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THE CALCIUM-SENSING RECEPTOR IN HUMAN DISEASE

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1. ABSTRACT

The discovery of the calcium-sensing receptor (CaR), a G protein–coupled receptor, has led to the elucidation of the pivotal roles of the CaR in systemic calcium homeostasis. The receptor is situated on the chief cells of the parathyroid glands, where it senses the extracellular Ca2+ concentration and in turn alters the rate of secretion of parathyroid hormone (PTH). The intracellular signal pathways to which the CaR couples include, but are not limited to, phospholipase C (PLC), and mitogen-activated protein kinases. The receptor is widely expressed in various tissues and likely serves important cellular functions beyond that of maintaining systemic calcium homeostasis. Functionally important mutations in the receptor have been found to cause disorders in calcium homeostasis due both to changes in the set point for PTH secretion and to the control of renal calcium excretion. These mutations cause hypercalcemia when the mutation inactivates the receptor and cause hypocalcemia when the mutation activates the receptor. Recent studies have revealed the presence of circulating autoantibodies to the calcium-sensing receptor in humans, with the clinical presentation the same as that for diseases caused by mutations in the CaR. In renal secondary hyperparathyroidism, a drug that stimulates the receptor (calcimimetic) shows great promise as a medical treatment for this condition.

2. INTRODUCTION

Maintaining near constancy of the extracellular ionized calcium concentration (Ca^{2+}_o) is critical because of myriad intra- and extracellular roles of calcium in vital bodily processes such as neuromuscular activity, clotting of the blood, and skeletal integrity (1,2). Ca^{2+}_o homeostasis is achieved through the carefully orchestrated translocations of calcium ions into and out of the extracellular fluids (ECF) via kidney, intestine, and bone (3). The parathyroid glands play a central role in this homeostatic system by virtue of their capacity to serve as "thermostats" for Ca^{2+}_o or "calciostats" by sensing small changes in Ca^{2+}_o and responding with oppositely directed alterations in parathyroid hormone (PTH) secretion. Since the cloning of the Ca^{2+}_o-sensing receptor, which mediates the capacity of parathyroid cells and a number of other cells to sense Ca^{2+}_o, our understanding of the role of extracellular calcium has advanced from considering it merely a physiologically indispensable ion to an awareness of its hormone-like role as an extracellular first messenger (4).

The parathyroid cell is a prototypical Ca^{2+}_o -sensing cell, having exquisite sensitivity to changes in Ca^{2+}_o, thereby acting as the prime regulator of systemic calcium homeostasis. Other cells involved in calcium metabolism also sense Ca^{2+}_o; these include thyroidal C-cells, which secrete calcitonin when the level of Ca^{2+}_o rises,

