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Invited Review

Role of the calcium-sensing receptor in parathyroid gland physiology

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Chen, Randolph A., and William G. Goodman. Role of the calcium-sensing receptor in parathyroid gland physiology. *Am J Physiol Renal Physiol* 286: F1005–F1011, 2004; 10.1152/ajprenal.00013.2004.—The calcium-sensing receptor (CaSR) represents the molecular mechanism by which parathyroid cells detect changes in blood ionized calcium concentration and modulate parathyroid hormone (PTH) secretion to maintain serum calcium levels within a narrow physiological range. Much has been learned in recent years about the diversity of signal transduction through the CaSR and the various factors that affect receptor expression. Beyond its classic role as a determinant of calcium-regulated PTH secretion, signaling through the CaSR also influences both gene transcription and cell proliferation in parathyroid cells. The CaSR thus serves a broad physiological role by integrating several distinct aspects of parathyroid gland function. The current review summarizes recent developments that enhance our understanding of the CaSR and its fundamental importance in parathyroid gland physiology.

hyperparathyroidism; parathyroid hormone secretion; vitamin D; parathyroid gland hyperplasia; calcimimetic

THE PARATHYROID GLANDS SERVE a pivotal physiological function by maintaining blood calcium levels, specifically blood ionized calcium concentrations, within a very narrow range. They do so by modulating the minute-to-minute release of parathyroid hormone (PTH) into the circulation. Such changes have almost immediate effects on calcium excretion in the urine and on calcium efflux from bone and, if sustained for hours or days, affect renal vitamin D metabolism and ultimately the efficiency of intestinal calcium absorption. The capacity of chief cells of the parathyroid to detect small changes in blood ionized calcium levels, modify PTH release accordingly, and initiate these adaptive responses is mediated by a calcium-sensing receptor (CaSR) located at the cell surface. The role of the CaSR as a modifier of various components of parathyroid gland function is the focus of the current review.

When characterized structurally in 1993, the CaSR was the first receptor described where the natural ligand was an ion, in this case the extracellular calcium ion, rather than a peptide hormone, a glycoprotein, or an extracellular organic molecule (9, 31). Activation of the receptor by raising extracellular calcium concentrations in various in vitro expression systems was shown to initiate signal transduction through pathways that had been demonstrated previously to be linked directly to decreases in PTH release from parathyroid cells (60, 63). Such findings together with evidence that alterations in the level CasR expression affected calcium-mediated PTH release from dispersed parathyroid cells in vitro thus established the molecular mechanism that regulates PTH secretion (9, 58). The importance of the CaSR as a general modifier of parathyroid gland function and systemic calcium homeostasis was underscored when distinct clinical disorders of mineral metabolism, including familial benign hypocalciuric hypercalcemia (FBHH), neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia, were shown to be caused by inactivating or activating mutations of the CaSR (18, 67–69).

In parathyroid tissue, vitamin D and calcium, both acting through negative regulatory elements located upstream from the gene for prepro-PTH, have been recognized for many years as key modifiers of PTH gene transcription, hormone synthesis, and parathyroid cell proliferation. Calcium may also affect PTH mRNA stability (59). A growing body of evidence indicates, however, that all the effects of extracellular calcium ions are mediated through the CaSR. The importance of the CaSR in parathyroid tissue thus extends beyond its traditional role as a modifier of calcium-regulated PTH secretion to involve other key components of parathyroid gland function that are frequently abnormal in clinical disorders characterized by excess parathyroid gland activity such as hyperparathyroidism. These include disturbances in the control of PTH gene transcription and hormone synthesis and the development of parathyroid gland enlargement due to tissue hyperplasia (33).

STRUCTURAL AND FUNCTIONAL COMPONENTS OF THE CaSR AND THE REGULATION OF CaSR GENE EXPRESSION

The CaSR is a member of subfamily C of G protein-coupled receptors (GPCRs) (11). This large family of GPCRs includes the metabotropic glutamate receptors (mGluR), $GABA_B$ receptors, and pheromone receptors (6). In humans, the CaSR is composed of 1,078 amino acids (31). It has a large extracellular domain, seven membrane-spanning regions, and an intracellular portion that couples to G proteins and other signal transduction pathways (Fig. 1). The gene encoding the protein in humans is located on chromosome 3q13.3-21 (45).

