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Review

Extracellular Calcium Sensing Receptor: An Overview of Physiology, Pathophysiology and Clinical Perspectives

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Abstract. *The identification and cloning of the extracellular calcium sensing receptor (CaR) has provided a new conceptual framework in which we can better understand the interactions between extracellular calcium and the many cell types which express the CaR. The role of the CaR in regulating extracellular calcium ion homeostasis has been well established, as has its role in genetically determined disorders such as Familial Hypocalciuric Hypercalcaemia (FHH). This recently acquired knowledge has incited the discovery of new compounds which function as positive allosteric modulators of the CaR (named calcimimetics) and which are under clinical investigation for potential use in primary and secondary hyperparathyroidism. Research into the properties of the CaR produced an overwhelming influx of data but key questions have remained unanswered. We summarize the currently available information about the function of the CaR, underlining the significant progress which has been made in deciphering its role in pathological disorders and in drug development.*

The calcium receptor (CaR), whose presence in parathyroid cells had been speculated on for several years before its first cloning in 1993, is a receptor expressed in a vast array of cells which demonstrates the ability to recognize changes in the extracellular Ca^{2+} ($[Ca^{2+}]_o$) and respond by generating intracellular Ca^{2+} ($[Ca^{2+}]_i$) increases. The CaR belongs to family C of the G-protein coupled receptors (GPCRs), whose members (metabotropic glutamate receptors (mGluRs) 1-8, vomeronasal receptors (VRs), mammalian taste receptors, fish odorant receptors and γ-aminobutyric acid type B receptors ($GABA_BRs$)) share the common topology of an enormous claw-like extracellular NH_2-terminus (1,2). GPCRs are receptors which interact with G-proteins and are characterized by the presence of a core consisting of 7 transmembrane α-helices, which form 3 intra- and 3 extra-cellular loops. Family C receptors, including CaRs, are believed to have evolved from the fusion of bacterial periplasmic binding proteins (PBPs) and nutrient transporting proteins (2).

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CaR: structure-function relationships

The human CaR gene (Figure 1) lies within the long arm of chromosome 3 and consists of 7 exons (3,4). CaRs can be either located intracellularly, as newly synthesized, non-glycosylated protein molecules, or as immature, non-fully processed receptors, or expressed on the plasma membrane, predominantly as dimers. In parathyroid cells the CaR resides within caveolae, which are defined as specialized, flask-shaped plasma membrane microdomains with a high concentration of signal transducing molecules and proteins such as caveolin 1 or the scaffolding protein filamin.

The CaR's extracellular domain (ECD) consists of ~612 amino acids, among which are 11 Asn residues which serve as N-glycosylation sites (Table I). At least 3 Asn residues must be glycosylated in order to ensure CaR expression on the plasma membrane (1,5). The enormous NH_2-terminus consists of a bilobed construction which resembles a Venus Flytrap and is thus called the VFT domain, and a cysteine-rich domain. The VFT lobes (I and II) are connected by 3 strands, forming a hinge, which enables the VFT to adopt a closed configuration post to the binding of the CaR's primary agonist, Ca^{2+}. The VFT domain's conformational change causes a subsequent rotation of each VFT of the dimer relative to the other around an axis perpendicular to the dimer interface (5-8).

Lobe I of the VFT domain contains 4 loops of undefined structure. Loop I is probably required for proper protein folding and for the formation of the VFT and forms part of

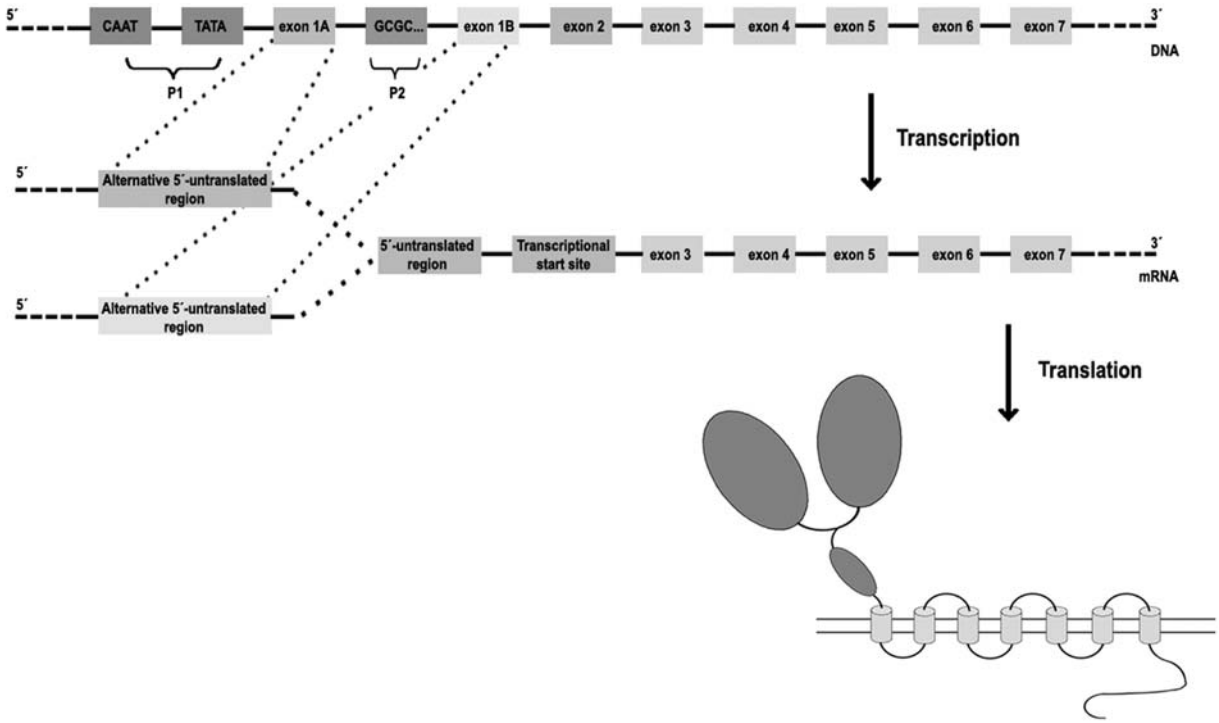

Figure 1. *Diagram depicting human CaR gene transcription and mRNA translation. Exons 1A and 1B code for alternative 5'-untranslated regions, which are spliced post-transcriptionally to the 5'-untranslated region coded for by exon 2. Exons 2-6 code for the gigantic CaR extracellular NH_2-terminus and only exon 7 codes for the receptor's transmembrane domain and intracellular COOH-terminus. P1 and P2 are promoters situated upstream of exons 1A and 1B, respectively. Each promoter's transcription is subjected to tissue-dependent regulation. Vitamin D-response elements (VDREs) reside within the CaR gene promoters and account for the basis of the CaR up-regulation by vitamin D.*

the dimer interface (5,7). Loop II, in addition to its essential role in protein folding and processing, contains residues Cys^{129} and Cys^{131}, which interact with their homologue residues in the other CaR of the dimer, resulting in the formation of intermolecular disulfide bonds, which account for the covalent basis of CaR dimerization. Furthermore, loop II is thought to impose structural constraints upon the dimer, which limit its ability to adopt the closed active configuration after Ca^{2+} binding (7-10). Loop III is of no functional significance, whereas the intramolecular disulfide bond between Cys^{437} and Cys^{449} within loop IV is critical for protein folding and VFT formation (7). The VFT domain contains a plethora of Cys residues, between which additional intramolecular disulfide bonds are believed to be formed, also contributing to protein folding (11,12).

The cysteine-rich domain is a short (84 aa) sequence enriched with 9 highly conserved Cys residues and plays a pivotal role in the transmission of the VFT rotation signal to the receptor's transmembrane domain (TMD) (5,11,13). Signal transmission through the Cys-rich domain is probably achieved as a result of non-covalent interactions between the latter and the VFT domain (12).

CaR dimerization (Table II) is primarily accomplished through the formation of 1 or 2 intermolecular disulfide bonds between Cys^{129} and Cys^{131} of each monomer (1,10,14,15). However, it is considered that intermolecular non-covalent interactions between the monomers' distal 6 transmembrane helices and proximal COOH-termini contribute to the CaR dimerization as well. Interestingly, CaR-mGluR1a heterodimers have been recognized and heterodimerization has been speculated to occur as a result of intermolecular reactions similar to those seen in homodimerization (16).

CaRs can be functional even when they are expressed as monomers. Nevertheless, some of the most interesting aspects of CaR activation are attributed to its constitutive expression as a dimer. Dimerization has been proved to confer functional complementation upon the CaR (*i.e.* only one fully functional ECD and a flawless TMD and COOH-terminus are required for the expression of the dimer's biological activity (5,17)). This remarkable property is partly responsible for the receptor's synergistic model of activation by Ca^{2+} and can ensure that heterodimers consisting of monomers with different inactivating mutations maintain their ability to sense $[Ca2^{+}]_o$ changes (1,18).