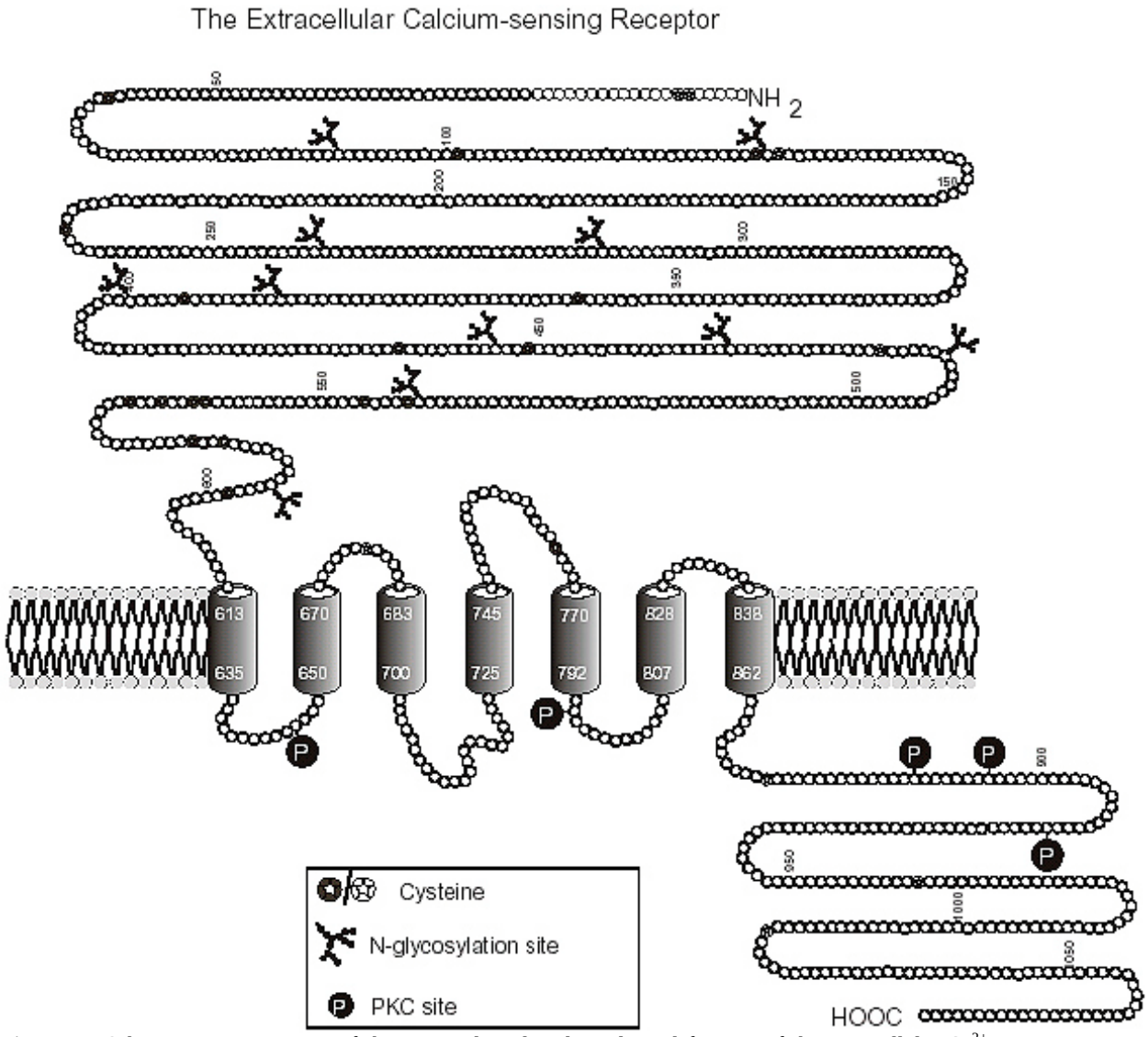

Figure 1. Schematic representation of the principal predicted topological features of the extracellular Ca^{2+}-sensing receptor cloned from human parathyroid glands. The extracellular domain contains 612 amino acids, and the transmembrane domain and intracellular domain each contain approximately 200 amino acids. Also shown are the PKC sites, N-glycolysation sites and conserved cysteines.

and renal proximal tubular cells, whose synthesis of 1,25-dihydroxy vitamin D_3 is inhibited by high levels of Ca^{2+}_o. The elevation of peritubular, but not luminal, Ca^{2+}_o in the tubules of the renal thick ascending limb inhibits the reabsorption of Ca^{2+} and Mg^{2+}, potentially providing a means for the autoregulation of renal handling of divalent cations. Finally, elevated levels of Ca^{2+}_o inhibit osteoclasts *in vitro* and potentially could regulate bone resorption *in vivo* via this mechanism. Thus, Ca^{2+}_o, by serving its hormone-like role as an extracellular messenger, acts in concert with PTH, calcitonin, and $1,25(OH)_2D_3$ to maintain mineralion homeostasis. The purpose of this review is to provide a brief delineation of the normal roles of the CaR in maintaining Ca^{2+}_o homeostasis and an update on recent progress in our understanding of hypo- and hypercalcemic states related to various CaR mutations.

3. BIOCHEMICAL AND PHYSIOLOGICAL FEATURES OF THE CAR

The human homologue of the CaR comprises 1,078 amino acid residues and has three structural domains characteristic of the superfamily of G protein–coupled receptors (GPCR)—a large, 612 residue, amino (N)-terminal extracellular domain that senses Ca^{2+}_o, a central core of 250 residues with seven transmembrane helices, and a 216-amino acid carboxyl (C)-terminal tail predicted to be cytoplasmic (figure 1) (5,6). The CaR has several N-linked glycosylation sites that are important for its expression on the cell surface, where the receptor resides primarily as a disulfide-linked dimer (7). The CaR has five protein kinase C (PKC) and two protein kinase A (PKA) phosphorylation sites within its intracellular domains (8). Phosphorylation of these PKC sites inhibits the coupling of

the CaR to phospholipase C (PLC), one of the principal intracellular signaling pathways of the CaR.

CaR agonists (i.e., high Ca^{2+}_o) activate phospholipases A_2, C, and D in parathyroid cells and in CaR-transfected human embryonic kidney (HEK-293) cells (9). High Ca^{2+}_o evokes a transient rise in the cytosolic calcium concentration (Ca^{2+}_i) in these cells caused by PLC-induced generation of inositol trisphosphate (IP3), which releases Ca^{2+} from intracellular stores, followed by a sustained increase in Ca^{2+}_i due to the opening of Ca^{2+}-permeable plasma membrane channels. The CaR also stimulates the p42/44 mitogen-activated protein kinases (MAPKs) by a mechanism involving activation of cytoplasmic tyrosine kinases, e.g., c-Src, and inhibits adenylate cyclase via the inhibitory G protein, $G\alpha_i$, in parathyroid and some kidney cells (10). The CaR inhibits accumulation of cAMP in some cells by increasing Ca^{2+}_i via the mechanisms noted above, which then inhibits a Ca^{2+}-inhibitable isoform of adenylate cyclase (11).

3.1. CaR agonists

Physiological fluids contain several positively charged small molecules that could bind to the CaR and transduce signals intracellularly (12). It is interesting that the principal physiological agonist of the CaR, Ca^{2+}, regulates its activity at millimolar concentrations, implying an extremely low affinity compared with the affinities of other receptors for their agonists (13). Mg^{2+} also inhibits PTH secretion via the CaR, although at generally supraphysiological levels, although Mg^{2+} may serve as a physiological ligand for the CaR in the thick ascending limb of the nephron (14). The CaR exhibits marked positive cooperativity in response to Ca^{2+}_o and other polyvalent cation agonists, such as Gd^{3+}, neomycin, and spermine (12,15). The Hill coefficient of the receptor is typically 3 to 4 (16), but the steepness of the physiological response of the parathyroid glands to Ca^{2+} is even more extreme: suppression of PTH release *in vitro* is minimal at 0.75 mM and maximal at ~2 mM. Unlike many receptors, the CaR is resistant to desensitization, a response that may optimize its capacity for continuous surveillance of extracellular levels of Ca^{2+}. Since the CaR is distributed in a variety of tissues (discussed later in this review), possibly the receptor senses local endogenous agonists or modulators in addition to Ca^{2+}_o. A recent report revealed that the CaR could serve as an "ionic-strength sensor" in dispersed bovine parathyroid cells, since exposing these cells to 40 mM NaCl shifts the median effective concentration producing 50% inhibition (EC_{50}) of PTH release by at least 0.5 mM (17). Furthermore, spermine and to a lesser extent spermidine have been noted to act as physiological agonists of CaR, a function that is particularly relevant in the central nervous system (CNS) (18).

