CHAPTER 6

THE CALCIUM-SENSING RECEPTOR: PHYSIOLOGY, PATHOPHYSIOLOGY AND CaR-BASED THERAPEUTICS

Physiology and pathophysiology of CaR

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Abstract: The extracellular calcium (Ca_o^{2+})-sensing receptor (CaR) enables the parathyroid glands and other CaR-expressing cells to sense alterations in the level of Ca_o^{2+} and to respond with changes in function that are directed at normalizing the blood calcium concentration. In addition to the parathyroid gland, the kidney is a key site for Ca_o^{2+}-sensing that enables it to make physiologically relevant alterations in divalent cation and water metabolism. Several disorders of Ca_o^{2+}-sensing arise from inherited or acquired abnormalities that "reset" the serum calcium concentration upward or downward. Inactivating mutations produce a benign form of hypercalcemia when present in the heterozygous state, termed Familial Hypocalciuric Hypercalcemia (FHH), while homozygous mutations produce a much more severe hypercalcemic disorder resulting from marked hyperparathyroidism, called Neonatal Severe Hyperparathyroidism (NSHPT). Activating mutations cause a hypocalcemic syndrome of varying severity, termed autosomal dominant hypocalcemia or hypoparathyroidism. Inactivating or activating antibodies directed at the CaR produce the expected hyper- or hypocalcemic syndromes, respectively. "Calcimimetic" CaR activators and "calcilytic" CaR antagonists have been developed. The calcimimetics are currently in use for controlling severe hyperparathyroidism in patients receiving dialysis treatment for end stage renal disease or with parathyroid cancer. Calcilytics are being evaluated as a means of inducing a "pulse" in the circulating parathyroid hormone (PTH) concentration, which would mimic that resulting from injection of PTH, an established anabolic form of treatment for osteoporosis

Keywords: Seven transmembrane receptor, calcium-sensing receptor, calcium homeostasis, calcimimetic, calcilytic, familial hypocalciuric hypercalcemia, autosomal dominant hypoparathyroidism, acquired hypoparathyroidism, osteoporosis, hyperparathyroidism

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1. INTRODUCTION

The calcium-sensing receptor (CaR) is a seven transmembrane receptor (7TM receptor–also termed G protein-coupled receptor) that is expressed widely throughout the body (Brown et al, 1993). It plays key roles in the maintenance of a nearly constant extracellular ionized calcium concentration (Ca_o^{2+}), particularly in the chief cells of the parathyroid gland. Here it regulates the synthesis and secretion of PTH as well as parathyroid cellular proliferation (Tfelt-Hansen and Brown, 2005), inhibiting all three when Ca_o^{2+} is high and stimulating them when Ca_o^{2+} is low (Tfelt-Hansen and Brown, 2005). PTH plays a central role in the acute regulation of Ca_o^{2+} and through its regulation by the CaR, maintains Ca_o^{2+} within a narrow range (1.1–1.3mM). Both very high and very low levels of Ca_o^{2+} can lead to serious clinical sequellae and in some instance can be life-threatening. Even minute alterations in Ca_o^{2+} from its normal level (e.g., of a few percent) promote immediate physiologic responses, especially reciprocal changes in PTH secretion that will normalize the level of Ca_o^{2+}. The CaR, cloned over a decade ago, is a central element in the maintenance of this delicate state of calcium homeostasis. It serves as the body's thermostat for Ca_o^{2+}, functioning as a "calciostat" and informing the parathyroid glands of the precise level of Ca_o^{2+}.

The CaR was cloned using the expression-cloning technique in Xenopus laevis oocytes (Brown et al, 1993). Analysis of its nucleotide and amino acid sequences place the CaR within family C of the superfamily of seven transmembrane, G protein-couple receptors (GPCRs). Other members of this family are the G protein -coupled, so-called metabotropic receptors for glutamate (mGluRs) and for gamma-aminobutyric acid (GABA), as well as GPCRs for sensing pheromones, taste, and odorants (in fish). Recently, another member of family C, GPRC6A, has been found to share several pharmacological properties with the CaR (Wellendorph and Brauner-Osborne, 2004; Wellendorph et al, 2005). Like the CaR, GPRC6A is sensitive towards certain L-amino acids, although unlike the CaR, which senses predominantly aromatic amino acids, GPRC6A is most responsive to basic amino acids (Wellendorph et al, 2005). Subsequent studies showed that this receptor is also activated by high concentrations of extracellular calcium (e.g., 10–20 mM) and calcimimetics (Pi et al, 2005), allosteric activators of the CaR that will be discussed later. These data have implicated GPRC6A as a second calcium-sensing receptor (CaSR2).

The physiological relevance of the CaR in humans was proven by the identification of inherited disorders caused by mutations in the receptor leading to either loss- or gain-of-function (Hendy et al, 2000). Heterozygous (e.g., the mutation is present in only one allele) gain-of-function mutations cause a form of autosomal dominant hypoparathyroidism (ADH). Heterozygous loss-of-function mutations are the cause of a disorder called familial hypocalciuric hypercalcemia—FHH, also termed familial benign hypocalciuric hypercalcemia (FBHH), which typically manifests as asymptomatic hypercalcemia with relative or absolute hypocalciuria. When present in the homozygous or compound heterozygous state, in contrast, inactivating CaR mutations produce neonatal severe primary hyperparathyroidism

(NSHPT), a severe, sometimes lethal disease if it is left untreated. Mouse models with disruption of one or both CaR genes produce biochemical and phenotypic features closely resembling those observed in FHH and NSHPT, respectively. Thus our increasing understanding of inherited disorders of calcium-sensing as well as animal models with knockout of the receptor have illuminated not only the pathophysiology but also the physiology of the CaR and is reviewed in a later section.

CaR expression is greatest in the parathyroid glands, calcitonin-secreting C-cells of the thyroid gland, and kidney, but the CaR is also found in the two other key organs that participate in calcium homeostasis: gut and bone (Brown and MacLeod, 2001). This review will focus on the structure and function of the CaR, its role in normal physiology and in various disorders of Ca_o^{2+}–sensing, and the development of CaR-based therapeutics.

2. BIOCHEMICAL AND PHYSIOLOGICAL FEATURES OF THE CaR

This section briefly introduces key aspects of the structure of the CaR and its downstream signaling pathways to provide sufficient background information to understand the molecular basis for both normal mineral ion homeostasis and for inherited diseases of the CaR. We also present an update regarding the growing number of naturally occurring and pharmacologic ligands of the receptor. The use of the latter as CaR-based therapeutics for various disorders of Ca_o^{2+}–sensing is covered in greater depth in section F, following the description of these ligands and disorders.

2.1. Structure and Signaling Pathways of the CaR

The 5.3-kb clone of the CaR isolated by expression cloning, when expressed in the oocytes, exhibited the same pharmacological properties as the Ca_o^{2+}-sensing mechanism previously characterized in dispersed bovine parathyroid cells, the prototypical calcium-sensing cell (Brown et al, 1993). The use of nucleic acid hybridization-based cloning then enabled the CaR to be cloned from humans (Garrett et al, 1995), rats (Riccardi et al, 1995), mice, rabbits (Butters et al, 1997) and, more recently, the dogfish shark (Nearing et al, 2002) and bony fish (Loretz et al, 2004). The nucleic acid sequences of the mammalian receptors are at least 85% identical to that of the original bovine parathyroid CaR. The amino acid sequences show even greater similarity [>90% identity using http://www.ncbi.nlm.nih.gov/BLAST/]. Therefore, only a limited amount of divergence from a putative primordial calcium-sensing receptor has taken place throughout evolution, and the functionally important structural features have presumably been retained.