Table I. *CaR structure-function relationships.*

	Structural determinants	Function
NH_2-terminal extracellular domain (ECD) ~612 amino acids	11 Asn residues – N-glycosylation sites	CaR expression on the plasma membrane
	Lobe I of the VFT domain	• proper protein folding • formation of the VFT structure • formation of the dimer's interface • constriction of CaR activation
	Cys^{129} and Cys^{131}	Covalent basis of CaR dimerization
	Ca^{2+} binding sites	CaR activation by Ca2+
	Acidic residues acting as binding sites	CaR activation by • divalent and trivalent cations (Mg^{2+}, Gd^{3+}) • cationic compounds (neomycin) • organic polycationic compounds (spermine, polyamine, amyloid-β-peptide, poly-L-arginine)
	L-amino acid binding sites	CaR allosteric activation by L-amino acids or enhancement of CaR's affinity for Ca^{2+}
	Cysteine-rich domain	Transmission of the "Ca^{2+} binding" signal to the TMD
Transmembrane domain (TMD) ~250 amino acids	Transmembrane helices	• Protein processing • CaR expression on the plasma membrane • CaR stability • Signal transduction along the receptor
	Distal 6 helices	Non-covalent basis of CaR dimerization
	Extracellular loops 2 and 3	Limitation of CaR activation
	"Calcimimetics" interaction sites	Signal magnification and increase of CaR's affinity for Ca^{2+}
	Acidic residues acting as binding sites	CaR activation by Ca^{2+}, Gd^{3+}, polycationic compounds (only in the presence of calcimimetics)
	PKA phosphorylation sites	Unknown
	PKC phosphorylation sites	Possibly similar to Thr^{888}
COOH-terminal intracellular domain (ICD) ~216 amino acids	Proximal COOH-terminus	Non-covalent basis of CaR dimerization
	His^{879} and Phe^{881}	• CaR expression on the plasma membrane • COOH-terminus interaction with cascades of intracellular signal transduction
	Thr^{888} – PKC phosphorylation site	Inhibition of the COOH-terminus interaction with G-proteins
	PKA phosphorylation sites	Unknown
	PKC phosphorylation sites	Possibly similar to Thr^{888}
	892-end sequence	Limitation of CaR expression
	907-997 sequence	Interaction with filamin
	868-886 sequence	CaR desensitization process
	877-888 sequence	Activation of Ca^{2+} influx

The CaR, whose affinity for Ca^{2+} is relatively low, binds its primary agonist in the VFT domain of the NH_2-terminus (1,5,19,20). Multiple Ca^{2+} binding sites are probably present in the receptor's ECD and there has been speculation that an additional binding site might exist in the TMD. Divalent and trivalent cations (Mg^{2+}, Gd^{3+}), cationic compounds (neomycin) and organic polycationic compounds (spermine, polyamine, amyloid-β-peptide, poly-L-arginine) can also activate the CaR *in vitro* by interacting with acidic residues of the ECD and TMD (3,21,22). In addition, L-amino acids are thought to interact with a binding site formed by residues of both monomers and situated very close to the Ca^{2+} binding site, resulting in the CaR's allosteric activation or stereoselective enhancement of the receptor's affinity for its physiological agonists (19,20). Although the CaR binds both Ca^{2+} and L-phenylalanine at similar sites within the ECD, it is unique in its ability to respond to these different agonists by generating different types of $[Ca^{2+}]_i$ oscillations, each of

Table II. *Important features of the CaR.*

Dimerization	-A result of 1 or 2 intermolecular disulfide bonds and intermolecular non-covalent interactions. -It confers functional complementation upon the CaR.
Agonist binding	-Multiple Ca^{2+} binding sites in the VFT domain and at least one Ca^{2+} binding site in the TMD -Polycationic compounds interact with acidic residues within the receptor's ECD and TMD -L-amino acids interaction site within the receptor's ECD
Ability to recognize distinct agonists	-CaR responds to L-amino acids binding with low frequency transient $[Ca^{2+}]_i$ oscillations -CaR responds to Ca^{2+} binding with higher frequency sinusoidal $[Ca^{2+}]_i$ oscillations
Synergistic model of activation	-Hill coefficient ~3, despite the receptor's low affinity for Ca^{2+} -The result of • the existence of multiple Ca^{2+} binding sites • G-protein synergistic activation • the CaR homodimerization -CaR's ability to sense minimal changes in the $[Ca^{2+}]_o$
PKC phosphorylation	-Thr^{888} phosphorylation by PKC inhibits COOH-terminus interaction with G-proteins -Basal PKC activity produces $[Ca^{2+}]_i$ oscillation motifs instead of permanent $[Ca^{2+}]_i$ increases -Generation of distinct responses to different agonists and protection from increased $[Ca^{2+}]_i$ cytotoxity are attained

which accounts for a distinct intracellular signal. In fact, CaR activation by Ca^{2+} causes high frequency sinusoidal $[Ca^{2+}]_i$ oscillations, whereas L-phenylalanine interaction with the CaR results in lower frequency transient $[Ca^{2+}]_i$ oscillations (23).

The CaR's TMD is necessary for the protein's processing, expression on the plasma membrane and stability, and plays a pivotal role in signal transmission along the receptor (24). The TMD receives the VFT rotation signal and alters the 7 α-helices' disposition in turn, passing on the signal to the COOH-terminus or leading to direct interaction of the 3 intracellular loops with G-proteins (25,26). Extracellular loops 2 and 3 contain four highly conserved acidic residues, which have been speculated to impose conformational limitations upon the TMD (25,27). In the presence of subthreshold $[Ca^{2+}]_o$, the so called "calcimimetics" (NPS R-568, NPS R-467) interact allosterically with the TMD, resulting in signal magnification and a subsequent increase in the CaR's affinity for Ca^{2+} (20, 21, 25, 28, 29). Besides interacting with calcimimetics, the CaR TMD is probably able to bind Ca^{2+}, Gd^{3+} and polycationic compounds (22,25).

The CaR COOH-terminus consists of 216 amino acids, but only a very short sequence (874-888) of the intracellular domain's (ICD) proximal region is required for the receptor's biological activity (1,24,30). This sequence contains residues His^{879} and Phe^{881}, which have been recognized as critical for the CaR expression on the plasma membrane and the COOH-terminus interaction with intracellular cascades of signal transduction. In addition, it bears sites responsible for the activation of Ca^{2+} influx as well as residues which participate in CaR's desensitization process (31,32). However, the remainder of the COOH-terminus should not be considered as lacking functional significance, for it contains a site which interacts with filamin, and residues without which the CaR would be overexpressed on the cell surface, having a major impact on Ca^{2+} homeostasis (24,33-35).

Thr^{888} is a protein kinase C (PKC) phosphorylation site within the CaR ICD (one should take into consideration the fact that the CaR intracellular loops and COOH tail contain 2 protein kinase A (PKA) phosphorylation sites of unknown physiological relevance and a total of 5 PKC phosphorylation sites) (1,21,31). Thr^{888} phosphorylation by PKC inhibits the COOH-terminus interaction with G-proteins and the subsequent mobilization of $[Ca^{2+}]_i$ stores (1,31). Thus, it is believed that basal PKC activity is responsible for the CaR's ability to respond to activation with $[Ca^{2+}]_i$ oscillation motifs rather than permanent $[Ca^{2+}]_i$ increases. This type of response is both a mechanism of intracellular signalling and a means of protection from the increased $[Ca^{2+}]_i$ cytotoxic effects (36).

The CaR owes its non-redundant role in Ca^{2+} homeostasis to its ability to sense very small changes of the $[Ca^{2+}]_o$, which is attributable to a highly synergistic model of receptor activation (Hill coefficient ~3-4) (1,21,32). This property, which is unique to the CaR and compensates for the low affinity Ca^{2+} binding by the VFT domain, has been suggested to be a consequence of CaR homodimerization or to result from the existence of multiple Ca^{2+} binding sites or to be the outcome of synergistic G-protein activation (18,22,32).

CaR expression in tissues involved in systemic calcium homeostasis

As has been extensively demonstrated, CaR is a widely distributed receptor (37,39-41), but its presence in tissues which participate in Ca^{2+} homeostasis makes further elucidation of its exact role compelling.

Parathyroid cells: CaR is present in parathyroid cells (37,38) and regulates the secretion of parathyroid hormone (PTH) (42), the proliferation of parathyroid cells (43,47), the transcription of PTH mRNA (42) through a variety of secondary messengers such as phospholipase C (PLC), phospholipase A_2 (PLA_2), phospholipase D (PLD), the mitogen-activated protein kinase (MAPK) pathway and inhibition of cyclic AMP (cAMP) (46,47,49,50).

C cells: The discovery of the presence of CaR in C cells (41) was followed by the clarification of the pathway leading to the secretion of calcitonin (CT), a hormone of secondary importance in systemic Ca^{2+} homeostasis. (51)

Kidney cells: CaR transcripts have been found in the renal tubules of rats, using reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry (40,55), and some of its possible functions have already been described: kidney homeostasis of Ca^{2+} is co-regulated by CaR (57,59); CaR activation specifically regulates PTH-suppressible phosphate absorption (56); CaR participates in the regulation of salt and water excretion (58); CaR affects $1,25(OH)D_3$ production in the cells of the proximal duct (75); CaR is responsible for the renal tubular damage caused by aminoglycosides (44); CaR is expressed in acid-transferring cells (55); CaR could play an important role in hypercalcaemia-induced polyuria (73), possibly through an increase in ET-1 production (71).

Intestine: Although CaR mRNA and the protein are expressed in several types of cells of the small and large intestine (53), its exact role is not clear. However, the fact that it could have an influence on cancer cells in the large intestine makes further investigation vital (60).

Placenta: Syncytiotrophoblasts and cytotrophoblasts express CaR (72) and their responses to elevations of $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ and their participation in the secretion of parathyroid hormone related peptide (PTHrP) (48), suggest the presence of intracellular secondary messengers similar to those of the parathyroid cells.

Bone cells

Osteoblasts: CaR is present in osteoblasts (54). It is well known that increased levels of Ca^{2+} induce proliferation and chemotaxis in osteoblasts, and a number of intracellular secondary messengers have been identified; activation of PLC (isoforms β, γ), which correlates CaR to the nucleus and to mitosis (45), expression of a Ca^{2+}-dependent Na^+ channel (61), regulation of the activity of a Ca^{2+}-activated K^+ channel (62), activation of the JNK pathway and up-regulation of several mitogenic genes (74) and its expression in stromal cells (63) which may secrete paracrine factors, are ways through which $[Ca^{2+}]_o$ may affect osteoblasts. Finally, an extracellular cation-sensing receptor with distinct cation specificity has been discovered, possibly explaining the response of osteoblasts to strontium (76).

Osteoclasts: Osteoclasts and their precursors express CaR and respond to Ca^{2+} agonists or elevated Ca^{2+} (64), but the intracellular pathway involved in transmitting these messages remains mostly unknown (65) and other calcium sensing pathways may play a more active role (70, 77).