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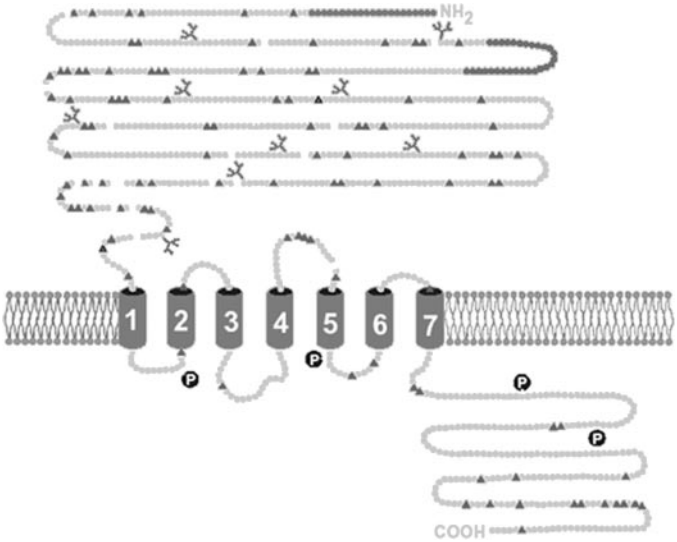

Fig. 1. Structure of the calcium-sensing receptor. *1–7*, Membrance-spanning regions.

Vitamin D response elements (VDREs) have been identified recently in each of two promoter elements that regulate the expression of alternate splice transcripts of the CaSR (12). Calcitriol, or 1,25-dihydroxy-vitamin D, enhances gene expression by both promoters (12). Such findings suggest that vitamin D plays an important role in modulating CaSR expression. They also suggest additional mechanisms to account for certain of the biological actions of vitamin D sterols, particularly with regard to their antiproliferative effects in parathyroid tissue.

Reductions in vitamin D receptor (VDR) and CaSR expression have been described both in parathyroid adenomas and in hyperplastic parathyroid tissues (26, 49, 55, 80). It is uncertain whether these changes are related mechanistically, but alterations in CaSR expression may contribute to disturbances in cell cycle regulation and to monoclonal cell growth in parathyroid tissue (80). The level of expression of one splice variant of the CaSR has been reported to be diminished in human parathyroid adenomas (17). The impact of this change on calcium-regulated PTH secretion and the more generalized role of variations in CaSR expression as a modifier of parathyroid cell proliferation in primary hyperparathyroidism have yet to be characterized fully.

The NH_2-terminal portion of the CaSR forms a very large extracellular domain containing ~600 amino acid residues (11). The extracellular domain interacts with extracellular calcium ions, possibly through clusters of acidic amino acids located in this portion of the molecule, thereby modifying the levels of receptor activation and signal transduction (7, 38, 61). Cysteine residues in the extracellular domain mediate the formation of dimers of the CaSR, whereas other loci in this region serve as sites for N-glycosylation (3, 4, 24, 66, 71, 73, 77). Both processes affect receptor expression at the cell surface and/or the ability of the receptor to undergo ligand activation.

Exactly how calcium and other extracellular cations interact with the extracellular portion of the CaSR is not understood. Studies of other members of this family of GPCRs indicate, however, that ligands for these receptors bind to a dimeric Venus flytrap-like structure formed by their extracellular domains (43). Based on three-dimensional assessments of the mGluR1 receptor, it is likely that the extracellular domain of the CaSR forms two adjacent lobes that are connected to the membrane-spanning region by a cysteine-rich segment (42, 43). It has been proposed that the binding of calcium ions at the cleft between lobes in the extracellular domain induces rotation along the dimer interface (43). Such a conformational change could thus alter the spatial configuration of the membrane-spanning portion of the receptor and modulate signal transduction. A careful analysis of mutations that result in either a loss or gain of receptor function indicates that these cluster along the margins of the dimer interface in a three-dimensional model of the CaSR, lending further credence to the proposal (43).

Although calcium ions represent the physiologically relevant ligand for the CaSR in vivo, a number of divalent, trivalent, and polyvalent cations and various amino acids can activate the CaSR in vitro (9). Recent studies have demonstrated that l-amino acids, particularly the aromatic l-amino acids tryptophan and phenylalanine, lower the threshold for CaSR activation by extracellular calcium (20, 21, 81). Serine residues located at positions 169, 170, and 171 in the extracellular domain of the CaSR appear to mediate this response (82). Such findings are noteworthy for several reasons.