The CaR is a member of family C II of the superfamily of seven transmembrane (7TM) receptors, also termed G protein-coupled receptors (GPCRs) (Brown and MacLeod, 2001). 7TM receptors are by far the largest group of cell surface receptors. They are very important in clinical medicine, since the 7TM receptors represent

the targets of about 50% of currently available drugs. The human CaR comprises 1078 amino acid residues and has three structural domains, as do all 7TM receptors (Figure 1). It has an unusually large extracellular domain (ECD) (612 residues), which is characteristic of the family C GPCRs; a transmembrane domain (TMD) of 250 amino acids containing the 7 membrane spanning helices, and an intracellular, C-terminal tail (ICD) of 216 amino acids (Figure 1). The receptor exhibits substantial N-linked glycosylation, which is important for the normal level of cell membrane expression of the receptor but does not appear to modify the function of the

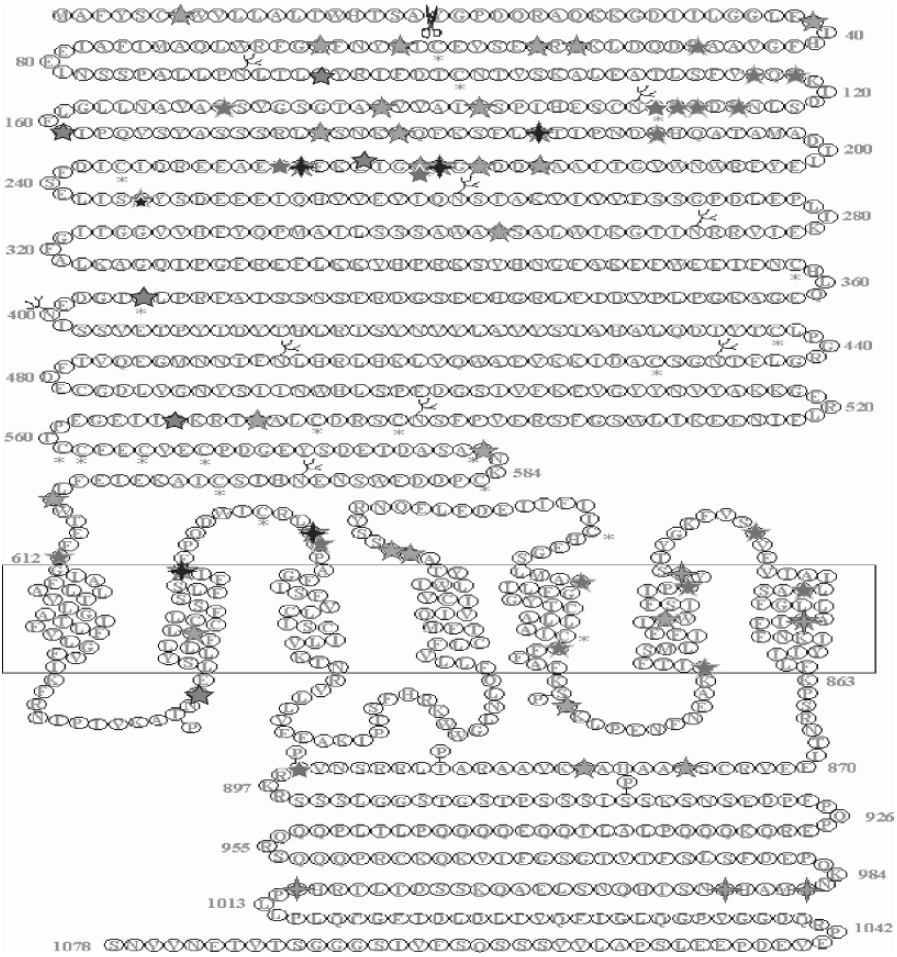

Figure 1. Topology of the CaR showing naturally occuring mutations
Scissors indicate end of signal peptide, circled P's at amino acids T646, S794, S915, S895, T888 are protein kinase C sites, blue symbols are polymorphisms, green are inactivating mutations, red are activating mutations, and black on top of green are two inactivating mutations (See Colour Plate 11) reproduced with permission from G.N. Hendy, Ph.D.

receptor per se (Rav et al, 1998). The cell surface form of the CaR is primarily a dimer, and the two monomers within the dimeric CaR are linked by disulfide bonds involving cysteine residues 129 and 131 within each monomer (Bai et al, 1998a). The location(s) of the Ca_o^{2+}-binding sites in the CaR have not been fully elucidated, but recent evidence suggests that one potential binding pocket is present within the cleft between the two lobes (Silve et al, 2005). The ECD of each CaR monomer probably contains more than one binding sites for Ca_o^{2+}, because the Hill coefficient for the activation of the receptor by Ca_o^{2+} is 3, 4; consistent with the presence of positive cooperativity amongst at least this number of binding sites within the dimeric CaR. The TMD is also apparently involved in Ca_o^{2+}-sensing, since a mutant CaR lacking the ECD also responds to Ca_o^{2+} and other polyvalent cations (Hu et al, 2005). The receptor contains five protein kinase C (PKC) phosphorylation sites (Figure 1) (Bai et al, 1998b). The PKC phosphorylation sites are part of a negative feedback system, whereby phosphorylation of one or more of the PKC sites, particularly T888, inhibits CaR-mediated activation of phospholipase C (PLC), which is a major downstream mediator of the receptor's biological responses. PLC is an important participant in CaR-mediated activation of PKC, particularly the calcium-dependent forms of the enzyme. Studies carried out prior to the cloning of the CaR demonstrated that incubation of parathyroid chief cells with high levels of Ca_o^{2+} inhibited hormone-dependent cAMP accumulation and activated PLC and, consequently, IP_3 production, documenting that the CaR activates both $G\alpha_q$ and $G\alpha_{i/o}$ (Brown, 1991). The ICD binds to the scaffolding proteins, filamin-A and caveolin-1 (anti-caveolin antibodies co-immunoprecipitate the CaR, but direct binding of caveolin-1 to the CaR has not been demonstrated) (Hjalm et al, 2001; Kifor et al, 1998); both of these proteins also bind signaling partners activated by the CaR, such as components of the MAPK pathways (see below). The interaction of the CaR with filamin-A was recently shown to protect the CaR from intracellular degradation (Zhang and Breitwieser, 2005); this finding may explain why some studies have found that the CaR exhibits limited internalization following binding to its ligands.

Studies utilizing a heterologous system of human embryonic kidney (HEK) cells with or without stably transfected CaR (HEK-CaR) have uncovered a plethora of intracellular signaling pathways regulated by the receptor (Awata et al, 2001; Brown and MacLeod, 2001). Most are also active in CaR-mediated signaling in other cells expressing the CaR endogenously. In HEK-CaR cells as well as in parathyroid cells, the CaR activates phospholipases (PL) A_2, C, and D. PLC hydrolyzes phosphatidylinositol bisphosphate to produce IP_3, which, in turn, activates the IP_3 receptor in the endoplasmic reticulum (ER), thereby releasing calcium from its internal stores within the ER; the attendant influx of calcium into the cytosol causes spikes in the cytosolic free calcium concentration (Ca_i^{2+}). An early step in the biosynthesis of polyphosphoinositides is conversion of phosphatidylinositol (PI) to phosphatidylinositol 4-phosphate (PI-4P) by phosphatidylinositol 4-kinase. The CaR stimulates phosphatidylinositol 4-kinase via $G\alpha_q$ in parallel with its concomitant activation of PLC in HEK-CaR cells (Huang et al, 2002). Another key group of intracellular

signaling pathways linked to the CaR is the mitogen-activated protein kinases (MAPKs). Activation of MAPKs takes place via phosphorylation by their respective upstream kinases. MAPKs are key intracellular signaling pathways that often produce changes in gene expression, i.e., in cell cycle regulation. But MAPKs can also regulate processes close to the cell membrane, such as the activity of potassium channels and the secretion of peptides. We have previously demonstrated that the CaR in parathyroid cells and HEK-CaR cells stimulates the activity of MAPKs (Kifor et al, 1997). Handlogten et al. also showed that the CaR activates MAPK in HEK-CaR cells; they used HEK cells stably transfected with a dominant-negative CaR (Arg796Trp) as a control (Handlogten et al, 2001). They likewise demonstrated that the CaR stimulates PLA_2 through $G\alpha_q$, PLC, calmodulin, and calmodulin-dependent kinase, but not through Ga_s or MAPK in HEK-CaR cells. The explanation(s) underlying the differing results of these two studies concerning the role of MAPK in PLA_2 activation has not yet been clarified. In cells that express the CaR at lower levels than parathyroid cells and HEK-CaR cells, including testicular cancer cells, MAPK and phosphatidylinositol 3-kinase (PI3-kinase), components of an important prosurvival pathway, have been shown to be activated by the CaR (Tfelt-Hansen et al, 2004).