Recently, a variety of amino acids have been demonstrated to serve as positive modulators of the CaR, potentiating the actions of the receptor's polycationic agonists in their presence (e.g., at >1 mM Ca^{2+}) but not in their absence (19). This action is stereoselective for most amino acids, with L-amino acids being several fold more potent than D-amino acids. Although individual amino acids exhibit relatively low potencies (in the millimolar range) for activating the CaR, a mixture of amino acids similar to that present in fasting persons has a substantial impact on the receptor's sensitivity to polycationic agonists, decreasing the EC_{50} for Ca^{2+} by 20% to 40%. Furthermore, the order of potency for the effects of amino acids on the CaR, with aromatic amino acids being most potent, is highly reminiscent of that for the known effects of various amino acids in stimulating the secretion of gastrin and gastric acid. Thus, it is possible that the CaR mediates these latter actions and functions *in vivo* more generally as a "nutrient receptor" than as a sensor responding solely to mineral ions (and other polycations). Given the growing list of CaR agonists, it is plausible that various positively charged small biomolecules, could be identified as CaR agonists.

3.2. Physiology

Three calciotropic hormones—PTH, calcitonin, and $1,25(OH)_2D_3$—are required for maintaining Ca^{2+}_o homeostasis (3,20). The inverse relationship between Ca^{2+}_o and PTH (a Ca^{2+}_o-elevating hormone) secretion and the positive relationship between Ca^{2+}_o and the release of calcitonin (CT) (a Ca^{2+}_o-lowering hormone; figure 2) release are both mediated by the CaR (21). The actions of PTH and CT are mediated by their respective GPCRs on specific kidney and bone cells. Studies performed subsequent to the cloning of the CaR have shown that it regulates not only the secretion of PTH but also its actions on target tissues, enabling extracellular calcium ions to act as another Ca^{2+}_o-regulating hormone. Similar to CT, the actions of extracellular calcium on its receptor lower Ca^{2+}_o.

The response of the Ca^{2+}_o homeostatic system to hypocalcemia illustrates nicely the central role of the CaR in calcium homeostasis. The resultant rise in circulating levels of PTH enhances distal renal tubular Ca^{2+} reabsorption. It is known that activation of distal tubular CaRs directly inhibits tubular reabsorption of Ca^{2+}. Thus, reducing Ca^{2+}_o has the opposite effect—directly stimulating Ca^{2+} transport—in addition to doing so indirectly via the CaR-mediated increase in PTH secretion. Both PTH and low Ca^{2+}_o directly enhance renal proximal tubular synthesis of $1,25(OH)_2D_3$ and therefore the ensuing increase in the efficiency of intestinal Ca^{2+}_o absorption. Further studies are needed to determine whether this action of Ca^{2+}_o on the proximal tubule is CaR-mediated.

PTH also acutely inhibits bone formation by a direct action on osteoblasts and indirectly—acting via the osteoblast—increases the activity of bone-resorbing osteoclasts. These actions are both potentiated by $1,25(OH)_2D_3$ and promote net efflux of Ca^{2+} from bone. Interestingly, high Ca^{2+}_o inhibits osteoclast formation and activity and increases osteoblastic activity via direct actions on the respective bone cells. Therefore, the effect of lowering Ca^{2+}_o, similar to that of increasing PTH, is to enhance skeletal Ca^{2+} release. The CaR is expressed on osteoblasts (22), as well as on some osteoclasts (23) and their precursors, and is a candidate for mediating, at least in part, these actions of Ca^{2+}_o on bone cells. Therefore, the CaR and the PTH receptor are expressed on key kidney and

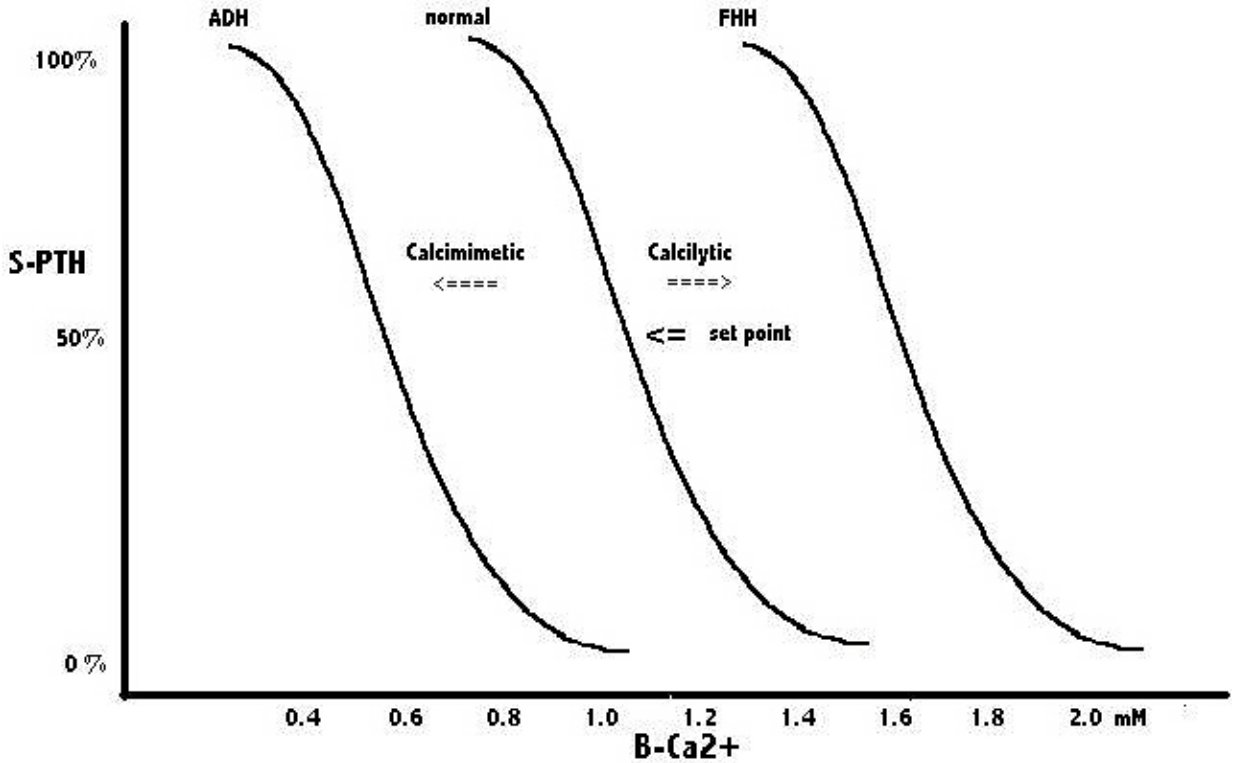

Figure 2. Sigmoid relation between the blood Ca^{2+} and serum PTH levels in normal state, in ADH autosomal dominant hypoparathyroidism, and in FHH familial hypocalcuric hypercalcemia and the therapeutic effect of the calcimimetic (shifting set point to the left) and the calcilytic (shifting set point to the right). Set point is defined as the calcium level that produces half-maximal inhibition of PTH secretion.