First, phenylalkylamines are small organic molecules that are structurally similar to certain l-amino acids. They enhance CaSR activation by extracellular calcium ions. Several of these compounds have thus been designated as calcimimetic agents because they mimic the effect of extracellular calcium ions to activate the CaSR (62). It is likely, however, that l-amino acids and phenylalkylamines interact with different portions of the CaSR. Whereas l-amino acids interact with specific loci in the extracellular domain as mentioned previously, calcimimetic agents bind most likely to the membrane-spanning portion of the receptor (39).

Second, parathyroid gland function is abnormal in many patients undergoing long-term total parenteral nutrition (36). Disruption of the normal diurnal variation in plasma PTH levels in such patients is due predominantly to nocturnal infusions of calcium-containing nutrient solutions, but changes in other aspects of PTH secretion appear to be unrelated to alterations in calcium metabolism (36). It is plausible, therefore, that interactions between the CaSR and l-amino acids, which are an integral component of parenteral nutritional solutions, affect parathyroid gland function in those managed by total parenteral nutrition.

The central portion of the CaSR contains ~250 amino acids. This region of the molecule forms the seven membrane-spanning domains that characterize GPCRs and the intracellular and extracellular loops that connect them (11). Selected portions of the second and third intracellular loops are involved in phospholipase C activation, but it is unclear whether this requires steric interactions with specific loci within the intracellular domain (see below) (14, 15).

The $COOH_2$-terminal region of the CaSR is composed of ~200 amino acids and forms the intracellular portion of the receptor. Consensus sequences in the intracellular domain represent sites for phosphorylation by PKC and PKA (11, 16). Phosphorylation by PKC diminishes CaSR activation (5, 16), but the physiological role of phosphorylation by PKA is not yet

known. Extensive truncation of the intracellular domain completely abrogates receptor activity, whereas lesser truncations in this portion of the molecule have diverse effects (72). These include localization of the receptor to the cell surface, the apparent affinity of the CaSR for extracellular calcium ions, the cooperative nature of signal transduction after activation by calcium, and desensitization of the receptor after ligand binding (2–4, 10, 15, 30, 78).

SIGNAL TRANSDUCTION BY THE CaSR

Activation of the CaSR produces rapid, transient increases in cytosolic calcium concentration by mobilizing calcium from thapsigargin-sensitive intracellular stores and by increasing calcium influx though voltage-insensitive calcium channels in the cell membrane (60, 63, 64, 79). Unlike most stimulus-regulated secretory systems, increments in intracellular calcium concentration in parathyroid cells are associated with decreases, rather than increases, in hormone release. The mechanisms responsible for the disparate second messenger signaling and divergent secretory behavior of parathyroid cells compared with other endocrine tissues are not known (19).

Parathyroid cells are exquisitely sensitive to changes in extracellular calcium concentration, as documented abundantly by in vitro studies of dispersed parathyroid cells and by in vivo studies of humans and several species of animals. Very small changes in blood ionized calcium concentration induce prompt and relatively large changes in PTH secretion and/or plasma PTH levels (Fig. 2). The steep slope of the relationship between blood ionized calcium and PTH concentrations along the midportion of the curve provides a robust mechanism for profoundly modifying PTH secretion in response to small changes in the level of ionized calcium in blood. It also suggests that signal transduction through the CaSR is amplified substantially either by the cooperative binding of calcium ions to the extracellular domain of the receptor or by cooperative, or facilitated, intracellular signaling and/or coupling of the receptor to G proteins.

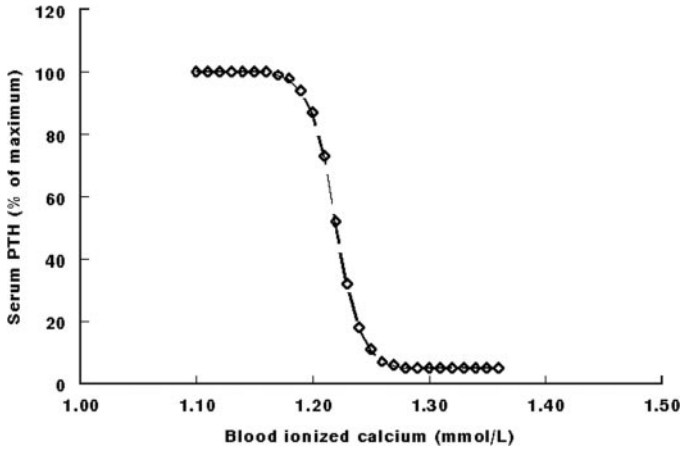

Fig. 2. Relationship between blood ionized calcium concentrations and plasma parathyroid hormone (PTH) levels in persons with normal renal and parathyroid gland function (adapted from Ref. 70). Very small changes from baseline in blood ionized calcium concentration along the midportion of the curve elicit large changes in plasma PTH levels. The response occurs within seconds to minutes and provides a robust mechanism for rapidly altering renal tubular calcium absorption and calcium efflux from bone to maintain constant blood ionized calcium levels.