Osteocytes: Osteocytes are osteoblasts which are no longer participating in bone formation and are encased within the substance of the bone. They too respond to Ca^{2+} agonists and antagonists (elevation of $[Ca^{2+}]_i$) (78), but it is not known whether CaR plays an important role in these processes. In fact, there are suggestions of a calcium sensing mechanism similar to that seen in osteoclasts.

Chondrocytes: The activation of CaR in the growth plate accelerates longitudinal bone growth by stimulating growth plate chondrogenesis (66). Increase of Ca^{2+} in the microenvironment of osteoblasts results in their differentiation, suppresses aggrecan, type II and type X collagen and alkaline phosphatase mRNA levels (67, 69), while concentrations of other molecules such as osteopontin, osteocalcin and osteonectin are increased (68). The role of chondrocytes in bone formation, especially during fetal development, is critical, so it is important that further research be conducted to discover whether Ca^{2+} agonists and antagonists have significant effects on the growth plate and especially the developing embryo.

CaR expression in tissues uninvolved in systemic calcium homeostasis

Although the brain is not directly involved in controlling the systemic level of $[Ca^{2+}]_o$, sensing of local changes in $[Ca^{2+}]_o$ is an important aspect of brain cellular physiology. The presence of CaR in numerous regions of the brain, such as the subfornical organ, olfactory bulbs, hippocampus, hypothalamus, corpus striatum, cerebellum and the ependymal zones of the cerebral cortex (39,79-81) implies that it has a role in local Ca^{2+} homeostasis and in other cellular processes. CaR-expressing brain cell populations include neurons, oligodendrocytes, astrocytes and microglia (82-86).

In addition, expression of CaR has been demonstrated in rodent fibroblasts and ovarian surface epithelial cells, suggesting the possibility that extracellular calcium levels might influence the rate of cell proliferation (87-88). CaR

is required to mediate calcium signaling involved in human keratinocyte differentiation (89-93) and modulates local calcium homeostasis in human lens epithelial cells (94). Cardiac tissue is known to be very sensitive to calcium homeostasis and recent CaR detection in rodent cardiac monocytes may imply an important role in the modulation of cardiac function under both normal and abnormal conditions (95). The presence of CaR in the ductal epithelial cells of the normal human breast has been demonstrated (96), mice mammary glands express high CaR levels during lactation, adjusting PTHrP and calcium secretion in response to CaR signalling (97). Moreover, CaR is involved in a mechanism which regulates adrenocorticotropic hormone (ACTH) secretion and the presence of an increased concentration of CaR may be of significance in the behaviour of human pituitary adenomas (98-99). A recent study revealed the presence of CaR in melanotrope cells of the South African clawed toad *Xenopus Laevis* (100). The receptor has also been localized in human gastrin-secreting G-cells and pancreatic β-cells (101). CaR expression throughout the gastrointestinal tract, including colon mucosa, suggests multiple potential roles for the receptor in gastrointestinal biology, such as epithelial cell differentiation and regulation of fluid transport (102-104).

Red blood cell precursors, megakaryocytes, blood platelets, monocytes and macrophages express relatively high CaR levels, while white cell precursors express relatively low levels of the receptor (105). It has been suggested that the presence of CaR on monocytes and macrophages may imply a role for it in chemotactic responses (106). Hepatic CaR expression may be important for a variety of liver functions such as bile and liprotein secretion, prevention of cholestasis, resistance to toxicity and regeneration (107).

Study of the CaR in non-mammals suggests that CaR had diverse functions earlier in evolution. It has been shown that, in fish, CaRs provide internal signals for sensing alterations in environmental water salinity (108). This suggests the possibility that the presence of CaR in some of the above human tissues may be nothing more than vestigial, especially at sites where expression is very low (109).

Diseases caused by abnormalities in the human CaR

In addition to the clarification of the normal mechanism of calcium ion homeostasis, the cloning of the CaR also contributed to the understanding of the pathophysiology of the receptor by facilitating the recognition of conditions in which the response of the CaR to Ca^{2+} is altered (110). Such conditions as Familial Hypocalciuric Hypercalcaemia (FHH), Neonatal Severe Hyperparathyroidism (NSHPT) and Autosomal Dominant Hypocalcaemia (ADH) are a consequence of structural or functional abnormalities of the CaR.

Familial Hypocalciuric Hypercalcaemia (FHH) is an autosomal dominant disorder which is characterized by mild, lifelong, asymptomatic hypercalcaemia. The hypercalcaemia is evident from the first week of life and may range anywhere from a borderline increase in the level of ionized calcium to marked hypercalcaemia (total serum calcium up to 3.5 mmol/L). Borderline hypermagnesaemia (0.95-1.1 mmol/L) is present in up to 50% of cases and serum phosphate values are normal or slightly reduced. Renal calcium excretion is at the lower range of normal or below normal with 75% of patients demonstrating 24h renal calcium excretion of less than 2.5 mmol and 95% less than 5.0 mmol (111,112). These low levels of renal calcium excretion in the face of elevated serum calcium levels contrast sharply with the high levels of excretion seen in patients with primary hyperparathyroidism.

The management of FHH is conservative. Surgical intervention, such us total parathyroidectomy, will not cure the hypercalcaemia and, since the disorder runs a benign course, surgery is not indicated and patients should not be put on a low calcium diet (112).

Aetiology: Most of the families with FHH have heterozygous mutations in the CaR gene which are inherited with an autosomal dominant pattern and almost 100% penetration. Many of them are point mutations which tend to cluster in the extracellular region of the receptor. *In vitro* studies of mutant CaRs have demonstrated either relative or total absence of receptor activity. Using linkage analysis, FHH was initially associated with genetic locus 3q21-24 and, for the majority of affected families, the abnormality is located on chromosome 3 (112-114). The remaining affected families, who do not have a mutation in the CaR coding location, may have an abnormality in a non-coding region or a mutation in one of the genetic loci connected with FHH and situated on chromosome 19 (some families have been shown to have abnormalities at locations 19p13.3 and 19q13) (115). This variety of causative abnormalities currently prevents the use of a genetic test for the diagnosis of the condition.

Differential diagnosis: A careful family history should be taken from any patient found to have asymptomatic hypercalcaemia with specific reference to abnormal blood test results and any history of parathyroid surgery amongst family members. To exclude the diagnosis of FHH, urinary excretion rates of calcium and creatinine are measured. In FHH the excretion of calcium is typically low and almost always less than 100mg/24h (variation: 30 to 80), while the clearance ratio of creatinine/calcium is usually less than 0.01 (111,117). The distinction of FHH from primary hyperparathyroidism is important in order to avoid unnecessary hyperparathyroidectomy.

Neonatal Severe Hyperparathyroidism (NSPHT) is a manifestation of homozygous CaR loss-of-function

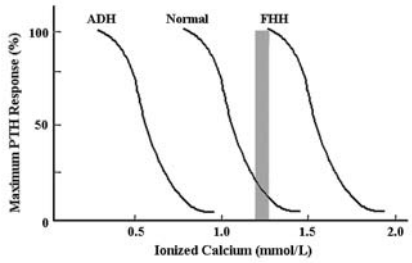

Figure 2. *Qualitative depiction of the steep inverse sigmoid relationship between plasma Ca^{2+} concentration and PTH secretion in normal individuals, in ADH and in FHH. The set-point is the plasma Ca^{2+} at which half the maximal PTH response occurred. The normal range of ionized calcium is 1.1-1.3 mmol/l (grey area). Changes in set-point or "calciostat" produce major changes in PTH release at any given Ca^{2+} level. In FHH the loss of function mutations in the CaR gene lead to generalized resistance of varying degrees to extracellular calcium, hence the set-point is elevated. The opposite phenotype, ADH is associated with gain of function mutations in the CaR gene leading to hyper-responsiveness to extracellular calcium. The PTH release is reduced in inappropriately low levels of Ca^{2+} and the set-point is resetting downwards producing stable hypocalcaemia.*

mutations (115) and is a rare disorder characterized by extreme hyperparathyroidism and its associated bony changes occurring in infancy. In such cases the first few days of life are marked by growth retardation, hypotonia, constipation and respiratory distress. Bony changes include undermineralisation, sub-periosteal erosion, metaphyseal destruction of the long bones and ribs, rib-cage deformity and craniotabes. There may be severe hypercalcaemia with total serum calcium levels oscillating between 5 and 7 mmol/L, however, some infants may present with lower values. The circulating PTH is elevated (often to five times the normal level), serum phosphate is normal or reduced, serum magnesium normal or increased, bony alkaline phosphatase elevated and urinary calcium excretion normal or reduced (112). Treatment of infants with severe NSHPT requires surgical ablation of all parathyroid tissue in the first or second month of life because delay results in increasingly severe bony abnormalities (including further deformity of the bones of the thorax) and eventually in death from emaciation and respiratory distress.

The clinical syndrome of NSHPT in a non-homozygous form has been noted sporadically in members of FHH-affected families, caused by a compound heterozygous mechanism whereby a different inactivating mutation was inherited from each parent, or as a result of *de novo* heterozygous mutations of the CaR gene exerting a 'dominant' negative effect (116).

Pathophysiology: In FHH and NSHPT, there is alteration of function of both the parathyroid glands and the kidneys as a result of altered sensitivity to serum calcium levels. This is the result of the partial or total lack, respectively, of the normal alleles controlling the development of the CaR receptor (117). As a consequence of altered receptor sensitivity to serum calcium levels, normal and elevated levels of serum calcium are perceived as low by the parathyroid glands which continue to secrete PTH, thus further raising the level. In other words the calcium set-point, *i.e.* the calcium level necessary to trigger PTH secretion, is set higher (Figure 2) (118). Similarly the kidneys see normal and elevated levels of serum calcium as low and fail to excrete appropriate amounts in the urine, leading to hypocalciuria and continued maintenance of high serum calcium levels. This renal insensitivity to elevated serum calcium levels explains the failure of total parathyroidectomy to offer significant clinical benefit in FHH. The different 'doses' of mutant CaR gene involved are responsible, on the one hand, for the relative mildness of the clinical features of FHH and, on the other hand, for the seriousness of the presentation of NSHPT (119).

