Calcium-Sensing Receptors

By William G. Goodman

It is now known that variations in extracellular calcium concentration exert diverse physiologic effects in a variety of tissues that are mediated by a calcium-sensing receptor (CaSRs). In parathyroid tissue, the CaSR represents the molecular mechanism by which parathyroid cells detect changes in blood ionized calcium concentration, modulate parathyroid hormone (PTH) secretion accordingly, and thus maintain serum calcium levels within a narrow physiologic range. In the kidney, the CaSR regulates renal calcium excretion and influences the transepithelial movement of water and other electrolytes. More generally, activation of the CaSR represents an important signal transduction pathway in intestine, placenta, brain, and perhaps bone. Some of these actions involve cell cycle regulation, changes that may be relevant to understanding the pathogenesis of parathyroid gland hyperplasia in secondary hyperparathyroidism caused by chronic kidney disease. The CaSR represents an appealing target for therapeutic agents designed to modify parathyroid gland function in vivo, offering the prospect of novel therapies for selected disorders of bone and mineral metabolism. Other receptors capable of responding to extracellular calcium ions also have been identified, but the functional importance of these interactions remains to be determined.

THE IDENTIFICATION OF the calcium-sensing receptor (CaSR) and its subsequent cloning in 1993 were seminal events that have enhanced our understanding of calcium metabolism in health and disease markedly.[1-3] These developments definitively established the molecular mechanism that accounts for the regulation of parathyroid hormone (PTH) secretion by calcium, and they made it possible to define the genetic basis of several clinical disorders including familial benign hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal-dominant hypocalcemia.[4-7] Apart from its role in controlling PTH secretion, the CaSR is now recognized as an important signal transduction pathway in a variety of tissues including kidney, gastrointestinal tract, placenta, pancreas, and brain.[8] In the kidney, CaSR activity not only regulates renal tubular calcium transport but also influences the transepithelial movement of water and other electrolytes in several nephron segments.[9] The level of activation of the CaSR is now known to modify cell proliferation, cell differentiation, and apoptosis, and disturbances in this signal-transduction pathway may contribute substantively to the proliferation of parathyroid cells and to the development of parathyroid gland hyperplasia in chronic renal failure.[10] As such, the CaSR affects several key components of parathyroid gland function.

The current discussion provides a brief overview of selected structural components of the CaSR and their impact on signal transduction and receptor expression, particularly in parathyroid cells. The central role of the CaSR in regulating PTH secretion is reviewed, giving special attention to changes in parathyroid gland function that occur in renal failure. The CaSR as a potential molecular target for therapeutic agents designed to modify bone and mineral metabolism and to influence PTH secretion also is discussed.

STRUCTURAL AND FUNCTIONAL COMPONENTS OF THE CaSR

The CaSR is a member of the C family of G-protein–coupled receptors and, in humans, is 1,078 amino acids in length.[2] The amino-terminal portion of the molecule contains approximately 600 amino acid residues and forms a very large extracellular domain.[1,2] Clusters of acidic amino acids in this portion of the CaSR are thought to interact with extracellular calcium ions, thereby modulating the levels of receptor activation and signal transduction.[11-13] But the details of ligand binding to the CaSR and the specific loci within the extracellular domain that are responsible for ligand-receptor interactions remain to be defined. Although calcium ions represent the physiologically relevant ligand for the CaSR in vivo, a number of divalent, trivalent, and polyvalent cations,

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and various amino acids, also can activate the CaSR in vitro.[1]

The extracellular domain of the CaSR undergoes N-glycosylation at several sites, a process that affects both receptor localization within the cell membrane and the level of receptor activation.[1,2,14,15] Cysteine residues in the extracellular domain participate in the formation of receptor dimers, further modifying receptor function.[16-18]

The central portion of the CaSR contains approximately 250 amino acids. This region of the molecule forms the 7 membrane-spanning domains that characterize G-protein–coupled receptors and the intracellular and extracellular loops that connect them.[1,2] Selected portions of the second and third intracellular loops are involved in receptor phosphorylation, but it is unclear whether this requires stearic interactions with the intracellular domain (vide infra).[19]