bone cells involved in Ca^{2+}_o homeostasis, and their respective ligands, Ca^{2+}_o and PTH, exert mutually antagonistic actions on these cells. Just the opposite chain of events occurs with hypercalcemia: there is high Ca^{2+}_o–induced, CaR-mediated inhibition of PTH secretion and renal Ca^{2+} reabsorption, as well as increased skeletal uptake of Ca^{2+}, although further studies are needed, as noted above, to define the role of the CaR in the direct regulation of bone-cell function. To some extent, CaR-mediated stimulation of CT secretion also participates in the defense against hypercalcemia by inhibiting bone resorption and increasing renal Ca^{2+} excretion.

Although the CaR is expressed on intestinal epithelial cells involved in absorbing dietary Ca^{2+}, its role in this process, if any, is currently unknown (24). The CaR is likewise present on the apical membrane of colon crypt cells, where it may inhibit their proliferation in response to increases in luminal Ca^{2+}_o. This action of the CaR does not contribute to Ca^{2+}_o homeostasis but may mediate a protective action of increased dietary Ca^{2+} intake against the development of colonic neoplasms.

The CaR also plays a crucial roles in the defense against and pathophysiology of hypercalcemic states. In response to a Ca^{2+} load arising from either excessive skeletal release or gastrointestinal absorption, the CaR plays a key role in defending against hypercalcemia. Even slight increases in Ca^{2+}_o, by virtue of the exquisite sensitivity of the CaR to changes in extracellular calcium, reduce PTH secretion, thereby indirectly (e.g., via associated decreases in PTH) and directly (i.e., via renal CaRs) increasing urinary Ca^{2+} excretion. Elevations in Ca^{2+}_o also decrease skeletal release of Ca^{2+}, probably also through both indirect (i.e., via changes in PTH) and direct (via CaRs and/or other Ca^{2+}_o -sensing mechanisms in bone cells) mechanisms. In PTH-dependent forms of hypercalcemia, such as primary hyperparathyroidism (PHPT) and familial hypocalciuric hypercalcemia (FHH), in contrast, the CaR participates actively in initiating and maintaining hypercalcemia. In PHPT, reduced expression of the mRNA and/or protein for an otherwise normal CaR, which has been shown in most but not all studies, resets pathological parathyroid gland(s) to maintain an elevated level of Ca^{2+}_o (25). In FHH, reduced levels of normally functioning CaRs in parathyroid and kidney, and perhaps in bone, promote hypercalcemia by resetting the way in which CaR-expressing tissues respond to Ca^{2+}_o. Therefore, available data strongly support important roles for the CaR in the normal defense against hypercalcemia and in the pathophysiology of PTH-dependent forms of hypercalcemia, to be discussed briefly later.

3.3. Non-homeostatic function of the CaR

While the physiological relevance of the CaR in cells uninvolved in mineralion homeostasis remains to be clarified, recent studies on the expression and functions of the CaR in the diverse cell types expressing it have greatly

expanded the range of its possible regulatory functions (26). Ca^{2+}_o-regulated processes likely mediated by the CaR include (1) secretion of PTH, CT, ACTH, gastrin, insulin, growth hormone, and parathyroid hormone–related peptide (PTHrP); (2) ion channel/transporter activity, e.g., nonselective cation channels (NCC), voltage-dependent Ca^{2+} channels, calcium-activated potassium (K), and aquaporin-2 water channels; (3) chemotaxis of preosteoblastic cells and macrophages; (4) proliferation of parathyroid, colon, and ovarian surface epithelial cells, as well as fibroblasts and keratinocytes; (5) differentiation of keratinocytes, goblet cells, and mammary epithelial cells; (6) protection agianst apoptosis of fibroblasts, HEK-293 cells stably transfected with the CaR, and prostate cancer cells; and (7) gene expression, e.g., of PTH, the vitamin D receptor, and the CaR. Thus, Ca^{2+}_o may serve as an extracellular messenger regulating diverse cellular functions, not only in cells directly involved in Ca^{2+}_o homeostasis but also in the variety of additional cell types expressing the CaR.

What is the role of the CaR in "non-homeostatic" cells, such as neurons, oligodendrocytes, astrocytes, and microglia in the CNS? The brain provides a location highly suited for local Ca2+o-sensing because it is separated from the systemic circulation by the blood-brain barrier. Furthermore, dynamic changes in Ca2+o occur in association with neuronal activation and attendant cellular uptake of Ca2+o, such as during impulse conduction and neurotransmission. In addition, synaptic vesicles contain large amounts of Ca2+. Release of this Ca2+ during neurotransmission could potentially modulate the activity of CaRs within the synaptic cleft, in effect enabling Ca2+o to serve as a neurotransmitter (Ca2+o and glutamate might function in this manner as "cotransmitters" in glutamatergic synapses). Furthermore, increases in neuronal activity and certain pathological states, such as seizures, ischemia, and hypoglycemia, cause increased cellular uptake of Ca2+, thereby producing reduced levels of Ca2+o within the brain extracellular fluid (ECF) that could potentially be sensed by CaRs in the cells in the immediate locale. Indeed, astrocytes and oligodendrocytes are key regulators of the extracellular ionic composition of the brain, which is crucial for regulating neuronal excitability. The CaR in glial cells could potentially contribute to maintaining local ionic homeostasis in the brain ECF through its known capacity to stimulate Ca2+-activated K+ channels and nonselective cation channels and to enhance the proliferation of oligodendrocytes and microglia.