Autosomal Dominant Hypocalcaemia (ADH), like FHH and NSHPT, results from genetically-determined mutation of the CaR, but, in contrast, in this case the CaR is abnormally sensitive to serum calcium levels, causing failure of PTH secretion even in the presence of low levels of serum calcium. In other words, the calcium set-point is set lower (120). In addition, the kidney also sees even low levels of serum calcium as elevated and continues to release calcium into the urine, resulting in hypercaliuria and perpetuating the hypocalcaemia. ADH-affected individuals may have only mild hypocalcaemia, but seizures can occur, especially in younger patients and especially during febrile episodes. There is a tendency towards hyperphosphataemia, although serum phosphate may be in the normal range. Serum magnesium levels may be at the lower end of the normal range or subnormal in untreated individuals, illustrating the fact that the CaR can also act as a magnesium sensor. Serum intact PTH levels are usually within the normal range. Urinary calcium excretion is higher than in typical hypoparathyroid patients, despite the fact that PTH levels in hypoparathyroid patients are lower than in ADH-affected individuals (121). Because of the marked hypercalciuria, there is a risk of renal complications such as nephrocalcinosis, renal stones and impaired renal function. Renal tubular cells, excessively inhibited from reabsorbing calcium by the overactive calcium receptors, sustain the hypercalciuria (112). Thus, caution should be used to avoid overtreatment of ADH with vitamin D (and its metabolites) or calcium supplements.

In addition to the inherited disorders and *de novo* mutations of the CaR known to alter calcium homeostasis, other mechanisms may be playing a part. Deranged extracellular calcium sensing may arise from antibodies

probably acting on an epitope in the extracellular region of the receptor. In some families affected by disordered calcium homeostasis, there may be circulating anti-CaR antibodies with *in vivo* blocking activity. In these families the linkage analysis for the detection of mutations was negative and parathyroid glands removed from one individual with autoimmune hypercalcaemia were reported to have no observable cellular infiltrate, suggesting that this type of antibody could have an isolated stimulatory effect on PTH secretion (122). There are also two reported cases of ADH and one of NSHPT which have occurred in patients with Bartter syndrome (123,124).

The above studies have greatly expanded awareness of the role played by the CaR in disease, including both genetically determined disorders (FHH, NSPT and ADH) and acquired autoimmune parathyroid conditions (125).

Activation of the CaR by Ca^{2+} and other agonists

Type I calcimimetics: Extracellular calcium does not constitute the sole activator of the CaR. On the contrary, years of research have led to the conclusion that the CaR constitutes a non selective receptor that responds to a wide variety of divalent and polyvalent cations (Table II) (126). These divalent/polyvalent cations were the first substances that were identified to activate the CaR (besides Ca^{2+}) and were thus named Type I calcimimetics due to their ability to mimic the action of extracellular calcium. In light of the new facts that have emerged, it seems that the CaR plays a key role in divalent mineral ion homeostasis and models have been proposed to explain the interactions, as demonstrated by Hebert *et al.,* who examined the putative role of the CaR in the distal nephron (127).

Investigation of the physiological role of Type I calcimimetics has also provided convincing explanations for the toxicity exhibited by certain polyvalent cation compounds such as aminoglycosides (AGA) and polyamines (44,128,130). Neomycin, tobramycin and gentamicin are notorious for their nephrotoxicity. Most AGA nephrotoxicity occurs in the proximal tubule where the CaR is located on the luminal membrane (129). McLarnon *et al.* convincingly demonstrated that CaR constitutes a target for various AGAs and that their potency correlates with their pH-dependent charge (130).

L-amino acids: As mentioned previously, the CaR belongs to the family of metabotropic glutamate receptors (mGluRs) (a subfamily of GPCRs). The CaR is closely related to mGluR subtypes 1α, 3 and 5 and $GABA_B$ receptor. Conigrave *et al.* demonstrated that the CaR can be allosterically modulated by specific L-amino acids (131,132). Specific amino acid residues in the extracellular domain of the CaR have been identified as having an important role in the detection of L-phenynalanine (19). Further investigation of the L-amino acid sensing properties of the CaR may reveal a role for this receptor in amino acid metabolism.

Type II calcimimetics: Unfortunately, Type I calcimimetics have no clinical significance in the sense that they cannot be used in the treatment of calcium homeostasis disorders due to their poor specificity for the CaR. The need for novel approaches to the treatment of primary and secondary hyperparathyroidism led researchers to the discovery of Type II calcimimetics which lack the polyvalent cation structure of their Type I counterparts. In this case, the term "calcimimetics" is not used literally since these new compounds do not mimic the action of extracellular calcium. Instead they function as positive allosteric modulators and therefore bind to the receptor causing an allosteric modulation, thereby increasing the receptor's sensitivity to Type I calcimimetics (133,137). Type II calcimimetics, therefore, have no effect without the presence of $[Ca^{2+}]_o$. It has been suggested that this action is mediated through their binding to the seventh transmembrane domain of the CaR. Research on related family 3 GPCRs, such as mGluRs and $GABA_B$, have strongly indicated that the site of action for positive allosteric modulators resides in the 7 transmembrane domain (TMD) of the receptor (22,25,152). Specifically, Knoflach *et al.* demonstrated that residues in the third and fifth transmembrane helices of the TMD were critical for selective response to positive allosteric modulators of mGluR1 (135). Recent findings have underscored the importance of the cell surface-proximal portion of the TMD in the action of the Type II calcimimetic NPS R-568 (136). Glu^{837} seems to play an important role in R-568 recognition by the CaR as demonstrated by Hu *et al.* (25).

Clinical trials using NPS R-568: First generation Type II calcimimetics are predominantly phenylalkylamine derivatives. Preclinical data suggested that compound R-568 inhibited the secretion of PTH from parathyroid cells (138). Silverberg *et al.* demonstrated that the administration of R-568 resulted in dose-dependent inhibition of PTH secretion and consequentially a reduction of serum ionized-calcium levels in postmenopausal women with primary hyperparathyroidism (138). Clinical studies were also conducted in patients suffering from secondary hyperparathyroidism (139,140). The first study was open-labelled and included 7 patients (139). The trial was conducted over a 2-day period. The patients were divided into a low and high dose group. Patients receiving 120 mg or 200 mg of NPS R-568 experienced a more than 60% decline in PTH levels after the administration of the drug. In a second study, Goodman *et al.* conducted a randomized double-blind, placebo-controlled trial to evaluate R-568's efficacy in patients suffering from severe secondary hyperparathyroidism (140). The serum PTH levels before

treatment in the study group varied between 303 and 1130 pg/mL. After administration of R-568, serum PTH levels declined in all patients and values continued to decrease with subsequent doses of R-568.

R-568 was also used for the treatment of hypercalcaemia due to a parathyroid carcinoma (141). Conventional treatment (saline hydration, furosemide, pamidronate and calcitonin) proved to be ineffective, so R-568 was added to the regimen and resulted in considerable improvement. After discharge, the patient received R-568 as a monotherapy and continued to do so for at least 2 years with no discernible side-effects. Doses under 600 mg/day failed to reduce serum calcium to acceptable levels, while a dose of 600 mg/day resulted in levels which were satisfactory overall, but tended to fluctuate rather than remain steady.

Despite impressive results achieved by using R-568 in patients with primary and secondary hyperparathyroidism, the development of the drug was eventually discontinued because of a problematic pharmacokinetic profile, including a very low bioavalaibility index and an unsatisfactory half-life (140). The second generation Type II calcimimetic AMG 073 is currently undergoing phase III clinical trials and is producing promising results.

Clinical trials using AMG 073: AMG 073 was introduced to bypass the shortcomings of R-568 treatment. Several studies have been conducted in hemodialysis patients suffering from secondary hyperparathyroidism (142-145,148). Lindberg *et al.* performed a double-blind, placebo-controlled study in 78 hemodialysis patients on conventional vitamin D sterols and calcium-containing or non-calcium-containing phosphate binders (143). Dose titration occurred during the first 12 weeks using daily doses of 10, 20, 30, 40 and 50 mg of AMG 073. During the maintenance period, a 7.5% decline in mean serum phosphorous was observed in patients receiving AMG 073, while the placebo group experienced a 10.9% increase. A 4.7% reduction in mean serum calcium levels occurred in patients treated with AMG 073, while no change was observed in the placebo group. This dose titration study also demonstrated a 11.9% decrease in the calcium x phosphorus product levels in patients given AMG 073 over the maintenance period and while a 10.9% increase was witnessed in the placebo group. High calcium x phosphorus levels are a common problem in the treatment of secondary hyperparathyroidism in patients suffering from chronic renal failure (146). These high levels are associated with the occurrence of metastatic calcifications.

The latest data from the phase III clinical trials currently being conducted support the previous findings and indicate that the major advantage of AMG 073 compared to conventional treatment is the normalization of calcium x phosphorus levels. On the other hand, Quarles *et al.* demonstrated an apparent beneficial effect of AMG-073 on bone metabolism as well (145). They assessed bone mineral density (BMD) in a study group treated with AMG-073 and in a control group. After 1 year of treatment, the study group experienced an increase in total body BMD of 0.5% and femoral neck BMD of 2%, whereas the control group showed BMD decreases of 1-1.6% in both zones. These increases in BMD correlated with parallel decreases in serum concentrations of bone-specific alkaline phosphatase, which is a reliable biomarker of bone formation.