2.2. Agonists of the Calcium-sensing Receptor

The CaR behaves in a promiscuous manner with regards to the considerable number of ligands that regulate it. CaR agonists are described as type I or type II (Nemeth et al, 1998b). Type I agonists are direct agonists, while type II serve as allosteric modulators, requiring the presence of calcium to stimulate the CaR; the type II modulators left-shift the calcium dose-response curve by sensitizing the receptor to this or other type I agonists. The type I ligands comprise a variety of polycations, both inorganic and organic, and their potencies generally parallel the number of positive charges that they possess. The rank order of potency for the stimulation of the CaR by various inorganic di- and trivalent cations is as follows: $Gd^{3+} \geq La^{3+} \gg Ca^{2+} = Ba^{2+} > Sr^{2+} > Mg^{2+}$ (Brown et al, 1990; Nemeth and Scarpa, 1987; Shoback et al, 1988). The best characterized type I organic polycationic CaR agonists are neomycin, spermine, and amyloid β-peptides (Brown et al, 1993; Quinn et al, 1997; Ye et al, 1997). Neomycin and gadolinium are often utilized to demonstrate that an action of Ca_o^{2+} is likely to be mediated through the CaR; however, they are far from specific for the CaR and newer, more specific approaches, such as the use of pharmacological activators or inhibitors of the receptor, dominant negative constructs or RNA silencing, offer great advantages in this regard. Slight alterations in Ca_o^{2+} (50–100 micromolar) within the physiological range *in vivo* regulate the CaR's activity, but the affinity of the receptor for its principal physiological regulator, calcium, is far lower than that of other GPCRs for their ligands. The CaR's low affinity, when considered within the context of the levels of calcium within the bodily fluids (e.g., millimolar) as well as the steepness of the curve relating Ca_o^{2+} to CaR activity, make the CaR a excellent "calciostat" for informing CaR-expressing cells of the precise level of Ca_o^{2+} within their immediate vicinity. The Hill coefficient, which provides a measure of how well the CaR responds to small alterations in the

concentrations of its agonists, is 3 to 4 in HEK-CaR cells (Bai et al, 1996), as noted above. In dispersed parathyroid cells *in vitro*, the CaR is even more sensitive to changes in Ca_o^{2+}: PTH release is maximal at 0.75 mM and fully suppressed at just below 2 mM ionized Ca_o^{2+} (Brown, 1991). HEK-CaR cells as well as other types of cells, which, in general, express the CaR at lower levels than do the chief cells of the parathyroid gland, e.g. leydig cancer cells derived from the testis, exhibit higher EC_{50} values ($\backsim$3–4 mM) (Bai et al, 1996; Sanders et al, 2000).

As noted earlier, type II agonists are allosteric modulators of the CaR, e.g., they potentiate the action of Ca_o^{2+} on the receptor. They comprise two classes: small molecule drugs and amino acids. Drugs that allosterically stimulate the CaR are called "calcimimetics" (Nemeth et al, 1998b). NPS R-467 and NPS R-568 and AMG 073 are calcimimetics that have been used in various experimental studies and clinical trials and, more recently, as treatments for the secondary hyperparathyroidism, which is not uncommonly encountered in patients with end stage renal disease receiving dialysis therapy (Block et al, 2004; Goodman et al, 2002). AMG 073 (also called cinacalcet or sensipar) is currently the drug of choice, because NPS R-467 and NPS R-568 are degraded by a cytochrome P-450 enzyme, CYP2D6 (Amgen, unpublished data). Five to seven percent of the general population expresses CYP2D6, which has reduced enzymatic activity, thereby resulting in higher blood levels and delayed metabolic clearance in this segment of the population. Calcimimetics interact with the CaR's TMD and in some manner increase the apparent affinity of the receptor for calcium. More details on CaR-based therapeutics and their application to human disease are given in Section F.

Some L-amino acids also act as type II agonists, in contrast to the respective D-amino acids, which are several-fold less potent in activating the receptor (Conigrave et al, 2000). This CaR's ability to be activated by both extracellular calcium and L-amino acids may permit it to sense nutrients in the gut, for example. Available data indicate that amino acids bind to the ECD of the receptor, interacting with a binding pocket homologous to those binding GABA and glutamate in the $GABA_B$ receptors and mGluRs, respectively (Silve et al, 2005; Zhang et al, 2002). This may be pharmacologically important, since L-phenylalanine and the calcimimetic NPS R-467 have synergistic actions in stimulating the CaR. Finally, calcilytics represent another type of pharmacological agent acting on the CaR, as described in more detail in Section F. They antagonize the action of Ca_o^{2+} on the receptor, and are being studied for use in the treatment of osteoporosis, as they stimulate a pulse of endogenous PTH secretion, which has the potential to exert an anabolic action on bone, similar to that of once daily injected PTH (Gowen et al, 2000).

2.3. Physiology

It is crucial for the body that Ca_o^{2+} is maintained within a narrow range (1.1–1.3 mM). Both very low and very high levels of Ca_o^{2+} can be dangerous and even life-threatening. Even very small alterations in the level of Ca_o^{2+}, on the order of

a few percent, produce immediate physiologic responses, which include alterations in PTH secretion that restore Ca_o^{2+} to its normal level. Rapid alterations in Ca_o^{2+} are more hazardous than slowly developing ones; thus the rapidity of the PTH response is essential as a homeostatic defense against both hypo- and hypercalcemia.

Parathyroid hormone (PTH), calcitonin, and $1,25(OH)_2D_3$ are the three most important Ca_o^{2+}-regulating hormones (Bringhurst et al, 1998). As noted earlier, there is a functionally critical inverse relationship between Ca_o^{2+} and PTH, a calcium-elevating hormone. This relationship is mediated by the CaR (Ho et al, 1995; Schwarz et al, 1993). In contrast, high Ca_o^{2+} stimulates the secretion of calcitonin (CT), a Ca_o^{2+}-lowering hormone; this action of calcium on CT secretion is likewise mediated by the CaR (Fudge and Kovacs, 2004). The rapidity with which the secretion of PTH and CT respond to changes in extracellular calcium usually normalizes Ca_o^{2+} within minutes to hours. Available data have demonstrated that the CaR is expressed not only in the organs that secrete calcium-regulating hormones (e.g., the parathyroid glands and C-cells of the thyroid glands), but also in target tissues for these hormones. These latter tissues regulate Ca_o^{2+} by translocating calcium ions into or out of the bodily fluids, and include the kidney, which expresses the CaR at robust levels in certain nephron segments, as well as bone and intestine, which express the receptor at lower levels (see below). By acting on both hormone-secreting and hormone-responsive tissues through its own cell surface receptor, Ca_o^{2+} acts in effect, as another Ca_o^{2+}-regulating "hormone" (in this case Ca_o^{2+}–lowering) or "first messenger". Elevations in the extracellular ionized calcium concentration stimulate the CaR and lower Ca_o^{2+} by enhancing CT secretion, promoting urinary calcium excretion and suppressing PTH release. In the remainder of this section, we will provide a brief discussion of the CaR's known and putative roles in parathyroid gland, kidney and bone, three out of the four main organs involved in calcium homeostasis. For a discussion of the CaR's localization and potential roles along the gastrointestinal tract, see (Hebert et al, 2004).