Numerous hematopoietic precursors within the bone marrow also express the CaR. While the role(s) of the receptor in these cells is uncertain, CaRs in the bone marrow would likely encounter substantial changes in Ca2+o related to bone turnover that may serve an informational role in the marrow microenvironment. Clearly, a great deal remains to be learned about the role of the CaR in the diverse cell types that express it that do not participate in systemic Ca2+o homeostasis.

3.4. Intracellular signal transduction

CaR agonists activate phospholipases C, A_2, and D in bovine parathyroid cells (10). These actions are likely to be CaR-mediated, because high Ca^{2+}_o no longer activates these phospholipases in parathyroid cells maintained in culture for 3 to 4 days, during which time expression of the CaR decreases by 80% or more. High Ca^{2+}_o also activates the same three phospholipases in CaR-transfected human embryonic kidney (HEK293) cells but not in nontransfected HEK cells. The high Ca^{2+}_o-evoked, transient rise in Ca^{2+}_i in bovine parathyroid and CaR-transfected HEK293 cells probably results from activation of PLC and resultant IP3-mediated release of Ca^{2+} from intracellular stores. In parathyroid and HEK293 cells, activation of PLC is insensitive to pertussis toxin and likely involves $G\alpha_{q11}$. In contrast, the CaR-mediated increase in inositol phosphates observed in the mouse pituitary AtT-20 cell line is sensitive to pertussis toxin, showing that the CaR also can activate PLC through a pertussis toxin–sensitive G protein (27).

In addition to evoking transient elevations in Ca^{2+}_i, the CaR elicits sustained increases in Ca^{2+}_i in parathyroid and CaR-transfected HEK293 cells through an incompletely understood Ca^{2+}_o influx pathway(s). Activation of a Ca^{2+}-permeable, nonselective cation channel (NCC) in CaR-transfected HEK cells has been shown with the patch-clamp technique (28). A similar NCC in parathyroid cells also is activated by high Ca^{2+}_o, likely via a CaR-dependent pathway, and may contribute to the high Ca^{2+}_o-evoked, sustained rise in Ca^{2+}_i in this cell type (29). High Ca^{2+}_o and other CaR agonists also markedly suppress agonist-stimulated accumulation of cAMP in parathyroid cells in a pertussis toxin–sensitive manner, suggesting that CaR-mediated inhibition of adenylate cyclase involves one or more isoforms of the inhibitory G protein, $G\alpha_i$(10).

How does the CaR regulate longer-term processes, such as cellular proliferation and differentiation? In some cells, the CaR stimulates cellular proliferation—as in rat-1 fibroblasts and human ovarian surface epithelial cells—and is thought to do so via activation of the p42/44 MAPK pathway in the first of these (30). In contrast, the CaR tonically inhibits parathyroid cellular proliferation, because infants with neonatal severe primary hyperparathyroidism (NSHPT) due to homozygous inactivating CaR mutations or mice homozygous for targeted disruption of the CaR gene exhibit marked parathyroid cellular hyperplasia. The signaling pathway(s) through which the CaR regulates parathyroid cell proliferation, however, is not known. As noted above, cellular differentiation *in vitro* of some CaR-expressing cell types (e.g., keratinocytes) is triggered by increases in Ca^{2+}_o (31). High Ca^{2+}_o-elicited, presumably CaR-mediated, changes in several intracellular signalling pathways may mediate this biological response, including the accumulation of inositol phosphates and elevations in Ca^{2+}_i resulting from both release of Ca^{2+} from its intracellular stores and Ca^{2+} influx through NCC. However, longer-term processes mediated by the CaR, such as cellular proliferation and differentiation, may involve activation of MAPKs such as p42/44 and p38 MAPK, which have recently been shown to be regulated by the CaR. We recently showed that p42/44 activation is mediated by the interaction of the CaRs with a scaffold protein, filamin-A, in CaR-transfected HEK cells (32).

4. DISEASES INVOLVING THE CAR

Diseases involving the CaR can be divided into the following categories: (1) Diseases in which mutations lead to altered receptor function, as in (a) familial hypocalciuric hypercalcemia (FHH), which arises from the presence of a mutation in one allele of the CaR gene that partially or completely inactivates the receptor, (b) neonatal severe primary hyperparathyroidism (NSHPT), which can arise when both alleles have been inactivated, and (c) autosomal dominant hypoparathyroidism (ADH) when the receptor is oversensitive to Ca^{2+}_o; (2) diseases in which the receptor is the target of autoimmune antibodies; (3) diseases in which the CaR protein is apparently functionally normal but is reduced in quantity, as in primary (PHPT) and in some cases of uremic secondary hyperparathyroidism (SHPT); (4) polymorphisms in the CaR gene that may alter its function; and (5) f some malignant diseases in which the CaR may contribute to the pathophysiology of hypercalcemia.

4.1. Genetic changes leading to FHH, NSHPT, and ADH

The CaR has been found to be responsible for the majority of cases of FHH, NSHPT, and ADH, and over 50 mutations have been reported (33). The mutations can be nonsense, insertion, missense, deletion, and splice-site mutations found throughout the entire CaR gene (33-36) (CaR Database at website http://www.casrdb.mcgill.ca/).