Safety profile and side-effects: Lindberg *et al.*, during their 18-week dose titration trial concluded that daily doses of 50 mg were well-tolerated while side-effects were similar among the AMG 073-treated patients and the placebo group (143). Similar results were found by Quarles *et al.* in another 18-week double-blind randomized dose titration study (144). The only adverse effect occurring with greater frequency in AMG 073-treated patients was nausea. No other significant side-effects have been demonstrated apart from transient episodes of hypocalcaemia during the dose titration phases. In another randomized, double-blind, placebo-controlled study evaluating the clinical use of AMG-073 in patients with primary hyperparathyroidism, 56% of the study group reported adverse effects. The most frequent adverse effect was paresthesiae occurring in 19% of patients (33% in the placebo group) (147). Consequences of overzealous treatment of secondary hyperparathyroidism, such as a dynamic bone disease, have not been identified in patients taking calcimimetics.

Antagonists of the CaR

Calcilytics: In most fields the discovery of receptor antagonists constitutes an easier task than the identification of receptor agonists, but this does not seem to be the case with the CaR. A small compound, NPS 2143 has been studied extensively in recent years and is currently under investigation (29). Recently Calhex 231, a new potent calcilytic compound, has been discovered (149). The site of action of calcilytic compounds has evaded researchers in the past but Petrel *et al.* have shed some light on this question. By creating a model of the CaR based on the crystal structure of bovine rhodopsin, they were able to identify specific residues necessary for the binding of calcilytics to the receptor which are located in TMD 6 and TMD 7 (150). Apparently these identical residues are crucial for the binding of calcimimetics such as R-568. In contrast, efforts to identify an endogenous antagonist have been unsuccessful (29). Antagonism of the CaR in the parathyroid gland elevates the serum levels of PTH. Intravenous infusion of NPS 2143 in healthy rats resulted in a sudden 4- to 5-fold increase in PTH levels, which reached a maximum 15 to 30 minutes after infusion (29). It has been speculated that NPS 2143 probably causes a release of stored PTH in the parathyroid glands and not increased

proteosynthesis of the hormone. The properties of calcilytics could be extrapolated to clinical usage. It has been known for some time that transient elevated levels of PTH or its 1-34 fragment have a paradoxical anabolic effect on bone formation (151) and this approach has been utilized recently in the treatment of osteoporosis (152,153). Despite the fact that the PTH peptide has proven its beneficial effect on bone mineral density and has been accepted as a part of the conventional treatment of osteoporosis, it suffers from serious disadvantages such as the high cost of the drug and the necessity for intravenous administration, which results in a low level of patient acceptance. An orally administered compound such as NPS 2143 or Calhex 231 obviously has comparative advantages.

Conclusion

The identification and cloning of the CaR has enabled researchers to delve deeper into the physiology and the disorders of calcium ion homeostasis and to ascertain its role as one of the key factors in divalent and polyvalent mineral ion homeostasis in general. Despite the success of clarifying the CaR's role in organs participating directly in calcium ion homeostasis, such as the parathyroid glands and the kidneys, details of the extracellular calcium sensing mechanism of bone cells remain sketchy and this is of great significance since the effects of prolonged calcimimetic treatment on bone metabolism will need further investigation. As mentioned earlier, one of the great achievements of CaR research was the discovery of calcimimetic agents which, according to the latest clinical data, will be at the forefront of hyperparathyroidism treatment in the near future. First generation Type II calcimimetics were the first compounds discovered after the cloning of the CaR which promised a novel approach to the treatment of a number of disorders of calcium homeostasis. Preclinical trials proved their ability to suppress PTH secretion and clinical trials demonstrated that phenylalkylamine derivative compounds such as R-568 were safe and could be used to counter primary and secondary hyperparathyroidism. Second generation Type II calcimimetics are still under evaluation but seem to offer a promising solution to the shortcomings of the first generation Type II drugs. Despite the relative success of AMG-073 in recent studies, questions concerning its clinical use still need to be addressed.

Although the role of the CaR is well understood in cells and tissues participating directly in the regulation of calcium levels in the body, many questions remain to be addressed concerning its presence in a wide variety of loci which in turn do not take part, at least directly, in calcium homeostasis. The identification of the CaR in many of these tissues is unequivocally accepted, but at the moment we can only speculate on its putative role in these sites. Does the CaR serve another purpose than the ones already described or should we treat its wide distribution in the human body as a relic of our evolutionary past? Will the use of calcimimetics or calcilytics have any discernible effects on these systems or should we retain our confidence in the long-term safety of these drugs? Either way, further investigation into these issues will be required which will hopefully, in the near future, provide us with convincing answers and explanations of the multidimensional role of the CaR in the human organism.

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References

1 Brown EM and MacLeod RJ: Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev *81*: 239-297, 2001.

2 Felder CB, Graul RC, Lee AY, Merkle HP and Sadee W: The Venus Flytrap of periplasmic binding proteins: an ancient protein module present in multiple drug receptors. AAPS PharmSci *1*: 1-20, 1999

3 Chikatsu N, Fukumoto S, Takeuchi Y *et al*: Cloning and characterization of two promoters for the human calcium-sensing receptor (CaSR) and changes of CaSR expression in parathyroid adenomas. J Biol Chem *275*: 7553-7557, 2000.

4 Canaff L and Hendy GN: Human calcium-sensing receptor gene: Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. J Biol Chem *277*: 30337-30350, 2002.

5 Hu J and Spiegel AM: Naturally occurring mutations of the extracellular Ca^{2+}-sensing receptor: implications for its structure and function. Trends Endocrinol Metab *14*: 282-288, 2003.

6 Goldsmith PK, Fan GF, Ray K *et al*: Expression, purification, and biochemical characterization of the amino-terminal extracellular domain of the human calcium receptor. J Biol Chem *274*: 11303-11309, 1999.

7 Reyes-Cruz G, Hu J, Goldsmith PK, Steinbach PJ and Spiegel AM: Human Ca^{2+} receptor extracellular domain: Analysis of function of lobe I loop deletion mutants. J Biol Chem *276*: 32145-32151, 2001.

8 Jensen AA, Spalding TA, Burstein ES *et al*: Functional importance of the Ala^{116}-Pro^{136} region in the calcium-sensing receptor: Constitutive activity and inverse agonism in a family C G-protein-coupled receptor. J Biol Chem *275*: 29547-29555, 2000.

9 Hu J, Mora S, Colussi G, Proverbio MC *et al*: Autosomal dominant hypocalcemia caused by a novel mutation in the loop 2 region of the human calcium receptor extracellular domain. J Bone Miner Res *17*: 1461-1469, 2002.

10 Ray K, Hauschild BC, Steinbach PJ, Goldsmith PK, Hauache O and Spiegel AM: Identification of the cysteine residues in the amino-terminal extracellular domain of the human Ca^{2+} receptor critical for dimerization: Implications for function of monomeric Ca^{2+} receptor. J Biol Chem *274*: 27642-27650, 1999.

11 Fan GF, Ray K, Zhao XM, Goldsmith PK and Spiegel AM: Mutational analysis of the cysteines in the extracellular domain of the human Ca^{2+} receptor: effects on cell surface expression, dimerization and signal transduction. FEBS Lett *436*: 353-356, 1998.

12 Hu J, Reyes-Cruz G, Goldsmith PK and Spiegel AM: The Venus's-flytrap and cysteine-rich domains of the human calcium receptor are not linked by disulfide bonds. J Biol Chem *276*: 6901-6904, 2001.

13 Hu J, Hauache O and Spiegel AM: Human Ca^{2+} receptor cysteine-rich domain: Analysis of function of mutant and chimeric receptors. J Biol Chem *275*: 16382-16389, 2000.

14 Pace AJ, Gama L and Breitwieser GE: Dimerization of the calcium-sensing receptor occurs within the extracellular domain and is eliminated by Cys → Ser mutations at Cys101 and Cys236. J Biol Chem *274*: 11629-11634, 1999.

15 Zhang Z, Sun S, Quinn SJ, Brown EM and Bai M: The extracellular calcium-sensing receptor dimerizes through multiple types of intermolecular interactions. J Biol Chem *276*: 5316-5322, 2001.

16 Gama L, Wilt SG and Breitwieser GE: Heterodimerization of calcium sensing receptors with metabotropic glutamate receptors in neurons. J Biol Chem *276*: 39053-39059, 2001.

17 Bai M, Triverdi S, Kifor O, Quinn SJ and Brown EM: Intermolecular interactions between dimeric calcium-sensing receptor monomers are important for its normal function. Proc Natl Acad Sci *96*: 2834-2839, 1999.

18 Miedlich S, Gama L and Breitwieser GE: Calcium sensing receptor activation by a calcimimetic suggests a link between cooperativity and intracellular calcium oscillations. J Biol Chem *277*: 49691-49699,2002.

19 Zhang Z, Qiu W, Quinn SJ, Conigrave AD, Brown EM and Bai M: Three adjacent serines in the extracellular domains of the CaR are required for L-amino acid-mediated potentiation of receptor function. J Biol Chem *277*: 33727-33735, 2002.

20 Zhang Z, Jiang Y, Quinn SJ, Krapcho K, Nemeth EF and Bai M: L-Phenylalanine and NPS-467 synergistically potentiate the function of the extracellular calcium-sensing receptor through distinct sites. J Biol Chem *277*: 33736-33741, 2002.

21 Hofer AM and Brown EM: Extracellular calcium sensing and signaling. Nat Rev Mol Cell Biol *4*: 530-538, 2003.

22 Ray K and Northup J: Evidence for distinct cation and calcimimetic compound (NPS 568) recognition domains in the transmembrane regions of the human Ca^{2+} receptor. J Biol Chem *277*: 18908-18913, 2002.

23 Young SH and Rozengurt E: Amino acids and Ca^{2+} stimulate different patterns of Ca^{2+} oscillations through the Ca^{2+}-sensing receptor. Am J Physiol Cell Physiol *282*: C1414-C1422, 2002.

24 Ray K, Fan GF, Goldsmith PK and Spiegel AM: The carboxyl terminus of the human calcium receptor: Requirements for cell-surface expression and signal transduction. J Biol Chem *272*: 31355-31361, 1997.