2.3.1. Parathyroid (PT) glands

The most important function of the CaR in minute-to-minute Ca_o^{2+} homeostasis lies in the CaR-mediated inhibition of PTH secretion. The steep inverse sigmoidal curve relating Ca_o^{2+} and PTH release was described long before the CaR was cloned. Studies utilizing dispersed parathyroid cells to investigate Ca_o^{2+}–regulated PTH release are mainly limited to those performed within a few hours of isolating the cells, because these cells, particularly bovine parathyroid cells, exhibit a marked, loss of their expression of the CaR on the cell membrane over 24–48 hours (Brown et al, 1995; Mithal et al, 1995). The receptor is normally expressed at high levels on the surface of the parathyroid chief cells. The principal functions of the CaR in the parathyroid are shown in Table 1. Relatively little is known about the control of its expression on the cell surface, but of note, the expression of the CaR in rat parathyroid and kidney was increased by $1,25(OH)_2D_3$, while Ca_o^{2+} had no effect (Brown et al, 1996). In more recent studies, however, raising Ca_o^{2+} increased CaR expression in avian parathyroid gland (Yarden et al, 2000), and a

Table 1. Key Roles of CaR in Parathyroid and Kidney

- Parathyroid
  (1) Inhibit PTH secretion
  (2) Inhibit PTH gene expression
  (3) Inhibit parathyroid cellular proliferation
- Kidney
  (1) Proximal tubule—blunt PTH-induced phosphaturia
  (2) MTAL—inhibit NaCl reabsorption
  (3) CTAL—inhibit reabsorption of Ca^{2+} and Mg^{2+}
  (4) IMCD—inhibit vasopressin-elicited water reabsorption

calcimimetic elevated the expression of the receptor in pathological parathyroid glands (Mizobuchi et al, 2004). The upregulation of the receptor following its activation could clearly serve as a positive feedback loop contributing to CaR-mediated actions in the parathyroid gland. Of note in this regard, Ca_o^{2+} also regulates the expression of the vitamin D receptor (VDR) (Bajwa et al, 2005), which could potentiate the action of vitamin D on the level of CaR expression and also enhance the biological actions of the CaR. Another case where there is upregulation of parathyroid CaR expression is in sheep subjected to burn injury, which was accompanied by a decrease in set-point for suppression of PTH secretion by calcium (Murphey et al, 2000). This increase in CaR expression and the associated change in Ca_o^{2+}–regulated PTH secretion may be a factor that contributes to the hypocalcemia and relative hypoparathyroidism in human burn patients. It might also participate in the hypocalcemia accompanied by inappropriately normal PTH levels, which can be observed in severely ill patients with inflammatory states.

In bovine parathyroid cells, the CaR is located on the cell membrane in caveolin-1-containing rich membrane domains (Kifor et al, 1998) that are called caveolae and can serve as "message centers" where several different types of signaling molecules are concentrated. The signaling systems downstream of the CaR that contribute to the regulation of PTH secretion are not known with certainty. However, a recent report showed that knockout of both G_q and G_{11} in mice resulted in biochemical features similar to those caused by homozygous knockout of the CaR in mice and humans, suggesting a critical role for these G proteins in CaR-induced suppression of PTH secretion (Wettschureck et al, 2006). Another report showed that activation of the CaR increases ERK1/2 activity via the PKC pathway and, to a lesser extent, the PI-3K pathway, in normal parathyroid cells. Thus, while more work is needed, a pathway comprising CaR $\rightarrow$ $G_{q/11}$ $\rightarrow$ PLC $\rightarrow$ PKC $\rightarrow$ ERK1/2 may participate in the modulation of PTH secretion by Ca_o^{2+}.

2.3.2. *Expression, function and regulation of the CaR in the kidney*

The kidney plays several critical roles in calcium homeostasis. The CaR is widely expressed along essentially the whole nephron. The cellular localization and putative function(s) of the CaR in the kidney seem to depend upon the region of the nephron in which the receptor resides (Ward and Riccardi, 2002). The CaR's

expression along the nephron has been studied using *in situ* hybridization as well as reverse transcriptase-polymerase chain reaction (RT-PCR) of micro-dissected nephron segments (Riccardi et al, 1996). Later, the cellular localization and regional distribution of receptor protein along the nephron was examined using immunofluorescence (Riccardi et al, 1998). One outcome of these studies was the recognition that the polarity of CaR protein varies along the nephron. In the proximal tubule the receptor is present on the apical surface of the proximal tubular epithelial cells. On the contrary, in the cells of the cortical thick ascending limb (CTAL), the receptor is localized in the basolateral membrane. Similarly, basolateral staining for the CaR was observed in the medullary thick ascending limb (MTAL), macula densa, and the distal convoluted tubule (DCT). In the cortical collecting duct, immunostaining for the CaR is located on some intercalated cells, while in the inner medullary collecting duct (IMCD) the receptor has primarily an apical distribution.

There has been relatively little work characterizing the factors that regulate the CaR's expression in the kidney. A recent report demonstrated that in rat kidney, C-cell and parathyroid *in vivo* as well as in a human proximal tubule cell line *in vitro*, transcription of the CaR gene was increased about two-fold following 8 and 12 h of treatment with $1,25(OH)_2D_3$ (Canaff and Hendy, 2002), acting via two promoters that lie upstream of the CaR gene. A low phosphate diet had no effect on CaR expression along the nephron in one study (Caride et al, 1998), while Riccardi et al. demonstrated *in vivo* in rats that a low phosphate diet as well as treatment with PTH downregulated CaR protein in the proximal tubule (Riccardi et al, 2000). Thus CaR expression in the proximal tubule of the rat kidney is modulated by $1,25(OH)_2D_3$ and PTH and, perhaps, by dietary phosphate. One other study on the regulation of the CaR's expression along the nephron demonstrated that the level of CaR protein in purified apical endosomes isolated from IMCD, which also contain aquaporin-2, the water channel that traffics to the apical membrane in response to vasopressin and enhances water reabsorption, was reduced in rats rendered hypercalcemic by vitamin D administration (Sands et al, 1998). To summarize, the CaR's roles along the nephron include: 1) diminishing the inhibitory effect of PTH on renal phosphate reabsorption in the proximal tubule (Ba et al, 2003); 2) inhibiting renal tubular reabsorption of calcium in the CTAL (Motoyama and Friedman, 2002); and 3) reducing urinary concentrating ability in the IMCD by antagonizing the action of vasopressin (Sands et al, 1997).

2.3.3. Bone

Abundant data indicate that Ca_o^{2+} inhibits the formation and activity of osteoclasts and stimulates the activity of osteoblasts. The first evidence for the existence of a G protein-coupled, cation-sensing mechanism in osteoblasts was presented shortly after the cloning of the CaR (Quarles et al, 1994). Since then some, but not all studies have found that the CaR is expressed in various osteoblastic cell lines and primary osteoblasts (Chang et al, 1999; Chattopadhyay et al, 2004;

Pi et al, 1999). A interesting study demonstrated that osteoblasts from CaR knockout mice still had a promitogenic response to Ca_o^{2+}, supporting the presence of a calcium-sensing mechanism other than the full length CaR. This mechanism could potentially be represented by the newly cloned GPRC6A, although the latter is responsive to calcimimetics, and the actions of extracellular calcium on the CaR knockout osteoblasts were not (Pi et al, 2005). The CaR is also present in articular and hypertrophic chondrocytes (Chang et al, 1999). Utilizing a type II CaR agonist in organ culture (fetal rat metatarsal bones) to study the possible role of the CaR in bone growth, Wu et al. (Wu et al, 2004) demonstrated that the receptor modulates chondrogenesis in the growth plate and enhances longitudinal bone growth.