4.1.1. Familial hypocalciuric hypercalcemia (MIM # 145980 (37)

Familial hypocalciuric hypercalcemia is an autosomal dominant disease. Alterations in the amino acid sequence of the CaR can change its sensitivity to Ca2+o (figure 2). In 1972 Foley et al. were the first to characterize in detail a syndrome later defined as familial hypocalciuric hypercalcemia (FHH) (38). The major FHH disease locus was initially mapped by linkage analysis to the long arm of chromosome 3 (band q21-24) using the hypercalcemic phenotype in four large FHH families (39). In 1993 Pollak et al. identified three different missense mutations in the CaR gene in the affected members of three unrelated families (40). Since the discovery of the first mutation, many additional mutations have been found (33,41). Different mutations may have distinct phenotypes. A single mutant allele can exert a dominant negative effect on the remaining normal CaR allelle, reflecting the specific properties of the mutant receptor protein that is produced. The normal CaR has been shown to homodimerize, and the heterodimerization of a nonfunctional mutant protein with the wild-type CaR may partially inactivate the heterodimeric receptor complex (7,42). Mutations that just inactivate the CaR gene and do not form heterodimers (e.g., deletions) may simply reduce the number of normally functioning CaR expressed on the cell surface (haploinsufficiency). FHH is, however, not always linked to chromosome 3q. In two families with clinical FHH, the mutation has been shown to be linked to the long and short arms of chromosome 19 (band 19p13.3: Oklahoma variant), respectively (43,44).

4.1.2. Neonatal Severe Primary Hyperparathyroidism (MIM 239200 (37))

NSHPT is an autosomal recessive disease in its most severe form. In some of these cases, it is caused by the presence of mutations in both alleles of the CaR gene (e.g., homozygous FHH). Pollak *et al.* reported that of 11 FHH families mapped to chromosome 3q, consanguineous FHH unions produced four children with NSHPT (45). In addition, a single case of NSHPT has been reported that is associated with two different mutations—one a mutation in exon 7 from the mother and the other a mutation in exon 4 from the father (46). NSHPT has also been reported to arise from a mutation in only one allele of the CaR gene, as described later.

4.1.3. Autosomal Dominant Hypoparathyroidism (MIM#601298 (37))

ADH is an uncommon autosomal dominant disease, although in index cases it may represent a sizeable proportion of cases of idiopathic hypoparathyroidism (47). It is caused by the presence of an activating mutation of the CaR gene, which resets the set-point of Ca^{2+}_o-regulated PTH secretion and renal reabsorption of calcium to the left (figure 2). In 1994 Finegold *et al.* showed that the disease is linked to a locus on chromosome 3 q13—the same region that contains the gene for the CaR (48). During the same year, a heterozygous missense mutation, E127A, was identified as a cause of ADH in an unrelated family (48). At least 24 activating mutations in the ECD and TMD of the CaR have by now been reported (33).

4.2. Clinical aspects of FHH, NSHPT, and ADH

FHH is a lifelong, generally asymptomatic, state of PTH-dependent hypercalcemia with an inappropriately low rate of urinary excretion of calcium. Because of the benign nature of the condition, often a diagnosis of FHH is not made until a routine serum calcium determination is serendipitously found to be elevated or until the birth of a child with NSHPT (49). Patients with FHH usually have an inappropriately normal circulating level of PTH despite the hypercalcemia, although in occasional patients or families, the PTH level can be frankly elevated (34,50). Since they are hypercalcemic, however, even a normal PTH value is inappropriately high and reflects a new, elevated set point for Ca^{2+}_o-regulated PTH secretion (51) (figure 2). Because of its benign clinical course and because parathyroid surgery is curative only if a total parathyroidectomy is carried out, patients with FHH should simply be followed expectantly. Recently, a family was described that had hypercalcemia caused by an inactivating FHH mutation; some family members also had hypercalciuria and even renal stone formation (34). Subtotal parathyroidectomy appeared to provide long-term remission of the hypercalcemia and hypercalciuria in this latter family, indicating that in occasional FHH families parathyroid surgery may be appropriate. The most important differential diagnosis in patients with PTH-dependent hypercalcemia is between FHH and primary hyperparathyroidism. This distinction is important because in the latter disorder the treatment of choice is parathyroidectomy in a substantial proportion of patients.

NSHPT presents within the first 6 months of life with symptomatic, PTH-dependent hypercalcemia and the bony changes characteristic of hyperparathyroidism (see below). Clinically, infants with NSHPT present with failure to thrive and may exhibit poor feeding, hypotonia, polyuria, and dehydration. Biochemically they have hypercalcemia, hyperparathyroidism, and relative hypocalcuria (52). In patients with severe hypercalcemia caused by homozygous or heterozygous inactivating CaR mutations, NSHPT can be a devastating neurodevelopmental disorder and may be fatal if not treated surgically (52). Infants with the most severe forms of NSHPT develop rib-cage deformities, skeletal undermineralization, fractures, and rachitic changes (53,54). Within the past decade and with the recent availability of genetic testing for the presence of CaR mutations, it has become apparent, however, that there is a broader clinical spectrum, with some infants having milder hyperparathyroidism. This latter condition is more properly called neonatal hyperparathyroidism (NHPT). Following intensive medical treatment of their hypercalcemia, patients with NHPT revert to a state resembling FHH and have been shown to harbor heterozygous inactivating mutations or to be homozygous for very mild FHH mutations. Indeed, in some cases, patients who would have been expected to have had NHPT or NSHPT have survived with the condition undetected into adulthood, when they were serendipitously found to have PTH-dependent hypercalcemia due heterozygous or homozygous FHH mutations (55).

Patients with ADH are for most part clinically healthy. Their condition is generally diagnosed biochemically as a result of their mild to moderate hypocalcemia, with serum PTH levels in the lower half of the normal range (48). They may also exhibit relative hypercalciuria (normal or even frankly elevated levels of urinary excretion of calcium despite their hypocalcemia). Some studies have found their rates of urinary excretion of calcium to be higher than those of patients with typical hypoparathyroidism, although other studies have found no differences between the levels of urinary calcium excretion in hypoparathyroidism and those in ADH (56,57). Symptoms of hypocalcemia and seizures can occur, however, with the latter sometimes occurring in younger patients during febrile episodes as a result of intercurrent infections. A treatment regimen of calcium supplements and vitamin D metabolites should be limited to patients with symptomatic hypocalcemia and to have as its goal the elevation of the serum calcium only to a level that ameliorates those symptoms. Urinary calcium excretion should be monitored very assiduously in these cases to reduce the risk of urinary complications such as nephrocalcinosis, renal stone disease, and impairment of renal function (58).