25 Hu J, Reyes-Cruz G, Chen W, Jacobson KA and Spiegel AM: Identification of acidic residues in the extracellular loops of the seven-transmembrane domain of the human Ca^{2+} receptor critical for response to Ca^{2+} and a positive allosteric modulator. J Biol Chem *277*: 46622-46631, 2002.

26 Hauache OM: Extracellular calcium-sensing receptor: structural and functional features and association with diseases. Braz J Med Biol Res *34*: 577-584, 2001.

27 Ray K, Ghosh SP and Northup JK: The role of cysteines and charged amino acids in extracellular loops of the human Ca^{2+} receptor in cell surface expression and receptor activation processes. Endocrinology (April 29, 2004, ahead of print)

28 Hauache OM, Hu J, Ray K, Xie R, Jacobson KA and Spiegel AM: Effects of a calcimimetic compound and naturally activating mutations on the human Ca^{2+} receptor and on Ca^{2+} receptor/metabotropic glutamate chimeric receptors. Endocrinology *141*: 4156-4163, 2000.

29 Nemeth EF, Delmar EG, Heaton WL *et al*: Calcilytic compounds: Potent and selective Ca^{2+} receptor antagonists that stimulate secretion of parathyroid hormone. J Pharmacol Exp Ther *299*: 323-331, 2001.

30 Chang W, Pratt S, Chen TH, Bourguignon L and Shoback D: Amino acids in the cytoplasmic C terminus of the parathyroid Ca^{2+}-sensing receptor mediate efficient cell-surface expression and phospholipase C activation. J Biol Chem *276*: 44129-44136, 2001.

31 Jiang YF, Zhang Z, Kifor O, Lane CR, Quinn SJ and Bai M: Protein kinase C (PKC) phosphorylation of the $Ca^{2+}{}_o$-sensing receptor (CaR) modulates functional interaction of G proteins with the CaR cytoplasmic tail. J Biol Chem *277*: 50543-50549, 2002.

32 Gama L and Breitwieser GE: A carboxyl-terminal controls the cooperativity for extracellular Ca^{2+} activation of the human calcium sensing receptor: A study with receptor-green fluorescent protein fusions. J Biol Chem *273*: 29712-29718, 1998.

33 Awata H, Huang C, Handlogten ME and Miller RT: Interaction of the calcium-sensing receptor and filamin, a potential scaffolding protein. J Biol Chem *276*: 34871-34879, 2001.

34 Hjälm G, MacLeod RG, Kifor O, Chattopadhyay N and Brown EM: Filamin-A binds to the carboxyl-terminal tail of the calcium-sensing receptor, an interaction that participates in CaR-mediated activation of mitogen-activated protein kinase. J Biol Chem *276*: 34880-34887, 2001.

35 Lienhardt A, Garabédian M, Bai M *et al*: A large homozygous or heterozygous in-frame deletion within the calcium-sensing receptor's carboxylterminal cytoplasmic tail that causes autosomal dominant hypocalcemia. J Clin Endocrinol Metab 85: 1695-1702, 2000.

36 Young SH, Wu SV and Rozengurt E: Ca^{2+}-stimulated Ca^{2+} oscillations produced by the Ca^{2+}-sensing receptor require negative feedback by protein kinase C. J Biol Chem *277*: 46871-46876, 2002.

37 Brown EM, Gamba G, Riccardi D *et al*: Cloning and characterization of an extracellular $Ca(^{2+})$-sensing receptor from bovine parathyroid. Nature *366*: 575-80, 1993.

38 Garrett JE, Capuano IV, Hammerland LG *et al*: Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. J Biol Chem *270*: 12919-12925, 1995.

39 Ruat M, Molliver ME, Snowman AM and Snyder SH: Calcium sensing receptor: molecular cloning in rat and localization to nerve terminals. Proc Natl Acad Sci USA *92*: 3161-3165, 1995.

40 Brown EM, Pollak M and Hebert SC: Sensing of extracellular Ca^{2+} by parathyroid and kidney cells: cloning and characterization of an extracellular $Ca(^{2+})$-sensing receptor. Am J Kidney Dis *25*: 506-513, 1995.

41 Garrett JE, Tamir H, Kifor O *et al*: Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. Endocrinology *136*: 5202-5211, 1995.

42 Toribio RE, Kohn CW, Capen CC and Rosol TJ: Parathyroid hormone (PTH) secretion, PTH mRNA and calcium-sensing receptor mRNA expression in equine parathyroid cells, and effects of interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha on equine parathyroid cell function. J Mol Endocrinol *31*: 609-620, 2003.

43 Roussanne MC, Lieberherr M, Souberbielle JC, Sarfati E, Drueke T and Bourdeau A: Human parathyroid cell proliferation in response to calcium, NPS R-467, calcitriol and phosphate. Eur J Clin Invest *31*: 610-616, 2001.

44 Ward DT, McLarnon SJ and Riccardi D: Aminoglycosides increase intracellular calcium levels and ERK activity in proximal tubular OK cells expressing the extracellular calcium-sensing receptor. J Am Soc Nephrol *13*: 1481-1489, 2002.

45 Godwin SL and Soltoff SP: Calcium-sensing receptor-mediated activation of phospholipase C-gamma1 is downstream of phospholipase C-beta and protein kinase C in MC3T3-E1 osteoblasts. Bone *30*: 559-566, 2002.

46 Dare E, Kifor O, Brown EM and Weber G: Characterization of the phosphatidylinositol-specific phospholipase C isozymes present in the bovine parathyroid and in human kidney HEK293 cells stably transfected with the human parathyroid Ca^{2+}-sensing receptor. J Mol Endocrinol *21*: 7-17, 1998.

47 Tfelt-Hansen J, Chattopadhyay N, Yano S *et al*: Calcium-sensing receptor induces proliferation through p38 mitogen-activated protein kinase and phosphatidylinositol 3-kinase but not extracellularly regulated kinase in a model of humoral hypercalcemia of malignancy. Endocrinology *145*: 1211-1217, 2004.

48 Tfelt-Hansen J, MacLeod RJ, Chattopadhyay N *et al*: Calcium-sensing receptor stimulates PTHrP release by pathways dependent on PKC, p38 MAPK, JNK, and ERK1/2 in H-500 cells. Am J Physiol Endocrinol Metab *285*: E329-337, 2003.

49 Kifor O, MacLeod RJ, Diaz R *et al*: Regulation of MAP kinase by calcium-sensing receptor in bovine parathyroid and CaR-transfected HEK293 cells. Am J Physiol Renal Physiol *280*: F291-302, 2001.

50 Quitterer U, Hoffmann M, Freichel M and Lohse MJ: Paradoxical block of parathormone secretion is mediated by increased activity of G alpha subunits. J Biol Chem *276*: 6763-6769, 2001.

51 McGehee DS, Aldersberg M, Liu KP, Hsuing S, Heath MJ and Tamir H: Mechanism of extracellular Ca^{2+} receptor-stimulated hormone release from sheep thyroid parafollicular cells. J Physiol *502(Pt 1)*: 31-44, 1997.

52 MacLeod RJ, Chattopadhyay N and Brown EM: PTHrP stimulated by the calcium-sensing receptor requires MAP kinase activation. Am J Physiol Endocrinol Metab *284*: E435-442, 2003.

53 Chattopadhyay N, Cheng I, Rogers K *et al*: Identification and localization of extracellular $Ca(^{2+})$-sensing receptor in rat intestine. Am J Physiol *274 (1 Pt 1)*: G122-130, 1998.

54 Yamaguchi T, Kifor O, Chattopadhyay N and Brown EM: Expression of extracellular calcium (Ca^{2+}_o)-sensing receptor in the clonal osteoblast-like cell lines, UMR-106 and SAOS-2. Biochem Biophys Res Commun *243*: 753-757, 1998.

55 Riccardi D, Hall AE, Chattopadhyay N, Xu JZ, Brown EM and Hebert SC: Localization of the extracellular Ca^{2+}/polyvalent cation-sensing protein in rat kidney. Am J Physiol *274(3 Pt 2)*: F611-622, 1998.

56 Ba J, Brown D and Friedman PA: Calcium-sensing receptor regulation of PTH-inhibitable proximal tubule phosphate transport. Am J Physiol Renal Physiol *285*: F1233-1243, 2003.

57 Kos CH, Karaplis AC, Peng JB *et al*: The calcium-sensing receptor is required for normal calcium homeostasis independent of parathyroid hormone. J Clin Invest *111*: 1021-8, 2003.

58 Wang D, Pedraza PL, Abdullah HI, McGiff JC and Ferreri NR: Calcium-sensing receptor-mediated TNF production in medullary thick ascending limb cells. Am J Physiol Renal Physiol *283*: F963-970, 2002.

59 Motoyama HI and Friedman PA: Calcium-sensing receptor regulation of PTH-dependent calcium absorption by mouse cortical ascending limbs. Am J Physiol Renal Physiol *283*: F399-406, 2002.

60 Speer G, Cseh K, Fuszek P *et al*: The role of estrogen receptor, vitamin D receptor and calcium receptor genotypes in the pathogenesis of colorectal cancer. Orv Hetil *142*: 947-951, 2001.

61 Yamaguchi T, Chattopadhyay N, Kifor O *et al*: Expression of extracellular calcium-sensing receptor in human osteoblastic MG-63 cell line. Am J Physiol Cell Physiol *280*: C382-C393, 2001.

62 Ye CP, Yamaguchi T, Chattopadhyay N, Sanders JL, Vassilev PM and Brown EM: Extracellular calcium-sensing-receptor (CaR)-mediated opening of an outward K(+) channel in murine MC3T3-E1 osteoblastic cells: evidence for expression of a functional CaR. Bone *27*: 21-27, 2000.

63 Yamaguchi T, Chattopadhyay N, Kifor O and Brown EM: Extracellular calcium (Ca^{2+}(o))-sensing receptor in a murine bone marrow-derived stromal cell line (ST2): potential mediator of the actions of Ca^{2+}(o) on the function of ST2 cells. Endocrinology *139*: 3561-3568, 1998.