The CaR is expressed by some osteoclasts (Kanatani et al, 1999) and by monocytes, which are of the same lineage as osteoclast precursors (Yamaguchi et al, 2000). In addition to inhibiting the formation and activity of osteoclasts, high extracellular calcium has been shown to promote osteoclast apoptosis (Lorget et al, 2000). However, the calcimimetic, AMG 073, produced none of the actions of elevated extracellular calcium on osteoblast proliferation or osteoclast formation and resorption in one study (Shalhoub et al, 2003). One possible mechanism that has been suggested to mediate calcium-sensing in osteoclasts is a plasma membrane, ryanodine-like receptor that couples to increases in the intracellular calcium concentration (Zaidi et al, 1999). Therefore, although the CaR and other calcium-sensing mechanisms may participate in the regulation of bone cell and cartilage function, further studies are clearly required to clarify the divergent results observed in the studies to date.

3. DISORDERS OF CALCIUM-SENSING THAT INVOLVE THE CaR

3.1. Clinical and Genetic Features of Familial Hypocalciuric Hypercalcemia (FHH) [OMIM 14598]

The principal disorders of extracellular calcium sensing are listed in Table 2. FHH is typically a benign form of hypercalcemia (Law Jr. and Heath III, 1985; Marx et al, 1981a). The diagnosis of FHH can be made in a patient with mild-to-moderate, PTH-dependent hypercalcemia averaging approximately 2.75 mM (total calcium), an autosomal dominant pattern of inheritance of a similar degree of hypercalcemia on family screening, and an inappropriately reduced rate of urinary calcium excretion in the face of hypercalcemia. Several families, however, have been identified with more marked hypercalcemia, averaging 3 and 3.4 mM. Several affected neonates from some of these kindreds have manifested a neonatal severe hyperparathyroid-like state (Bai et al, 1997). It is thought that the mutant receptors harboring these mutations may in some cases exert a dominant negative action on the wild type partner in mutant-wild type heterodimers.

Because the disorder is benign in most cases, patients with FHH are frequently not diagnosed until a routine measurement of the blood calcium concentration shows

Table 2. Disorders of Calcium-Sensing

- Conditions with reduced sensitivity to Ca_o^{2+}
  (1) FHH/NSHPT
  (2) Primary hyperparathyroidism
  (3) Inactivating antibodies to the CaR
- Conditions with increased sensitivity to Ca_o^{2+}
  (1) Autosomal dominant hypocalcemia
  (2) Activating antibodies to the CaR

an unexpectedly high value, or family screening is carried out owing to the birth of a child with NSHPT (Marx et al, 1982). Patients with FHH commonly have normal serum levels of PTH despite their hypercalcemia, although in about 15–20% of cases, PTH levels are frankly elevated (Heath, 1989b).

The hypercalcemia in FHH, when viewed in the context of a normal level of PTH that is inappropriately high for that serum calcium concentration, reflects the presence of a right-shifted set-point for Ca_o^{2+}-regulated PTH release (Auwerx et al, 1984). An additional important finding is the fact that there is typically a normal or even frankly reduced urinary calcium excretion in spite of the coexistent hypercalcemia (Marx et al, 1981a). This alteration in renal calcium handling reflects "resistance" of the kidney to the usual hypercalciuric action of hypercalcemia and is the equivalent of the resistance of PTH secretion to the normal inhibitory effect of high calcium in FHH. Of note, administration of a loop diuretic (e.g., ethacrynic acid) promotes renal excretion of calcium in hypoparathyroid subjects with FHH (Attie et al, 1983). This observation points towards a key role of the thick ascending limb—the site where this class of diuretics acts–in the anomalous renal calcium handling in FHH.

Short of carrying out mutational analysis, the most useful means of distinguishing FHH from other forms of hypercalcemia, particularly primary hyperparathyroidism, is to determine the ratio of the renal clearance of calcium to that of creatinine (Ca/Cr). A value less than 0.01 is found in about 80% of individuals with FHH, while a similar proportion of cases of primary hyperparathyroidism have levels higher than this (Fuleihan et al., 2002). Another biochemical finding in patients with FHH is their capacity to concentrate their urine normally, in contrast to patients with primary hyperparathyroidism, in whom maximal urinary concentration elicited by dehydration is reduced (Marx et al, 1981b). While this finding is not used diagnostically, it likely reflects renal resistance of FHH patients to the hypercalcemia-induced diminution in urinary concentrating ability that is observed in other forms of hypercalcemia. Individuals with FHH usually manifest serum magnesium concentrations that are in the upper normal range or mildly elevated. Finally, while differentiating FHH from primary hyperparathyroidism is usually straightforward, a recent study of the genetic basis for familial isolated hyperparathyroidism showed that four of 22 unrelated probands harbored inactivating mutations of the CaR (Warner et al, 2004). Therefore the clinician should bear in mind that FHH can be an underdiagnosed but important cause of familial isolated hyperparathyroidism (Simonds et al, 2002;

Warner et al, 2004). Moreover this study points out that there can be overlap in the clinical presentations of FHH and primary hyperparathyroidism, particularly in familial forms of the latter.

Additional FHH families have also highlighted the existence of atypical presentations of this condition that initially led to a diagnosis of primary hyperparathyroidism. One such kindred manifested hypercalcemia, high PTH levels, hypercalciuria and even renal stone formation, but was then shown to harbor an inactivating FHH mutation (Carling et al, 2000). Subtotal parathyroidectomy in most affected family members provided long-term remission of their biochemical abnormalities, demonstrating that parathyroid surgery, while typically ineffective in curing hypercalcemia in FHH, may be appropriate in an occasional kindred. Recent studies have described the presence of single and multiple parathyroid "adenomas" in several patients with FHH, although the parathyroid glands in FHH typically are of normal size and histology, or in some cases exhibit mild chief cell hyperplasia with one or more enlarged glands (Burski et al, 2002; Carling et al, 2000). A few cases of parathyroid lipohyperplasia have been described in FHH, and some authors have suggested that finding should prompt a search for inactivating mutations of CaR (Fukumoto et al, 2001).

While FHH most commonly presents as an asymptomatic form of hypercalcemia, a few kindreds exhibit more severe hypercalcemia. Nevertheless, even in these cases the natural history of the disorder is usually so benign that the great majority of these patients should be followed without intervention, with a few exceptions (see above). In the unusual individual with FHH and symptomatic hypercalcemia, the new calcimimetics–described in more detail below—could potentially provide a useful form of treatment; indeed the use of this treatment was recently described in this setting.

The mutations in the CaR gene in FHH or NSHPT cause a loss-of-function of the CaR that result in a rightward shift in the set-point for Ca_o^{2+}-regulated PTH secretion. In 1972, Foley et al. (Foley Jr et al, 1972) first described the characteristic clinical features of the hereditary condition now known as familial hypocalciuric hypercalcemia (FHH) (it was initially called familial benign hypercalcemia). Nearly two decades later, linkage analysis showed that the predominant locus of the FHH disease gene resided on the long arm of chromosome 3 (band q21–24) in four large FHH families (Chou et al, 1992). However, FHH is not always linked to chromosome 3q. Notably, two families with a clinical features similar to FHH showed linkage to the short and long arms of chromosome 19 (Heath et al, 1993; Lloyd et al, 1999), respectively; one of these was called the Oklahoma variant and exhibited a tendency for the biochemical abnormalities to be progressive with time (Lloyd et al, 1999). FHH that is linked to these latter two loci may be present in a minority of the $\backsim$30% of FHH cases without an identifiable mutation in the CaR gene. The remaining cases of FHH without an identifiable mutation presumably harbor mutations in regulatory regions of the CaR gene that control its expression, but this remains to be shown directly.