The carboxy-terminal region of the protein is comprised of approximately 200 amino acids and forms the intracellular portion of the receptor. Consensus sequences in the intracellular domain serve as phosphorylation sites for both protein kinase C and protein kinase A.[1,2] Phosphorylation of the CaSR by protein kinase C diminishes the level of receptor activation, but the physiologic role of phosphorylation by protein kinase A is not yet known.[20] Extensive truncation of the intracellular domain completely abrogates receptor activity, whereas smaller structural changes in this portion of the molecule have diverse effects.[21] Such modifications influence localization of the receptor to the cell surface, the apparent affinity of the CaSR for extracellular calcium ions, cooperativity of the receptor in mediating signal transduction after ligand binding, and desensitization of the receptor after ligand-induced activation.[16,17,22]

THE CaSR AND PTH SECRETION

In parathyroid cells, increases in extracellular calcium concentration activate the CaSR and diminish PTH release. In contrast, decreases in extracellular calcium concentration inactivate the CaSR, triggering the release of PTH that has been sequestered in secretory granules within the parathyroid cell.[23,24] Activation of the CaSR induces transient increases in cytosolic calcium concentration owing to the mobilization of calcium ions from intracellular stores.[25,26] Unlike most other stimulus-coupled secretory systems, rapid cytosolic calcium transients in parathyroid cells are associated with decreases, rather than increases, in hormone release. The mechanisms that account for the disparate second messenger signaling and divergent hormone secretory behavior of parathyroid cells compared with other endocrine tissues are not understood.[27]

The time course of CaSR-mediated changes in PTH secretion by parathyroid cells is consistent with other responses mediated by G-protein–coupled receptors, and it is measured in seconds or minutes. Rapid changes in PTH secretion in response to small changes in extracellular ionized calcium concentration together with the steep reciprocal relationship between extracellular calcium levels and the amount of PTH released provide a robust mechanism for regulating blood-ionized calcium values within a narrow physiologic range.[28] Short-term PTH-mediated changes in calcium excretion in the urine and in calcium efflux from bone are primarily responsible for maintaining constant serum calcium concentrations over time. In this context, pulsatile PTH secretion and minute-to-minute fluctuations in plasma PTH levels in vivo may reflect ongoing physiologic variations in the level of CaSR activation.[29]

In patients with primary hyperparathyroidism (1°HPT) caused by parathyroid adenoma and in those with secondary hyperparathyroidism (2°HPT) caused by parathyroid gland hyperplasia associated with end-stage renal disease, the levels of expression of the CaSR in parathyroid tissue are reduced by 30% to 70% as measured by immunohistochemical methods.[30] Such changes theoretically could result in defective calcium sensing by parathyroid cells in either disorder, leading to excess PTH secretion and to persistently increased plasma PTH levels.

Careful assessments using in vivo dynamic tests of parathyroid gland function in patients with surgically proven 1°HPT confirm the existence of a calcium-sensing defect that can be distinguished from changes in secretory behavior attributable to parathyroid gland enlargement.[31] The defect in calcium sensing in 1°HPT is qualitatively similar to that seen in patients with familial benign hypocalciuric hypercalcemia using the same in vivo methods. Despite such findings, it remains uncertain whether the previously described modest reductions in CaSR expression in parathyroid adenomas are sufficient to account for this abnormality. Decreases in receptor expression exceeding 90% or

more are required to attenuate signal transduction in many ligand-receptor interactions. Although the extent to which CaSR expression must be diminished to abrogate CaSR-mediated signaling in parathyroid cells is not known, plasma PTH levels consistently decrease in patients with 1°HPT after the administration of calcimimetic agents, a response that is mediated through the CaSR.[32] Such in vivo findings indicate that signaling through the CaSR is largely preserved in 1°HPT despite modest reductions in CaSR expression.