64 Kameda T, Mano H, Yamada Y *et al*: Calcium-sensing receptor in mature osteoclasts, which are bone resorbing cells. Biochem Biophys Res Commun *245*: 419-422, 1998.

65 Lorget F, Kamel S, Mentaverri R *et al*: High extracellular calcium concentrations directly stimulate osteoclast apoptosis. Biochem Biophys Res Commun *268*: 899-903, 2000.

66 Wu S, Palese T, Mishra OP, Delivoria-Papadopoulos M and De Luca F: Effects of Ca^{2+} sensing receptor activation in the growth plate. FASEB J *18*: 143-145, 2004.

67 Chang W, Tu C, Bajra R *et al*: Calcium sensing in cultured chondrogenic RCJ3.1C5.18 cells. Endocrinology *140*: 1911-1919, 1999.

68 Dvorak MM, Siddiqua A, Ward DT *et al*: Physiological changes in extracellular calcium concentration directly control osteoblast function in the absence of calciotropic hormones. Proc Natl Acad Sci USA *101*: 5140-5145, 2004.

69 Chang W, Tu C, Chen T *et al*: Expression and signal transduction of calcium-sensing receptors in cartilage and bone. Endocrinology *140*: 5883-5893, 1999.

70 Moonga BS, Li S, Iqbal J *et al*: $Ca(^{2+})$ influx through the osteoclastic plasma membrane ryanodine receptor. Am J Physiol Renal Physiol *282*: F921-932, 2002.

71 Shiraishi N, Kitamura K, Kohda Y *et al*: Increased endothelin-1 expression in the kidney in hypercalcemic rats. Kidney Int *63*: 845-852, 2003.

72 Bradbury RA, Cropley J, Kifor O *et al*: Localization of the extracellular Ca(2+)-sensing receptor in the human placenta. Placenta *23*: 192-200, 2002.

73 Wang W, Li C, Kwon TH *et al*: Reduced expression of renal Na+ transporters in rats with PTH-induced hypercalcemia. Am J Physiol Renal Physiol *286*: F534-545, 2004.

74 Chattopadhyay N, Yano S, Tfelt-Hansen J *et al*: Mitogenic action of calcium-sensing receptor on rat calvarial osteoblasts. Endocrinology. Apr 7 2004 [Epub ahead of print]

75 Carpenter TO, Insogna KI, Boulware SD and Mitnick MA: Vitamin D metabolism in chronic childhood hypoparathyroidism: evidence for a direct regulatory effect of calcium. J Pediatr *269*: 252-257, 1990.

76 Pi M and Quarles LD: A novel cation-sensing mechanism in osteoblasts is a molecular target for strontium. J Bone Miner Res *19*: 862-869, 2004.

77 Shalhoub V, Grisanti M, Padagas J *et al*: *In vitro* studies with the calcimimetic, cinacalcet HCl, on normal human adult osteoblastic and osteoclastic cells. Crit Rev Eukaryot Gene Expr *13*: 89-106, 2003.

78 Kamioka H, Sumitani K, Tagami K *et al*: Divalent cations elevate cytosolic calcium of chick osteocytes. Biochem Biophys Res Commun *204*: 519-524, 1994.

79 Chattopadhyay N, Legradi G, Bai M *et al*: Calcium-sensing receptor in the rat hippocampus: a developmental study. Brain Res Dev Brain Res *100*: 13-21, 1997.

80 Simpson JB and Routenberg A: Subfornical organ lesions reduce intravenous angiotensin-induced drinking. Brain Res *88*: 154-161, 1975.

81 Chattopadhyay N: Biochemistry, physiology and pathophysiology of the extracellular calcium-sensing receptor. Int J Biochem Cell Biol 32: 789-804, 2000.

82 Ye C, Ho-Pao CL, Kanazirska M, Quinn S *et al*: Deficient cation channel regulation in neurons from mice with targeted disruption of the extracellular Ca^{2+}-sensing receptor gene. Brain Res Bull *44*: 75-84, 1997.

83 Ye C, Kanazirska M, Quinn S, Brown EM and Vassilev PM: Modulation by polycationic Ca21-sensing receptor agonists of nonselective cation channels in rat hippocampal neurons. Biochem Biophys Res Commun *224*: 271-280, 1996.

84 Chattopadhyay N, Ye CP, Yamaguchi T *et al*: Extracellular calcium-sensing receptor in rat oligodendrocytes: expression and potential role in regulation of cellular proliferation and an outward K1 channel. Glia *24*: 449-458, 1998.

85 Chattopadhyay N, Evliyaoglu C, Heese O, Carroll R, Sanders JPB and Brown EM: Regulation of secretion of PTHrP by Ca^{2+}-sensing receptor in human astrocytes, astrocytomas, and meningiomas. Am J Physiol *279*: 691-699, 2000.

86 Chattopadhyay N, Ye C, Yamaguchi T *et al*: The extracellular calcium-sensing receptor is expressed in rat microglia and modulates an outward K1 channel. J Neurochem *72*: 1915-1922, 1999.

87 McNeil SE, Hobson S, Nipper V and Roland KD: Functional calcium-sensing receptors in rat fibroblasts are required for activation of SRC kinase and mitogen-activated protein kinase in response to extracellular calcium. J Biol Chem *273*: 1114-1120, 1998.

88 McNeil L, Hobson S, Nipper V and Roland KD: Functional calcium-sensing receptor expression in ovarian surface epithelial cells. Am J Obstet Gynecol *178*: 305-313, 1998.

89 Bikle D, Ratnam A, Mauro T, Harris J and Pillai S: Changes in calcium responsiveness and handling during keratinocyte differentiation. Potential role of the calcium receptor. J Clin Invest *97*: 1085-1093, 1996.

90 Tu CL, Chang W and Bikle D: The extracellular calcium-sensing receptor is required for calcium-induced differentiation in human keratincytes. J Biol Chem *276*: 41079-41085, 2001.

91 Bikle D, Ng D, Tu CL, Oda Y and Xie Z: Calcium- and vitamin D-regulated keratinocyte differentiation. Mol Cell Endocrinol *177*: 161-171, 2001.

92 Komunes L, Oda Y, Tu C *et al*: Epidermal expression of the full-length extracellular calcium-sensing receptor is required for normal keratinocyte differentiation. J Cell Physiol *192*: 45-54, 2002.

93 Fatherazi S, Belton C, Cai S *et al*: Calcium receptor message, expression and function decrease in differentiating keratinocytes. Pflugers Arch *448*: 93-104, 2004.

94 Chattopadhyay N, Ye C, Singh DP *et al*: Expression of extracellular calcium-sensing receptor by human lens epithelial cells. Biochem Biophys Res Commun *233*: 801-805, 1997.

95 Wang R, Xu C, Zhao W, Zhang J, Cao K, Yang B and Wu L: Calcium and polyamine regulated calcium-sensing receptors in cardiac tissues. Eur J Biochem *270*: 2680-2688, 2003.

96 Cheng I, Klingensmith ME, Chattopadhyay N *et al*: Identification and localization of the extracellular calcium-sensing receptor in human breast. J Clin Endocrinol Metab *83*: 703-707, 1998.

97 VanHouten J, Dann P, McGeoch G *et al*: The calcium-sensing receptor regulates mammary gland parathyroid hormone-related protein production and calcium transport. J Clin Invest *113*: 598-608, 2004.

98 Emanuel RL, Adler GK, Kifor O *et al*: Calcium-sensing receptor expression and regulation by extracellular calcium in the AtT-20 pituitary cell line. Mol Endocrinol *10*: 555-565, 1996.

99 Romoli R, Lania A, Mantovani G, Corbetta S, Persani L and Spada A: Expression of calcium-sensing receptor and characterization of intracellular signaling in human pituitary adenomas. J Clin Endocrinol Metab *84*: 2848-2853, 1999.

100 Van den Hurk MJ, Ouwens DT, Scheenen WJ *et al*: Expression and characterization of the extracellular Ca^{2+}-sensing receptor in melanotrope cells of *Xenopus laevis*. Endocrinology *144*: 2524-2533, 2003.

101 Squires PE: Non-Ca+2-homeostatic functions of the extracellular Ca^{+2}-sensing receptor (CaR) in endocrine tissues. J Endocrinol *165*: 173-177, 2000.

102 Gama L, Baxendale-Cox LM and Breitwieser GE: Ca^{2+}-sensing receptors in intestinal epithelium. Am J Physiol Cell Physiol *273*: C1168-C1175, 1997.

103 Sheinin Y, Kallay E, Wrba F, Kriwanek S, Peterlik M and Cross H: Immunocytochemistry localization of the extracellular calcium-sensing receptor in normal and malignant human large intestinal mucosa. J Histochem Cytochem *48*: 595-601, 2000.

104 Cheng S, Okuda M, Hall AE, Geibel JP and Hebert S: Expression of calcium-sensing receptor in rat colonic epithelium: evidence for modulation of fluid secretion. Am J Physiol Gastrointest Liver Physiol *283*: G240-G250, 2002.

105 House MG, Kohlmeier L, Chattopadhyay N *et al*: Expression of an extracellular calcium-sensing receptor in human and mouse bone marrow cells. J Bone Miner Res *12*: 1959-1970, 1997.

106 Yamaguchi T, Ye CP, Chattopadhyay N, Sanders JL, Vassilev PM and Brown EM: Enhanced expression of extracellular calcium sensing receptor in monocyte-differentiated *versus* undifferentiated HL-60 cells: Potential role in regulation of a nonselective cation channel. Calcified Tissue Int *66*: 375-382, 2000.

107 Canaff L, Petit JL, Kisiel M, Watson P, Gascon-Barre M and Hendy GN: Extracellular calcium-sensing receptor is expressed in rat hepatocytes coupling to intracellular mobilization and stimulation of bile flow. J Biol Chem *276*: 4070-4079, 2001.