In this regard, selected regions of the intracellular domain of the CaSR play a crucial role in several aspects of signal transduction. In studies using site-directed mutagenesis and truncated forms of the receptor in transfected human embryonic kidney (HEK-293) cells, a portion of the CaSR between amino acid residues 868 and 886 has been shown not only to influence localization of the receptor to the plasma membrane but also to affect phospholipase C activation (15, 72). The same localized region of the molecule determines cooperativity of the responses to receptor activation by calcium, and it appears to be responsible for initiating low-frequency oscillations in cytosolic calcium levels in response to changes in extracellular calcium concentration or after exposures to calcimimetic agents (8, 56).

With respect to the cooperative behavior of the CaSR in signal transduction, it has been suggested that this particular portion of the intracellular domain of the CaSR is crucial for interacting with G proteins. Mutations in this region may thus simply interfere with signaling through this pathway (56). Alternatively, ligand-induced activation of only one member of an obligate receptor dimer has been reported to enhance G protein interactions for both components of dimeric receptor complexes (28, 29, 53, 54). Such findings were noted originally in studies of the heterodimeric $GABA_B$ receptor, another member of the larger family of GPCRs to which the CaSR belongs. It remains uncertain, however, whether this mechanism could account for enhanced receptor-activated signaling by homodimeric receptor structures like the CaSR (56).

Activation of GPCRs often induces oscillations in intracellular calcium concentration. Both the frequency and amplitude of such oscillations represent discreet mechanisms for receptor-initiated signal transduction in target tissues (23, 50). Although most studies designed to examine the relationship between CaSR activation and variations in cytosolic calcium concentration have utilized transfected cells unrelated to chief cells of the parathyroid, the results may be directly relevant to understanding calcium-regulated PTH secretion by parathyroid tissue. Oscillating cytosolic calcium concentrations have been reported not only to diminish PTH secretion but also to reduce the fraction of cells that release PTH into the extracellular environment (57). Activation of the CaSR may thus inhibit PTH secretion by several distinct mechanisms. Moreover, there appears to be heterogeneity among parathyroid cells with respect to the suppressive effects of calcium on PTH release. Such findings might explain, at least in part, substantial variations in the response to standardized intravenous infusions of calcium in lowering plasma PTH levels among patients with either primary or secondary hyperparathyroidism (37). They could also account for differences in the nonsuppressible component of PTH secretion between patients with primary hyperparathyroidism due to parathyroid adenoma and those with secondary hyperparathyroidism due to parathyroid gland hyperplasia (37).

At the cellular level, the CaSR is found in greatest abundance within caveolae in the plasma membrane of parathyroid cells (47). Caveolae are discreet regions in the cell membrane where receptors aggregate with other molecules, or scaffolding proteins, capable of modifying signal transduction (65). Several isoforms of caveolin promote the assembly of signaling proteins within these structures, and the caveolins themselves can affect both signal transduction and gene transcription.

In bovine parathyroid cells, the CaSR colocalizes with caveolin-1, alkaline phosphatase, several isoforms of PKC, and G protein subunits (46). Variations in extracellular calcium concentration modify tyrosine phosphorylation of calveolin-1, and decreases in caveolin-1 expression attenuate CaSR-mediated responses. Indeed, reduced levels of caveolin-1 have been reported to blunt the effect of calcium to diminish PTH release from parathyroid cells. Changes in caveolin-1 expression may thus contribute to the abnormal in vitro secretory behavior of dispersed bovine parathyroid cells and of cells obtained from human parathyroid adenomas (48).