4.3. Autoimmune diseases

Acquired hypoparathyroidism (AH) is a disease with hypocalcemia that results from deficient PTH secretion of unknown cause. It was the first disorder of the CaR that was linked to autoimmunity. Autoantibodies to the parathyroid glands were first reported by Blizzard *et al.* (59). In that study, 38% of 74 patients with autoimmune hypoparathyroidism were found to be positive for autoantibodies to the parathyroid as compared with only 6% of 245 healthy control subjects. The antiparathyroid autoantibodies in patients with sporadic, adult-onset hypoparathyroidism have been reported to be directed at the cell surface of human parathyroid cells and have been shown in some cases to inhibit PTH secretion (60). In a more recent study, 14 of 25 patients with AH were shown to have antibodies directed at the CaR, while a control group of 50 patients with various other autoimmune diseases and 22 normal controls did not have antibodies to CaR (61). The sera from the patients with antibodies to CaR, however, did not cause detedtable activation of the CaR in CaR-transfected HEK293 cells, suggesting that they did not produce hypoparathyroidism via direct stimulation of the receptor. Kifor *et al.* have recently shown that a clinical picture resembling FHH can be caused by autoantibodies directed at the CaR in four patients with other autoimmune conditions (e.g., sprue and Hashimoto's thyroiditis) (62). In this study the patients' sera were found to stimulate PTH secretion, presumably as a result of antibody-mediated inhibition of activation of the CaR by high Ca^{2+}_o. Further studies are needed to determine the incidence of autoimmune FHH in patients with various types of autoimmunity and PTH-dependent hypercalcemia.

4.4. Hyperparathyroidism (HPT) and CaR

In primary hyperparathyroidism (PHPT), the calcium set point of the pathological parathyroid gland(s) is shifted to the right as is observed in patients with FHH. However, it has been shown that the defective Ca^{2+}_o-regulated PTH secretion is not caused by inactivating mutations in the CaR gene (63). Studies have shown that there is a downregulation of both the amount of the CaR protein expressed and its mRNA in hyperplastic parathyroid glands from patients with secondary hyperparathyroidism (SHPT) and in adenomas in PHPT (25,64-67). A Japanese group studied 56 parathyroid glands and found an association of decreased CaR protein with enhanced proliferation of parathyroid cells in SHPT (68). However, Ritter *et al.* recently showed that parathyroid cellular hyperplasia precedes the downregulation of the CaR in a rat model of SHPT (69). This result suggested that the reduction in CaR expression might be related to the parathyroid cellular proliferation, perhaps because a reduction in the level of the CaR facilitates cell division in the parathyroid. The cause-and-effect relationships between the CaR, parathyroid hyperplasia, and the altered set point in PHPT and SHPT, however, is still not fully understood.

4.5. CaR polymorphism

Since the discovery of three apparently benign polymorphisms in the COOH tail of the CaR (70), there has been an ongoing search for a clinical relevance of such polymorphisms. The A986S, G990R, and Q1011E polymorphisms were found to present in 30%, 15%, and 10%, respectively, of more than 100 persons in the United States with no apparent disturbance in calcium homeostasis (71). In 1999 Cole *et al*. showed that the A986S polymorphism was present in 16% of 163 Canadian individuals and that this group of patients exhibited a small increase in total serum calcium concentration within the

normal range (72). The same relationship between this particular polymorphism and serum calcium concentration was again found in a larger study by the same group in 2001 (73). In a cohort of 122 Japanese patients undergoing hemodialysis, an association was found between two polymorphic CaR alleles in codon 990 in the intracellular C-tail and in intron 4 and PTH secretion, suggesting that analyzing CaR polymorphisms in patients on chronic hemodialysis could predict the progression of secondary hyperparathyroidism (74). Half a year earlier, another Japanese group studied the relationship between bone mineral density (BMD) of the radial bone and a CA-repeat in the CaR gene in 472 postmenopausal women. In this cohort, 247 women with the A3 allele (228 base pairs, containing 18 repeats of CA) had a significantly lower adjusted BMD (75), suggesting a role for the CaR in the determination of BMD. A Swedish study examined the relationship of the A986S CaR polymorphism to BMD and found that girls with the S allele had higher calcium levels (76); however, the relationship to BMC was not significant. An association of the CaR polymorphism G990R and that within intron 5 to secretion and/or PTH degradation and to clinical severity in 105 patients with PHPT patients has also been reported (77). No doubt further studies will elucidate the pathophysiologic significance of these polymorphisms in the CaR.

4.6. CaRs role in cancer and HHM

A growing body of literature suggests a role for the CaR in the regulation of several types of cancers. In 1998 Lin *et al*. showed that increasing Ca^{2+}_o levels could prevent apoptosis in AT-3 prostate cancer cells induced by Sindbis virus (78). Not long afterward, Chattopadhyay *et al.* showed that the CaR induces cellular proliferation in U373 human astrocytoma cells (79). A potential role for the CaR has been documented in reversing the proliferative phase of the human colon adenocarcinoma-derived cell line Caco-2 (80,81). PTHrP is the main mediator of humoral hypercalcemia of malignancy caused by the release of cytokines or hormones into the systemic blood by malignant tumors and thereby causing hypercalcemia. Two groups have found that the rat Leydig H-500 cancer cell line expresses the CaR on the cell membrane and that increases in Ca^{2+}_o, as well as in other polycationic CaR agonists, stimulate PTHrP secretion (82,83). These results with H-500 cells suggest a role for the CaR in humoral hypercalcemia of malignancy as a mediator in a malignant positive feedforward loop in some tumors. Similar results have also been found with human astrocytes, astrocytomas, meningiomas (84), and breast cancer cells (85).