For patients with 2°HPT, in vivo dynamic tests of parathyroid gland function consistently have failed to show a calcium-sensing defect when results are compared with those obtained in persons with normal renal and parathyroid gland function except in subjects with very advanced disease requiring surgical intervention.[33-36] Disturbances in calcium-regulated PTH secretion in 2°HPT thus appear to be caused largely by increases in the mass of parathyroid tissue rather than by defects in calcium sensing by parathyroid cells.[36,37] Moreover, the response to calcimimetic agents does not differ according to the severity of 2°HPT as judged by pretreatment plasma PTH levels in patients with chronic kidney disease.[38,39] The percentage decrease in plasma PTH levels 1 or 2 hours after single oral doses of calcimimetic compounds is quite similar among patients whose baseline values range from approximately 300 pg/mL to more than 1,800 pg/mL, results that nearly encompass the full spectrum of disease severity.[39] In contrast to these findings, the magnitude of the reduction in plasma PTH levels after the administration of calcimimetic compounds would be expected to be attenuated in patients with advanced disease if marked decreases in CaSR expression were an integral component of the disorder. Currently available data indicate, therefore, that modest reductions in CaSR expression, as reported previously in hyperplastic parathyroid tissues obtained from patients with end-stage renal disease, do not substantively impede calcium sensing by parathyroid cells in 2°HPT.

THE CaSR AND PARATHYROID GLAND HYPERPLASIA

Very rapid CaSR-mediated changes in PTH secretion represent one important component of parathyroid gland function (Fig 1).[40] In contrast, 2 other key determinants of aggregate PTH output from the parathyroid glands are the rate of hormone synthesis by parathyroid cells and the amount of parathyroid tissue available for hormone production.[41] At the cellular level, PTH synthesis is controlled by factors such as vitamin D and calcium that influence pre-pro-PTH gene transcription primarily (Fig 1). Posttranscriptional mechanisms that involve the stability and half-life of PTH messenger RNA also affect PTH synthesis.[42,43] The total number of cells available for hormone production is affected profoundly, however, by the development of parathyroid gland hyperplasia. Enlargement of the parathyroid glands markedly increases the capacity for hormone production, providing much greater amounts of PTH for release into the peripheral circulation in response to secretory stimuli.[41]

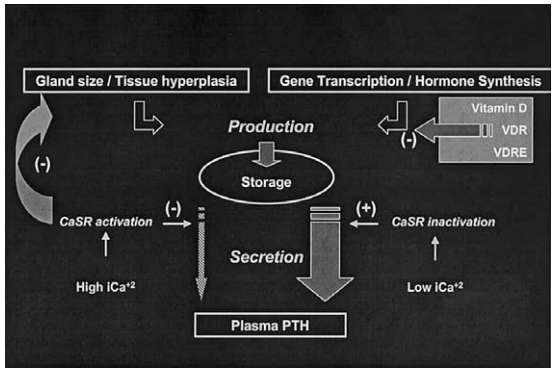

Fig 1. Vitamin D and calcium affect different components of parathyroid gland function. Vitamin D sterols inhibit pre-pro-PTH gene transcription and thus reduce the amount of hormone that is synthesized and stored in parathyroid tissue. Vitamin D sterols do not, however, affect calcium-regulated PTH secretion directly. In contrast, changes in blood-ionized calcium concentration modulate PTH release directly by affecting the level of activation of the CaSR. Signaling through the CaSR also appears to be critically important in the prevention of parathyroid gland hyperplasia, whereas a role for vitamin D as a modifier of parathyroid hyperplasia in vivo is less certain.

Recent evidence suggests that signaling through the CaSR plays a pivotal role in the evolution of parathyroid gland hyperplasia. Parathyroid gland enlargement is a prominent finding in mice that are heterozygous for inactivating mutations of the CaSR, a murine model of familial benign hypocalciuric hypercalcemia.[6] More extensive parathyroid gland hyperplasia occurs in animals that are homozygous for this mutation, the murine equivalent of neonatal severe hyperparathyroidism.[6] Both disorders are characterized by defects in calcium

sensing by parathyroid cells, leading to persistent and excessive PTH secretion despite varying degrees of hypercalcemia. As such, molecular defects that impede calcium sensing in parathyroid tissue are associated with parathyroid gland hyperplasia.