Since the initial discovery of three unique missense mutations in the CaR gene in 1993 in the families with FHH (Pollak et al, 1993), more than 150 additional mutations have been described. Most are unique to individual families, although a few apparently unrelated kindreds have identical mutations (e.g., gly552arg) (see web site http://www.casrdb.mcgill.ca). Most are missense mutations and reside in the first half of the ECD or within the TMD of the receptor (Hauache, 2001). However, truncation, insertion, deletion and splice site mutations have also been described (Carling et al, 2000; D'Souza-Li et al, 1998; Hendy et al, 2000; Janicic et al, 1995). An unusual type of mutation was described in an FHH family that exhibited a mutation in the acceptor splice site at position-1 of intron 2 of the CaR gene, which resulted in a frame shift and a truncated protein of 153 amino acids (D'Souza-Li et al, 1998). Although the mRNA for the receptor was stable, the truncated protein, lacking any of the transmembrane domain, was never found on the cell membrane. Individual mutations are associated with distinct phenotypes: In some instances, a mutant receptor exerts a dominant-negative action on the remaining wild type receptor within mutant-wild type heterodimers, as noted above, thereby rendering the phenotype more severe (Bai et al, 1997). Such a dominant-negative action presumably reflects the specific properties of the mutant receptor protein residing on the cell surface. In the case of mutant receptors exerting a dominant negative action, normally functioning, wild type CaR homodimers would only represent about one-fourth of all the cell surface receptors (reflecting the theoretically expected ratio of 1:2:1 of wild type homodimer, wild-type-mutant heterodimer, and mutant homodimers, respectively). Truncations in the CaR gene, in contrast, may simply reduce the number of normally functioning CaRs expressed on the cell surface, producing so-called haploinsufficiency, which results from a decrease in receptor expression/activity due to loss of one CaR allele, analogous to mice heterozygous for knockout of the CaR gene (Ho et al, 1995), where levels of the CaR protein are about 50% of normal. It should be kept in mind while evaluating the literature describing in vitro characterization of mutant CaRs that the cells used for these experiments differ substantially from the chief cells of the parathyroid glands or CaR-expressing kidney cells. Thus the experimental data likely represent only an approximation of how the receptors would function in their native environment.

3.2. Clinical and Genetic Features of Neonatal Severe Primary Hyperparathyroidism (NSHPT) [OMIM 239200]

NSHPT in most cases presents within the first six months of life. Affected infants have severe, symptomatic, PTH-dependent hypercalcemia, along with the bony changes of severe hyperparathyroidism. Infants with NSHPT can exhibit polyuria, dehydration, hypotonia, and failure to thrive (Brown et al, 1997; Eftekhari and Yousefzadeh, 1982; Grantmyre, 1973; Heath, 1989a; Marx et al, 1985). A prominent feature of the disease is the associated hyperparathyroid bone disease, which can be associated with multiple fractures. Rib fractures can in some cases produce a

"flail chest" syndrome that causes respiratory difficulties, owing to a decreased capacity of the affected infant to expand its chest wall and generate the negative intrathoracic pressure needed for normal respiration (Grantmyre, 1973).

The mass of the parathyroid glands in NSHPT is generally increased several-fold, and they exhibit prominent chief cell hyperplasia. Biochemical evaluation shows hypercalcemia, hyperparathyroidism, and relative hypocalciuria (Cole et al, 1997). Total serum calcium concentrations range from moderately elevated (e.g., 3–3.25 mM) to levels as high as 7.7 mM in the most severely affected cases (Brown, 2000; Heath, 1989a). PTH levels are often 10-fold higher than the upper limit of normal. Early diagnosis is critical, as untreated NSHPT can be a devastating neurodevelopmental disorder, which in some cases is lethal without parathyroidectomy to alleviate the hyperparathyroidism and hypercalcemia (Cole et al, 1997). As noted earlier, the most severe cases of NSHPT develop ribcage deformities, as well as rachitic changes, skeletal undermineralization, and fractures of the long bones and other skeletal sites (Eftekhari and Yousefzadeh, 1982; Grantmyre, 1973).

The older literature on NSHPT (e.g., prior to 20 years ago) describes substantial mortality in infants with NSHPT; therefore, treatment for the condition in its severe form has traditionally been total parathyroidectomy (Eftekhari and Yousefzadeh, 1982). However, more recently, a broader clinical spectrum for NSHPT has become apparent; particularly given the availability of genetic testing of the CaR gene. As a result a number of studies have now shown that some infants have milder hyperparathyroidism and a substantially milder clinical presentation and natural history (Heath, 1989a; Pearce et al, 1995b). This latter form of the disease might better be termed neonatal hyperparathyroidism (NHPT), to emphasize this milder phenotype in these infants, most of whom harbor heterozygous inactivating CaR mutations. In these latter cases, the condition can revert with time to a phenotype resembling FHH with medical management alone (Heath, 1989a). Therefore, at the moment parathyroidectomy should be reserved for the most severely affected infants, in whom intensive medical therapy (e.g., with aggressive hydration and, if appropriate, bisphosphonates) has failed to stabilize the patient, and there is concern for the infant's survival.

Recent reports have described patients with homozygous mutations in the CaR gene, who escaped detection until adulthood, at which time they did not have the usual symptoms and signs of hypercalcemia and were only identified serendipitously by routine biochemical screening. One such patient, a 35-year-old woman had two copies of the missense mutation pro39ala from related parents. She was asymptomatic, despite a serum calcium concentration of 3.75 to 4.25 mM (Aida et al, 1995). Another such patient, who was homozygous for a distinct inactivating CaR mutation was likewise not diagnosed until adulthood (Fukumoto et al, 2001). Both mutations produced relatively mild defects of their function when expressed heterologously, perhaps enabling a sufficient of control PTH release by calcium to be compatible with a relatively normal life, despite quite marked hypercalcemia. Indeed the seeming lack of hypercalcemic symptoms in the face of moderate to severe hypercalcemia supports the notion that at least some of these symptoms

are mediated by the CaR. That is, these patients seem to be resistant not only to the effects of calcium on parathyroid and kidney but also to the development of hypercalcemic symptoms. In these patients a calcimimetic might represent a means of lowering the serum calcium concentration–assuming the mutant CaRs were responsive to the drug–thereby providing not only a diagnostic test to determine whether the patient obtained any symptomatic benefit from parathyroidectomy but also, potentially, an effective long term, medical therapy.

NSHPT is most commonly an autosomal recessive condition; that is, the CaR genes from both of the parents is mutated (e.g., homozygous FHH). Pollak et al. studied 11 kindreds with FHH, in whom consanguineous unions engendered four infants with NSHPT (Pollak et al, 1994b). It should be recognized, however, that NSHPT is quite uncommon in FHH families considered as a whole. In one case of NSHPT, two distinct mutations—one a mutation in exon 7 from the mother and the other a mutation in exon 4 from the father—caused the disease, e.g., as a result of the compound heterozygosity in the proband, who thereby lacked any normal CaRs (Kobayashi et al, 1997). In theory, NSHPT can result from (1) homozygosity from a consanguineous FHH union, (2) two mutant alleles of the CaR gene arising from two distinct FHH kindreds or (3) from a de novo mutational event, with or without an inherited, mutant parental allele (Pearce et al, 1995b). In addition, an investigation of a girl with phenotypic NSHPT and her family revealed a single mutant allele (present in exon 6, Gly552Arg) in her CaR gene, while her sister, despite having the same genotype, had phenotypic FHH (Schwarz et al, 2000). Thus factors leading to this degree of phenotypic variation are still only partly understood.