5. CAR AS A DRUG TARGET

There are two groups of drugs that act on the CaR: calcimimetics and calcilytics. Calcimimetics mimic or potentate the action of Ca^{2+} at the CaR. NPS R-568 is a drug that increases the sensitivity of the CaR to activation by Ca^{2+}. It increases Ca^{2+}_i and inhibits PTH secretion from bovine parathyroid cells *in vitro* at concentrations between 3 and 100 nM (86). NPS R-568 shifts the curve for Ca^{2+}_o-regulated changes in Ca^{2+}_i and PTH release to the left without affecting the maximal or minimal response (figure 2) (87). The drug is metabolized by hepatic cytochrome P_{450}, specifically the CYP2D6 enzyme, and the bioavailability is less than 5%. CYP2D6 is an enzyme that 5% to 7% of the population express as an isoenzyme with reduced enzymatic activity. Another drug, AMG073, has the same allosteric effects on the CaR but has the advantage of not being metabolized by CYP2D6. The major focus of the use of the calcimimetics has been in PHPT and SHPT.

A new group of agents, the calcilytics, have been found to act as CaR antagonists (88). The first compound, NPS 2143, affects the agonist concentration-response curve in a converse manner: there is a shift to the right that again is unaccompanied by changes in either the minimal or maximal response (figure 2) (88).

5.1. Primary hyperparathyroidism (PHPT)

The recommended treatment of PHPT is surgical removal of the pathological parathyroid gland(s) in symptomatic patients or in asymptomatic patients with evidence of end-organ damage. However in some cases surgery is not possible because of the patient's poor health or unwillingness to undergo a surgical procedure. PHPT due to carcinoma is a condition in which surgery is only curative in about two-thirds of the cases. In these three clinical settings, the availability of a drug that controls hypercalcemia would represent a very helpful therapeutic advance. The oral administration of R-568 to postmenopausal women with PHPT caused a rapid and dose-dependent decrease in plasma levels of PTH and Ca^{2+} (89). Paralleling this reduction in the blood Ca^{2+} was an increase in urinary Ca^{2+} excretion. Similar findings have been reported in more extensive studies of patients with PHPT treated with AMG073 (all published in abstract form) (90-92). In a single case report, an 80-year-old man with severe PHPT caused by a parathyroid carcinoma was treated with R-568, which lowered the plasma PTH level and returned the plasma Ca^{2+} level to a near-normal value. This treatment was continued for 2 years and was well tolerated (93). These results suggest that calcimimetics might represent a good alternative to parathyroidectomy for the treatment of PHPT.

5.2. Secondary hyperparathyroidism (SHPT)

The first prospective pilot study examining patients with SHPT caused by renal disease found that basal levels of PTH fell 40% to 60% within 1 hour after their receiving a single oral dose of 40 to 80 mg of NPS R-568. This was accompanied by lowering of blood Ca^{2+}, which remained within the lower range of normal (1.1 mM) (94). A randomized, double-blind, placebo-controlled, multicenter study examined the safety and efficacy of repeated oral doses of 100 mg of NPS R-568 once a day for 24 days in patients with SHPT and end-stage renal disease. The trial showed that, in all patients in the treated group, the PTH level fell immediately, reached a nadir by 2 hours, and returned to 80% of the baseline level by 24 hours. In the control group, PTH levels remained unchanged (95). The main adverse effect observed was hypocalcemia, and with an oral dose of 100 mg of NPS R-568 daily, almost half of the treated patients developed hypocalcemia and did not complete the trial. Five withdrew after experiencing

symptoms consistent with hypocalcemia, including paresthesias and muscle weakness. Three patients completed the protocol after the dose was reduced to 50 mg daily. Gastrointestinal symptoms were more common in subjects receiving NPS R-568 than in the placebo group and included self-limited episodes of anorexia, nausea, or abdominal pain, although none of the participants withdrew due to these gastrointestinal symptoms. The efficacy and safety of AMG073 have been evaluated in several clinical trials (all published in abstract form) (96-98), and its biochemical effects to date are similar to those of NPS R-568 (99). The use of calcimimetics in the treatment of SHPT appears to be very promising, and it is likely that a gradual titration of the dose will provide the optimal means of lowering the serum PTH and blood Ca^{2+} level, especially when patients present with hypercalcemia, either occurring spontaneously or following high-dose vitamin D therapy.

5.3. Osteoporosis

PTH is an effective bone anabolic agent but must be administrated parenterally (100). Orally administered calcilytic compounds might provide an alternative approach to systemic administration of PTH by increasing the circulating level of endogenous PTH. Daily oral administration of the calcilytic NPS 2143 for 5 weeks increased circulating levels of PTH and increased bone turnover in an osteopenic, ovariectomized rat model of osteopenia (101). The compound caused an acute increase in serum PTH levels, sustained for at least 8 hours (figure 2). NPS 2143 given with 17ß-estradiol stimulated new bone formation in the proximal tibial metaphysis and increased bone mass in the distal femur. These results may provide the basis for a novel class of drug in the treatment of osteoporosis.

6. SUMMARY AND PERSPECTIVE

The calcium-sensing receptor is pivotal for maintaining serum calcium homeostasis. There is also an emerging body of knowledge on the function of the CaR in other tissues. The CaR has been shown to regulate cell-cycle events and growth-factor secretion. The importance of the receptor is illustrated by the existence of diseases resulting from mutations with either loss-of-function (i.e., familial hypocalciuric hypercalcemia in the heterozygous state and neonatal severe hyperparathyroidism in the homozygous state) or gain-of-function (e.g., autosomal dominant hypoparathyroidism). Recent reports have shown that the CaR is also a target for autoantibodies, causing acquired, PTH-dependent hypercalcemia. Trials of new drugs targeting the CaR have been very promising, particularly for the treatment of hyperparathyroidism with calcimimetics. These compounds will most likely be available in the clinic in the near future.

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