Results obtained in mice with inactivating mutations of the vitamin D receptor further implicate the CaSR as an important determinant of parathyroid gland hyperplasia. The tissues of vitamin D receptor knock-out mice are unable to respond to vitamin D because the molecular mechanism for vitamin D–dependent signaling has been disrupted.[44] As a result, hypocalcemia develops when the mice are weaned from their mothers at approximately 3 weeks of age, and the animals subsequently develop classic features of vitamin D deficiency in key target tissues. Changes include osteomalacia in bone, rickets in epiphyseal growth plate cartilage, strikingly increased serum PTH levels, marked parathyroid gland hyperplasia, and hypophosphatemia caused by PTH-mediated decreases in renal tubular phosphorus reabsorption and increases in phosphorus excretion in the urine.[44]

Serum calcium levels can be kept within the normal range in vitamin D receptor knock-out mice by maintaining them on a 2.5% calcium diet that is supplemented with lactose to facilitate passive, vitamin D–independent intestinal calcium transport.[45] This experimental intervention prevents nearly all of the manifestations of vitamin D deficiency. Notably, there is no evidence of parathyroid gland hyperplasia.[45] Such findings indicate that signaling through the CaSR is sufficient to prevent parathyroid gland hyperplasia even in tissues that are incapable of responding to vitamin D. The level of activation of the CaSR thus appears to be a more important regulator of parathyroid cell proliferation than signaling through vitamin D–dependent pathways. The finding that calcimimetic agents retard the development of parathyroid gland hyperplasia in rats with renal failure further highlights the functional importance of the CaSR in cell-cycle regulation in parathyroid tissue.[46]

In this regard, variations in CaSR expression may contribute substantively to vitamin D–mediated effects on cell proliferation and/or differentiation. Calcitriol, or 1,25-dihydroxy-vitamin D, inhibits cell proliferation in a variety of tissues, and it generally promotes cell differentiation. Recent evidence indicates that CaSR gene transcription is regulated by vitamin D through 2 distinct vitamin D response elements located in the promoter region.[47] Thus, calcitriol increases CaSR messenger RNA expression. Such a mechanism could account, at least in part, for the well-documented antiproliferative effects of vitamin D by enhancing signal transduction through the CaSR. These observations are relevant to understanding the pathogenesis of parathyroid gland hyperplasia in chronic renal failure, a disorder characterized by inadequate renal 1,25-dihydroxy-vitamin D production.[10]

THE CaSR IN TISSUES OTHER THAN PARATHYROID

As noted previously, the CaSR is expressed abundantly in tissues other than the parathyroids. In kidney, CaSR messenger RNA can be detected in the glomerulus and in most tubular segments.[48] Activation of the CaSR diminishes sodium and water reabsorption in the proximal tubule and also may affect phosphate transport in this portion of the nephron. In the thick ascending limb, CaSR activation diminishes calcium and magnesium reabsorption by lowering the transepithelial potential gradient generated by the apical Na^+-K^+-$2Cl^-$ transporter.[9] This effect is mediated by cytochrome P-450 and involves changes in arachidonic acid metabolism. Signaling through the CaSR also may antagonize PTH-mediated increases in calcium reabsorption in the distal convoluted tubule and cortical collecting duct.[9]

In intestine, the level of activation of the CaSR not only influences transepithelial solute and water transport but also modulates cell proliferation and differentiation in colonic epithelial cells.[49] In keratinocytes, the CaSR appears to mediate cell differentiation that is triggered by increases in extracellular calcium concentration.[50-52] In placenta, the CaSR serves to maintain fetal calcium homeostasis by influencing calcium-regulated PTH release, renal calcium excretion, and fetal bone turnover.[53]

In skeletal tissue, signaling through the CaSR may affect the proliferation and/or differentiated cellular function of osteoblasts and osteoclasts.[54,55] It has been suggested that CaSR activity can modify bone cell function in microenvironments where the concentration of extracellular calcium ions differs substantially from that in serum or plasma.[8] For example, the concentration of extracellular calcium ions probably far exceeds systemic levels in

localized areas where bone is being reabsorbed actively. Signaling through the CaSR thus may retard the recruitment of additional osteoclasts to sites of ongoing bone resorption and/or diminish osteoclastic activity, providing an additional localized level of regulatory control over osteoclast-mediated bone resorption. Despite such considerations, controversy persists about CaSR expression in bone and its function in bone cell metabolism.[56]

In growth plate chondrocytes, signaling through the CaSR affects the level of expression of several genes involved in extracellular matrix synthesis and endochondral bone formation.[57] The level of CaSR expression thus may serve to modulate chondrocyte proliferation and/or differentiation in epiphyseal growth plate cartilage.

