presence of NPS R-568 as well as an elevated level of $[Ca^{2+}]_o$ (127). The reduction in the level of PTH mRNA was greater than that seen with high calcium alone. The capacity of calcimimetics to suppress PTH synthesis could have broad implications both therapeutically as well as for understanding CaR-PTH interactions.

XI. Summary

The recent cloning of a $[Ca^{2+}]_o$-sensing receptor from several different tissues in several species directly demonstrates that a variety of cells can directly recognize and respond to small changes in their ambient level of $[Ca^{2+}]_o$ through a G protein-coupled, cell surface receptor. This finding directly documents that $[Ca^{2+}]_o$ can act as an extracellular, first messenger in addition to subserving its better known role as an intracellular second messenger. Several of the tissues expressing the CaR are important elements in the calcium homeostatic system that have long been known to be capable of sensing $[Ca^{2+}]_o$, such as parathyroid and thyroidal C cells. The presence of the receptor in the kidney, however, provides strong evidence that several of the long-recognized but poorly understood direct actions of $[Ca^{2+}]_o$ on renal function could be mediated by the CaR. These actions include the up-regulation of urinary calcium and magnesium excretion in the setting of hypercalcemia, which complements the indirect inhibition of renal tubular reabsorption of calcium that results from high $[Ca^{2+}]_o$-mediated inhibition for PTH secretion. The impaired renal concentrating capacity in hypercalcemia is likely a manifestation of a homeostatically important interaction between the regulation of renal calcium and water handling that reduces the risk of pathological deposition of calcium in the kidney when there is a need to dispose of excess calcium in the urine. In this regard, the availability of human syndromes of $[Ca^{2+}]_o$ "resistance" or "overresponsiveness" due to loss-of-function or gain-of-function mutations in the CaR, respectively, have provided useful experiments in nature that have clarified the importance of the receptor in both abnormal and normal physiology. Much remains to be learned, however, about the role of the CaR in locations, such as the brain, where it likely responds to local rather than systemic levels of $[Ca^{2+}]_o$. In such sites, it may represent an important modulator of neuronal function, responding to $[Ca^{2+}]_o$ as a neuromodulator or even neurotransmitter. The development of therapeutics that either activate or inhibit the function of the CaR may be useful for treating a variety of conditions in which the receptor is either under- or overactive. Finally, it would not be surprising to discover additional receptors for $[Ca^{2+}]_o$ or for other ions (the CaR may, in fact, be an important $[Mg^{2+}]_o$-sensor) that could function abnormally in certain disease states and be amenable to pharmacological manipulation with ion receptor-based therapeutics.

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