108 Nearing J, Betka M, Quinn S *et al*: Polyvalent cation receptor proteins (CaRs) are salinity sensors in fish. Proc Natl Acad Sci *99*: 9231-9236, 2002.

109 Quarles LD: Extracellular calcium-sensing receptors in the parathyroid gland, kidney and other tissues. Curr Opin Nephrol Hypertens *12*: 349-355, 2003.

110 Pearce SHS and Brown EM: Disorders of calcium ion sensing. J Clin Endocrinol Metab *81*: 2030-2035, 1996.

111 Heath H: Familial benign (hypocalciuric) hypercalcaemia, a troublesome mimic of mild primary hyperparathyroidism. Endocrinol Metab Clin North Am *18*: 723-740, 1989.

112 Pearce SHS: Clinical disorders of extracellular calcium-sensing and the molecular biology of the calcium-sensing receptor. Ann Med *34*: 201-206, 2002.

113 Chou YH, Pollak MR, Brandi ML *et al*: Mutations in the human Ca^{2+}-sensing receptor gene that cause familial hypocalciuric hypercalcaemia. Am J Hum Genet *56*: 1075-1079, 1995.

114 Bai M, Quinn S, Trivedi S *et al*: Expression and characterization of inactivating and activating mutations of the human Ca^{2+} receptor. J Biol Chem *271*: 19537-19545, 1996.

115 Hendy NG, De Souza L,Yang B *et al*: Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hyper-calcemia, neonatal severe hyper-parathyroidism and autosomal dominant hypocalcemia. Human Mutation *16*: 281-296, 2000.

116 Bai M, Pearce SHS, Kifor O *et al*: *In vivo* and *in vitro* characterization of neonatal hyperparathyroidism resulting from a *de novo*, heterozygous mutation in the Ca^{2+}-sensing receptor gene: normal maternal calcium homeostasis as a cause of secondary hyperparathyroidism in familial benign hypocalciuric hypercalcemia. J Clin Invest *99*: 88-96, 1997.

117 Brown EM: Physiology and pathophysiology of the extracellular calcium-sensing receptor. Am J Med *106*: 238-253, 1999.

118 Khosla S, Ebeling PR, Firek AF *et al*: Calcium infusion suggests a "set-point" abnormality of parathyroid gland function in familial benign hypercalcemia and more complex disturbances in primary hyperparathyroidism. J Clin Endocrinol Metab *76*: 715-720, 1993.

119 Pollak M, Chou YH, Marx SJ *et al*: Familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Effects of mutant gene dosage on phenotype. J Clin Invest *93*: 1108-1112, 1994.

120 Pearce SHS, Williamson C, Kifor O *et al*: A familial syndrome of hypocalcaemia with hypercalciuria due to mutations in the calcium-sensing receptor gene. N Engl J Med *335*: 1115-22, 1996.

121 Yamamoto M, Akatsu T, Nagase T and Ogata E: Comparison of hypocalcemic hypercalciuria between patients with idiopathic hypo-parathyroidism and those with gain-of-function mutations in the calcium sensing receptor: is it possible to differentiate the two disorders? J Clin Endocrinol Metab *85*: 4583-4591, 2000.

122 Kifor O, Moore FD, Delaney M *et al*: A syndrome of hypocalciuric hypercalcaemia caused by autoantibodies directed at the calcium-sensing receptor. J Clin Endocrinol Metab *88*: 60-72, 2003.

123 Vargas-Poussou R, Huang C, Hulin P *et al*: Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter –like syndrome. J Am Soc Nephrol *13*: 2259-2266, 2002.

124 Watanabe S, Fukumoto S, Chang H *et al*: Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. Lancet *360*: 692-694, 2002.

125 Sayer JA and Pearce SHS: Extracellular calcium-sensing receptor dysfunction is associated with two new phenotypes. Clin Endocrinol *59*: 419-421, 2003.

126 Handlogten ME, Shirashi N, Awata H *et al*: Extracellular Ca^{2+}-sensing receptor is a promiscuous divalent cation sensor that responds to lead. Am J Physiol Renal Physiol *279*: F1083-F1091, 2000.

127 Herbert SC, Brown EM and Harris HW: Role of the Ca^{2+}-sensing receptor in divalent mineral ion homeostasis. J Exp Biol *200*: 295-302, 1997.

128 Quinn SJ, Ye CP, Diaz R *et al*: The Ca^{2+}-sensing receptor: a target for polyamines. Am J Physiol *273*: C1315-C1323, 1997.

129 Molitoris BA: Cell biology of aminoglycoside nephrotoxicity: newer aspects. Curr Opin Nephrol Hypertens *6*: 384-388, 1997.

130 McLarnon S, Holden D, Ward DT *et al*: Aminoglycoside antibiotics induce pH-sensitive activation of the calcium-sensing receptor. Biochem Biophys Res Commun *297*: 71-77, 2002.

131 Conigrave AD, Quinn SJ and Brown EM: L-amino acid sensing by the extracellular Ca^{2+}-sensing receptor. Proc Natl Acad Sci USA *97*: 4814-4819, 2000.

132 Kobilka B: Allosteric activation of the CaR by L-amino acids. Proc Natl Acad Sci USA *97*: 4819-4820, 2000.

133 Hammerland LG, Garrett JE, Hung BC, Levinthal C and Nemeth EF: Allosteric activation of the Ca^{2+} receptor expressed in *Xenopus laevis* oocytes by NPS 476 or NPS 568. Mol Pharmacol *53*: 1083-1088, 1998.

134 Brauner-Osborne H, Jensen AA, Sheppard PO, O'Hara P and Krogsgaard-Larsen P: The agonist-binding domain of the calcium-sensing receptor is located at the amino-terminal domain. J Biol Chem *274*: 18382-18386, 1999.

135 Knoflach F, Mutel V, Jolidon S *et al*: Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. Proc Natl Acad Sci USA *98*: 13402-7, 2001.

136 Fox J, Lowe SH, Conklin RL *et al*: Calcimimetic compound NPS R-568 stimulates calcitonin secretion but selectively targets parathyroid gland Ca^{2+} receptor in rats. J Pharmacol Exp Ther *290*: 480-486, 1999.

137 Nemeth EF, Steffey ME, Hammerland LG *et al*: Calcimimetics with potent and selective activity on the parathyroid calcium receptor. Proc Natl Acad Sci USA *95*: 4040-4045, 1998.

138 Silverberg SJ, Bone HG 3rd, Marriott TB *et al*: Short-term inhibition of parathyroid hormone secretion by a calcium-receptor agonist in patients with primary hyperparathyroidism. N Eng J Med *337*: 1506-1510, 1997.
139 Antonsen JE, Sherrard DJ and Andress DL: A calcimimetic agent acutely suppresses parathyroid hormone levels in patients with chronic renal failure. Rapid communication. Kidney Int *53*: 223-227, 1998.
140 Goodman WG, Frazao JM, Goodkin DA, Turner SA, Liu W and Coburn JW: A calcimimetic agent lowers plasma parathyroid hormone levels in patients with secondary hyperparathyroidism. Kidney Int *58*: 436-445, 2000.
141 Collins MT, Skarulis MC, Bilezikian JP, Silverberg SJ, Spiegel AM and Marx SJ: Treatment of hypercalcemia secondary to parathyroid carcinoma with a novel calcimimetic agent. J Clin Endocrinol Metab *83*: 1083-1088, 1998.
142 Goodman WG, Hladik GA, Turner SA *et al*: The calcimimetic agent AMG 073 lowers plasma parathyroid hormone levels in hemodialysis patients with secondary hyperparathyroidism. J Am Soc Nephrol *13*: 1017-1024, 2002.
143 Lindberg JS, Moe SM, Goodman WG *et al*: The calcimimetic AMG 073 reduces parathyroid hormone and calcium x phosphorus in secondary hyperparathyroidism. Kidney Int *63*: 248-254, 2003.
144 Quarles LD, Sherrard DJ, Adler S *et al*: The calcimimetic AMG 073 as a potential treatment for secondary hyperparathyroidism of end-stage renal disease. J Am Soc Nephrol *14*: 575-583, 2003.
145 Quarles LD, Spiegel D, Curzi M *et al*: The effects of one year treatment with the calcimimetic AMG-073 on bone health in ESRD patients with secondary hyperparathyroidism (SHPT). J Am Soc Nephrol *13*: 572A-573A, 2002.
146 Goodman WG: Medical management of secondary hyperparathyroidism in chronic renal failure. Nephrol Dial Transplant *18*: [Suppl 3] iii2-iii8, 2003.
147 Shoback DM, Bilezikian JP, Turner SA *et al*: The calcimimetic cinacalcet normalizes serum calcium in subjects with primary hyperparathyroidism. J Clin Endocrinol Metab *88*: 5644-5649, 2003.
148 Block GA, Martin KJ, de Francisco AL *et al*: Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. N Engl J Med *350*: 1565-7, 2004.
149 Petrel C, Kessler A, Maslah F *et al*: Modeling and mutagenesis of the binding site of Calhex 231, a novel negative allosteric modulator of the extracellular Ca^{2+}-sensing receptor. J Biol Chem *278*: 49487-49494, 2003.
150 Petrel C, Kessler A, Dauban P, Dodd RH, Rognan D and Ruat M: Positive and negative modulators of the Ca^{2+}-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. J Biol Chem *279*: 18990-7, 2004.
151 Kimmel DB, Bozzato RP, Kronis KA *et al*: The effect of recombinant human (1-84) or synthetic human (1-34) parathyroid hormone on the skeleton of adult ovariectomized rats. Endocrinology *132*: 1577-1584, 1993.
152 Black DM, Greenspan SL, Ensrud KE *et al*: The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. N Eng J Med *349*: 1207-15, 2003.
153 Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H and Neer RM: The effects of parathyroid hormone, alendronate, or both in men with osteoporosis. N Eng J Med *349*: 1216-26, 2003.

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