Apart from its effects on PTH secretion, caveolin-1 inhibits expression of cyclin D (27), an important general determinant of cell cycle regulation and a factor implicated in the pathogenesis of some parathyroid tumors (44). Variations in caveolin-1 expression thus appear to be involved in the regulation of parathyroid cell proliferation. Signaling through MAPKs represents yet another mechanism by which changes in caveolin-1 expression can affect cell cycle regulation and cell proliferation in parathyroid tissue (48).

Filamin is a scaffolding protein that interacts directly with cell-surface receptors and the cytoskeletal protein actin. It too localizes to caveolae. The COOH-terminal region of filamin has been shown to interact with the intracellular domain of the CaSR, and activation of the MAPK ERK by the CaSR is eliminated in cells lacking filamin (1). Interactions among the CaSR, caveolin-1, filamin, and other scaffolding proteins thus provide numerous pathways for modulating signal transduction through the CaSR with divergent effects on hormone secretion, gene expression, and cell proliferation (40). Additional second-messenger signaling pathways affected by CaSR activation include phospholipase A_2 and phospholipase D, p38 MAPK, and JNK. The finding that the cysteine proteinase m-calpain localizes to caveolae and participates in degradation of the CaSR suggests yet another mechanism for regulating the level of CaSR expression (47).

THE CaSR AND PTH SECRETION

Decreases in CaSR expression ranging from 30 to 70% or greater have been documented by immunohistochemical methods both in parathyroid adenomas removed surgically from patients with primary hyperparathyroidism and in hyperplastic parathyroid tissues obtained from patients with secondary hyperparathyroidism due to chronic kidney disease (49). Such changes are thought generally to account for disturbances in calcium-regulated PTH secretion in both disorders. Indeed, preoperative in vivo assessments of calcium-regulated PTH secretion have been reported to differ according to the level of CaSR expression in parathyroid tissue. Set point estimates were highest in patients with primary hyperparathyroidism whose adenomas had little or no detectable CaSR by immunostaining (13).

Reductions in CaSR expression are also thought to explain the moderate hypercalcemia in mice heterozygous for inactivation of the CaSR gene, in which receptor expression in parathyroid cells is reduced by ~50% (41).

Despite such results, it is not entirely clear whether modest reductions in protein expression alone are sufficient to attenuate signal transduction by the CaSR in hyperplastic or adenomatous parathyroid tissues or whether such changes explain fully the defect in calcium sensing that characterizes both clinical conditions. It is similarly uncertain whether abnormal calcium sensing by parathyroid cells develops early in the course of parathyroid cell proliferation or whether the defect is limited to later stages of the disease when tissues are markedly hyperplastic or have undergone adenomatous transformation.

Despite well-documented decreases in CaSR expression in parathyroid adenomas, plasma PTH levels fall abruptly and consistently after single oral doses of calcimimetic agents in subjects with primary hyperparathyroidism (75). This pharmacological response is mediated through the CaSR, and attenuated responses would be expected if reductions in CaSR expression accounted fully for the calcium-sensing defect in this clinical disorder (75). Moreover, equivalent doses of calcimimetic compounds induce similar reductions in plasma PTH levels regardless of disease severity, as judged by baseline plasma PTH levels, in dialysis patients with secondary hyperparathyroidism due to chronic kidney disease (34, 35). The percent decrease in plasma PTH values 1 or 2 h after single oral doses of the calcimimetic compound cinacalcet hydrochloride (AMG 073) does not differ among patients whose baseline levels range from ~300 to more than 1,800 pg/ml, results that span the full range of disease severity (35). Even subjects with advanced disease in whom CaSR receptor expression is reduced and in whom calcium-sensing is presumably abnormal experience substantial reductions in plasma PTH levels. Such findings suggest that signaling through the CaSR is substantially preserved despite low levels of CaSR receptor expression both in patients with parathyroid adenomas and in those with marked parathyroid gland hyperplasia due to chronic kidney disease. The observation that the potency of the calcimimetic NPS R-568 is similar in animals with mild or severe secondary hyperparathyroidism is consistent with this view (25).

Recent observations in transgenic mice with parathyroid-targeted overexpression of the cyclin D_1 oncogene provide further evidence that both a calcium-sensing defect and reduced levels of CaSR expression are direct, but late, consequences of parathyroid cell proliferation (44). Additional studies using this experimental model should be useful in characterizing the relationship between variations in CaSR expression and calcium-regulated PTH secretion in vivo at different stages of disease progression. In vitro studies using parathyroid cells obtained from these animals are likely to provide valuable information about specific signal transduction pathways that account for abnormal calcium-regulated PTH secretion in this murine model of primary hyperparathyroidism.