3.3. Clinical and Genetic Features of Autosomal Dominant Hypoparathyroidism (OMIM) [#601298]

Patients with this inherited form of hypocalcemia/hypoparathyroidism are commonly asymptomatic, similar to the majority of patients with FHH. Some patients, however, can exhibit neuromuscular irritability, seizures and basal ganglia calcification. Patients generally exhibit mild to moderate hypocalcemia, with serum PTH levels that are inappropriately low given the hypocalcemia, e.g., within the lower half of the normal range or frankly subnormal (Pollak et al, 1994a). Affected individuals often exhibit relative or absolute hypercalciuria, with normal or frankly elevated urinary calcium excretion, respectively, in spite of their low serum calcium concentration. Some studies have shown that renal calcium excretion in ADH is higher than that in typical hypoparathyroidism. However, not all studies have shown this difference in renal calcium excretion (Okazaki et al, 1999; Yamamoto et al, 2000). During febrile episodes patients, particularly children, with ADH may present with symptoms of hypocalcemia and, in some cases, seizures. It is important to prevent renal complications, including nephrocalcinosis, nephrolithiasis, and renal impairment, during treatment of ADH patients with calcium and vitamin D (Pearce et al, 1995a). These renal complications are generally seen in a setting in which

the clinician has tried to correct the serum calcium concentration to or close to the normal range. Treatment with calcium supplements and vitamin D metabolites should be reserved for those patients with symptomatic ADH; the goal should be to increase the serum calcium concentration only to a level sufficient to render the patient asymptomatic (Lienhardt et al, 2001). Renal excretion of calcium requires monitoring in treated patients in order to minimize the risk of urinary complications. If a serum calcium concentration high enough to ameliorate symptoms cannot be achieved with calcium and vitamin D supplementation without inducing frank hypercalciuria (generally 4 mg/kg/24h), it may be necessary to co-administer a hypocalciuric agent, such as a thiazide diuretic or injectable PTH (Winer et al, 1998).

ADH is a rare syndrome, although in index cases it may comprise a sizeable fraction of cases of idiopathic hypoparathyroidism, perhaps representing as many as a third of such cases (Lienhardt et al, 2001). Patients with this condition harbor an activating or gain-of-function mutation of the CaR gene that resets the set-point of Ca_o^{2+}-regulated PTH secretion leftward and lowers renal calcium reabsorption. Within a year after the cloning of the CaR, Finegold et al. (1994) showed linkage of ADH to a locus on chromosome 3 q13—the same locus containing the gene for the CaR. Shortly afterward, a heterozygous missense mutation, Glu127Ala, was shown to be the cause of ADH in an unrelated family (Pollak et al, 1994a). Since these first reports, more than 30 mutations have been characterized causing ADH (see CaR Database at **http://www.casrdb.mcgill.ca/**). Most of these are missense mutations within the CaR's ECD and TMD. When expressed in heterologous systems, these mutations cause a left-shift in the activation of the CaR by Ca_o^{2+}, and they only rarely induce constitutive activation of the receptor (Baron et al, 1996; D'Souza-Li et al, 2002; Hauache, 2001; Pearce et al, 1996; Pollak et al, 1994a). A recent report identified a family with a large deletion of 181 amino acids within the C-terminus of the CaR, which increased the sensitivity of the receptor for Ca_o^{2+} (Lienhardt et al, 2000). This family contained the only individual to date known to be homozygous for an activating mutation, but this individual exhibited a phenotype very similar to that of the heterozygous family members. Thus one mutated allele may be enough to induce a maximal shift in the set-point of Ca_o^{2+}-regulated PTH secretion, and the presence of the second mutated allele does not alter the biochemical properties of the receptor dimers any further, perhaps due to a "dominant positive" effect of the mutant receptor on its wild type partner within the heterodimeric CaR. Another activating mutation of the CaR changed a cysteine at amino acid 129 to a serine (Cys129Ser) (Hauache, 2001; Hirai et al, 2001). Because this cysteine participates in dimerization of the CaR, this result suggests that this cysteine constrains the receptor in its inactive state.

4. AUTOIMMUNE DISEASES

Recent studies have identified autoimmune, acquired forms of hypo- and hypercalcemia analogous to ADH and FHH, respectively, but which result from activating and inactivating antibodies, respectively, rather than activating and inactivating mutations.

Although these conditions are rare, they are important to be aware of in considering the differential diagnosis of the inherited diseases of calcium homeostasis just described.

4.1. Anti-CaR Antibodies and PTH-Dependent Hypercalcemia

Kifor et al. (2003) found autoantibodies to the CaR in four patients who had a clinical picture resembling that of FHH in the setting of other autoimmune conditions (e.g., Hashimoto's thyroiditis and sprue). The patients' sera stimulated PTH secretion and inhibited high calcium-stimulated inositol phosphate accumulation and MAPK activation, presumably owing to antibody-mediated inhibition of the CaR. Further studies of a larger number of patients are required to determine the incidence of autoimmune, PTH-dependent hypocalciuric hypercalcemia in the presence of various types of autoimmunity.

4.2. Anti-CaR Antibodies and Hypoparathyroidism

Idiopathic hypoparathyroidism is a condition with hypocalcemia caused by insufficient PTH secretion to maintain normocalcemia that is of unknown cause (e.g., hypomagnesemia or prior neck surgery). Blizzard et al. reported the presence of autoantibodies to the parathyroid glands in the sera of patients with idiopathic hypoparathyroidism in 1966 (Blizzard et al, 1966). They showed that 38% of 74 patients with idiopathic hypoparathyroidism had demonstrable anti-parathyroid antibodies, compared with only 6% of 245 healthy controls, thereby establishing the existence of parathyroid autoimmunity. A later study showed that autoantibodies to the parathyroid glands in patients with sporadic, adult-onset hypoparathyroidism bound to the cell surface of dispersed human parathyroid cells and inhibited PTH release (Posillico et al, 1986). This result supported the presence of a cell surface moiety that participated in regulating PTH secretion, which in retrospect may have been the CaR. Li et al. (Li et al, 1996) more recently reported that 14 of 25 patients with autoimmune hyperparathyroidism had antibodies directed at the CaR. In contrast, none of 50 control patients with other autoimmune diseases and 22 normal subjects had antibodies to the receptor. Recently, Kifor at al. reported two patients with hypoparathyroidism and anti-CaR antibodies that activated the receptor as assessed by simulation of MAPK activity and inositol phosphate accumulation and inhibition of PTH release (Kifor et al, 2004). Two further studies have shown conflicting results concerning the presence of anti-CaR antibodies in patients presenting with autoimmune hypoparathyroidism. One study examined 90 patients with autoimmune polyendocrine syndrome type 1 and found no anti-CaR autoantibodies (Soderbergh et al, 2004). Another study evaluated 51 patients with idiopathic hypoparathyroidism–most with only hypoparathyroidism–and 45 healthy controls; Forty nine percent of the patients had serologic evidence of anti-CaR antibodies (Goswami et al, 2004), and there was an association between antibodies to the CaR and HLA-DR, suggesting an autoimmune component to

the disease. Of note, the two patients in the study of Kifor et al. with activating autoantibodies to the CaR also had Graves' disease and Addison's disease, respectively, further supporting the hypothesis of an autoimmune disease. It remains to be determined whether the differences in the results of these two studies reflect the fact that the incidence of anti-CaR antibodies in patients with type 1 APS and those with isolated hypoparathyroidism differs or is due to other factors.