THE CaSR AND GENE TRANSCRIPTION

Although signaling though the CaSR is most closely associated with changes in PTH secretion, the level of CaSR activation also influences prepro-PTH gene transcription, and ultimately the rate of PTH synthesis, in parathyroid cells (32, 74). As mentioned previously, vitamin D and the VDR modulate prepro-PTH gene transcription by interacting with a VDRE located upstream from the transcriptional start site. A separate calcium response element (CaRE) that is located ~3.6 kb upstream from the gene for prepro-PTH also negatively regulates gene transcription. CaREs are not unique to the PTH gene and have been shown to influence the transcription of other genes including renin and atrial naturietic polypeptide.

Exactly how calcium ions interact with various CaREs is not known. It is possible, however, that steady-state intracellular calcium concentrations, rapid changes in cytosolic calcium levels, and variations in both the frequency and amplitude of oscillations in intracellular calcium concentration represent distinct mechanisms for regulating gene expression (23, 50). Signaling through the CaSR has been shown to affect each of these four components of intracellular calcium metabolism and thus may have diverse effects on gene expression in parathyroid cells.

THE CaSR, CELL CYCLE REGULATION, AND PARATHYROID GLAND HYPERPLASIA

Parathyroid gland enlargement markedly increases hormone production capacity, providing much greater amounts of PTH for release into the peripheral circulation in response to secretory stimuli (33). Recent evidence suggests that signaling through the CaSR plays a pivotal role in the development of parathyroid gland hyperplasia. Glandular hyperplasia is a prominent finding in mice heterozygous for inactivating mutations of the CaSR, a murine model of FBHH (41). More extensive parathyroid gland hyperplasia occurs in animals homozygous for this mutation, the murine equivalent of neonatal severe hyperparathyroidism (41). As such, molecular defects that interfere with calcium sensing by parathyroid cells are associated with tissue hyperplasia.

Results obtained in mice with inactivating mutations of the VDR further implicate calcium-dependent signaling, probably through the CaSR, as an important determinant of parathyroid gland hyperplasia (52). Untreated VDR null mice develop hypocalcemia after weaning and exhibit all of the classic manifestations of vitamin D deficiency, including osteomalacia in bone, rickets in epiphyseal growth plate cartilage, strikingly elevated serum PTH levels, and marked parathyroid gland enlargement (52). Hypocalcemia can be prevented, however, when VDR null mice are fed a diet containing 2.0% calcium together with lactose to promote passive, vitamin D-independent intestinal calcium absorption (51). As a result, plasma PTH levels remain normal and parathyroid gland hyperplasia does not occur. Such findings indicate that calcium-dependent signaling, probably through the CaSR, is sufficient to prevent parathyroid gland hyperplasia even in tissues incapable of responding to vitamin D (51).

The same dietary manipulations effectively prevent the development of biochemical evidence of secondary hyperparathyroidism in mice incapable of producing 1,25-dihydroxyvitamin D due to inactivating mutations of the gene encoding 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) (22). However, unlike VDR null mice, parathyroid hyperplasia is retarded only partially in calcium-supplemented 1-α-hydroxylase null mice, whereas exogenous calcitriol administration fully prevents parathyroid gland enlargement in this experimental model (66a). Whether vitamin D-dependent pathways influence CaSR expression in the parathyroid and thus influence parathyroid gland hyperplasia under these conditions is not yet known.

Evidence that calcimimetic agents retard the development of parathyroid gland hyperplasia in rats with renal failure further underscores the functional importance of signaling through the CaSR as a key determinant of parathyroid cell proliferation (76). The antiproliferative actions of vitamin D sterols in parathyroid tissue may thus reflect both indirect effects mediated by increases in serum calcium concentration and direct effects mediated by upregulation of CaSR expression.

SUMMARY

The CaSR is integrally involved in each of three key components of parathyroid gland function: hormone secretion, hormone synthesis, and cell proliferation. Activation of the CaSR is linked to a broad array of intracellular signaling cascades that mediate diverse physiological responses in parathyroid tissue. Information about the details of signal transduction by the CaSR continues to expand, and discreet modifiers of receptor expression and receptor activation have been identified. These developments provide a more precise understanding of parathyroid gland physiology and a much broader recognition of the crucial role of the CaSR in mediating various parathyroid gland functions.

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