5. CaR-BASED THERAPEUTICS

The development of allosteric activators ("calcimimetics") (Nemeth et al, 1998b) and antagonists ("calcilytics") (Gowen et al, 2000) of the CaR has made possible CaR-based therapy of disorders of extracellular calcium homeostasis (Table 3). AMG073 (known as Cinacalcet hydrochloride or Sensipar) has recently been approved by the Food and Drug Administration (FDA) for use in treating secondary hyperparathyroidism in patients receiving dialysis therapy for end stage kidney disease (Block et al, 2004) as well as in parathyroid cancer (see website: www.amgen.com). The drug also shows efficacy in mild primary hyperparathyroidism, as described below, but has not yet received FDA approval for this indication (Peacock et al, 2005).

Beneficial effects of calcimimetics in treating 2^o HPT were first reported in animal models in which this condition was induced by subtotal nephrectomy. In these studies parathyroidectomy or NPS R-568 slowed the progression of renal failure and decreased heart-risk factors (Ogata et al, 2003). In both these animal models and *in vitro*, the calcimimetics decreased circulating PTH levels (Fox et al, 1999; Roussanne et al, 2001; Wada et al, 1998; Wada et al, 2000). It is noteworthy that NPS R-568 suppressed not only PTH release but also parathyroid cellular growth in rats with experimentally induced renal insufficiency that were receiving a normal-phosphate diet (Wada et al, 2000), indicating that the receptor regulates parathyroid growth as well as secretion. This study found no induction of parathyroid cell apoptosis.

Table 3. Use of CaR-Based Therapeutics

- Calcimimetics
  - (1) Approved by FDA
    - (a) Secondary hyperparathyroidism in dialysis patients
    - (b) parathyroid cancer
  - (2) Not yet approved
    - (a) Primary hyperparathyroidism
    - (b) Possibly FHH/NSHPT or inactivating CaR antibodies
- Calcilytics
  - (1) Not yet FDA-approved
    - (a) Osteoporosis
    - (b) Possibly activating CaR mutations or antibodies

Several studies have examined the efficacy of calcimimetics in treating 2^o HPT in human subjects on dialysis treatment (Antonsen et al, 1998; Block et al, 2004; Goodman et al, 2002; Ohashi et al, 2004). These studies demonstrated that both NPS R-568 and cinacalcet are effectively in lowering serum levels of PTH as well as serum total and ionized calcium concentrations, and, in studies of long enough duration, the calcium-phosphate product (e.g., the product of the calcium and phosphorous concentrations in the serum, which provide an index of the risk of pathological calcification). Because the calcimimetic-induced decrease in serum PTH at times induces hypocalcemia, it may be necessary to administer an active analogue of vitamin D along with the calcimimetic. The utility of an analogue of vitamin D in this setting makes physiological sense as a replacement for the decreased synthesis of $1,25(OH)_2D_3$ by the damaged kidneys.

In a recent 26 week multicenter study of 741 patients with end stage renal disease receiving hemodialysis, administration of cinacalcet reduced mean PTH concentration by 43% in patients, while PTH increased by 9% in the placebo group (Block et al, 2004). As has been observed in other clinical trials, the only consistent adverse effect in cinacalcet-treated subjects was generally self-limited nausea and vomiting. Notably, the serum calcium-phosphate product decreased by 15% in the patients receiving cinacalcet and was unchanged in the placebo group. The use of some traditional modes of treatment for 2^o HPT, e.g., vitamin D analogues and calcium-containing phosphate binders, can lead to hypercalcemia and/or hyperphosphatemia, which are associated with an increased risk of death as well as greater arterial stiffness, and calcification of the coronary arteries, aorta and cardiac valves. Thus the cinacalcet-induced decrease in the calcium-phosphate product might be expected to reduce these complications and perhaps prolong life in dialysis patients. A recent small meta analysis summarized the initial evidence for beneficial long term effects of cinacalcet, showing that adminstration of the drug reduces risk of parathyroidectomy, fracture and cardiovascular hospitalization and also improves quality of life (Ogata et al, 2006). There is a potential risk of low turnover bone disease if doses of cinacalcet are utilized that produce too great a decrease in serum PTH; further studies are needed to assess the frequency of this complication.

A recent, 52 week trial of cinacalcet in 78 patients with mild primary hyperparathyroidism found that 73% of the patients treated with the drug achieved normocalcemia, while only 5% of controls reached this endpoint (Peacock et al, 2005). There was no increase in urinary calcium excretion, suggesting that there was no excess activation of the CaR in the kidney that might have promoted hypercalciuria. Despite the biochemical improvements in these subjects, however, there was no increase in bone mineral density, unlike what is seen following surgical removal of parathyroid tumors in PHPT. Therefore, cinacalcet is safe and efficacious for treating the hypercalcemia in patients with primary hyperparathyroidism, although it may not be as effective as parathyroid surgery in treating patients with reduced bone mineral density. However, cinacalcet hasn't yet been approved by the FDA for the treatment of PHPT, except in the case of parathyroid cancer, for which

there is no other effective therapy of the biochemical abnormalities. Administration of the calcimimetic in the latter setting enables some amelioration of the severe hyperparathyroidism that not uncommonly is the cause of death in this condition. Based on experience in a small number of patients, there is about a 60% response rate, although progression of the disease eventually overwhelms the drug's efficacy in most cases (see www.amgen.com).

Calcium receptor antagonists, so-called calcilytics, have been developed, and their clinical utility is being explored. In the presence of the calcilytic, a higher than usual calcium concentration is needed to suppress PTH levels to a given extent (Gowen et al, 2000; Nemeth et al, 1998a). As a result, the calcium receptor reads normocalcemia as hypocalcemia and secretes a pulse of PTH. Parathyroid hormone, when administered in a once daily regimen, exerts an anabolic action on the skeleton, and is currently being used in the setting of osteoprosis (Neer et al, 2001). Once daily adminsitration of the calcilytic, therefore, could promote a similar effect owing to release of endogenous PTH, if the pharmacokinetics of the endogenous PTH secretion mimicked sufficiently well that resulting from exogenous PTH. Proof-of-principle has been obtained in a rat model of bone loss resulting from removal of the ovaries, but further studies, particularly those in humans, are needed to establish the role of calcilytics in the treatment of osteoporosis (Gowen et al, 2000).

6. SUMMARY AND FUTURE ISSUES

The CaR is a membrane bound 7TM receptor expressed in all of the tissues regulating extracellular calcium homeostasis. It "senses" even minute (on the order of a few percent) alterations in the level of calcium in the blood; thus it acts as the body's "calciostat". The CaR, in turn, regulates the functions of the cells that express it so as to normalize the level of blood calcium concentration. CaR-mediated control of the release of PTH plays an especially important role in calcium homeostasis, since it directly or indirectly regulates the functions of all of the tissues that are involved in regulating blood calcium. Patients who have loss-of-function mutations in the CaR gene exhibit a form of hypercalcemia that is accompanied by absolute or hypocalciuria. In the heterozygous form, it produces a benign hypercalcemic condition, FHH. In the homozygous form (NSHPT), the hypercalcemia may be lethal if it is not treated surgically. Gain-of-function mutations produce a generally benign state of hypocalcemia with relative or absolute hypercalciuria, ADH; It will be of interest in future studies to collect detailed clinical information in large kindreds to study the possible implications of the CaR's altered sensitivity to calcium in these patients and the resulting alterations in the levels of the serum calcium concentration. This could be even more relevant than previously considered, since the CaR is expressed in numerous organs, such as the breast brain, intestine and cardiovascular system, which are not thought to be involved in systemic calcium metabolism. Finally, symptomatic patients may derive benefit from the new calcimimetic CaR activators and, perhaps in the future, calcilytics.

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