THE CaSR AS A THERAPEUTIC TARGET

Because of its pivotal role in the regulation of PTH secretion, the CaSR represents an appealing target for therapeutic agents designed to modify parathyroid gland function.[58] Drugs capable of activating the CaSR would be potentially useful in the medical management of clinical disorders characterized by excess PTH secretion, particularly when complicated by hypercalcemia. Among these are primary hyperparathyroidism and advanced secondary hyperparathyroidism caused by chronic kidney disease and parathyroid carcinoma. Conversely, compounds capable of inactivating the CaSR and promoting endogenous PTH secretion might be beneficial in clinical conditions associated with hypocalcemia caused by inadequate PTH secretion such as idiopathic or postsurgical hypoparathyroidism. Controlled increases in endogenous PTH secretion also may be beneficial in osteoporosis in which the intermittent administration of exogenous PTH has been shown to have anabolic effects on trabecular bone mass.[59]

As discussed previously, a number of cations are capable of interacting with the extracellular domain of the CaSR in parathyroid cells. These ligands activate the receptor to varying degrees and thus mimic the effect of extracellular calcium ions to diminish PTH release. Agents that activate the CaSR through interactions with the extracellular domain have been categorized as type I calcimimetic agents.[12]

In contrast, type II calcimimetic compounds function as allosteric activators of the CaSR.[60] These are small organic molecules, or phenylalkylamines, that probably bind to the membrane-spanning region of the CaSR and induce conformational changes in protein structure. Type II calcimimetic compounds lower the threshold for CaSR activation by extracellular calcium ions and thus enhance signal transduction. In parathyroid cells, calcimimetic compounds magnify the inhibitory effect of calcium on PTH release across a wide range of extracellular calcium concentrations.[12]

Compounds that inactivate the CaSR and promote endogenous PTH secretion are called calcilytic agents because they lyse, or abrogate, signal transduction through the CaSR.[61] These compounds affect signal-transduction through the CaSR in a manner directly opposite to that of calcimimetics. By disrupting the inhibitory effect of extracellular calcium ions on PTH release, they lead to the release of PTH from parathyroid cell, thus promoting endogenous PTH secretion. As with calcimimetics, calcilytic agents act as allosteric modifiers of the CaSR.

These novel classes of drugs may in the future make it possible to precisely modulate parathyroid gland function and PTH secretion in vivo. Whether it is possible to specifically target the CaSR in other tissues such as kidney or gut is not known.

OTHER CALCIUM-SENSING RECEPTORS

The response of certain receptors to their primary agonists can be modified substantially by variations in extracellular calcium concentration. As such, these receptors have the capacity to sense extracellular calcium ions. The metabotropic glutamate receptors (mGluRs) share considerable homology with the CaSR, and both reside within the same superfamily of G-protein–coupled receptors.[8] Several mGluRs respond to changes in extracellular calcium concentration, although the physiologic importance of this finding remains uncertain. Similar to the CaSR, it is the extracellular domain of the mGluR that mediates its interaction with extracellular calcium ions. Differences in amino acid composition at key positions within the extracellular domain probably account for variations among the mGluRs in their responsiveness to extracellular calcium.[62]

Megalin, or gp330, is a very large protein belonging to the superfamily of low-density lipoprotein receptors.[63] It is expressed at high levels in parathyroid tissue and in cells of the proximal

nephron. Antibodies to megalin have been shown to modify PTH secretion from dispersed parathyroid cells in vitro, and early work suggested that this protein might indeed represent a calcium-sensing receptor. It is now apparent, however, that megalin functions primarily to promote the endocytosis of a variety of proteins by proximal tubular cells.[64] The uptake of vitamin D and its binding protein from tubular fluid into epithelial cells of the proximal nephron by megalin-dependent pathways is crucial for the renal synthesis of 1,25-dihydroxyvitamin D, the most active metabolite of vitamin D.[65] Megalin thus plays a key physiologic role in mineral homeostasis, but the functional importance of calcium binding to megalin as a modifier of receptor function is uncertain.

SUMMARY

The CaSR has emerged as a central factor in the regulation of calcium metabolism in particular and in the control of bone and mineral metabolism in general. It serves as a key signal transduction pathway in a variety of tissues, most notably parathyroid and kidney. Information continues to accumulate about structure-function relationships, mechanisms of signal-transduction, and factors that regulate CaSR gene transcription and expression. In the future, pharmacologic agents that target the CaSR may offer new therapeutic strategies for several clinical disorders that are characterized by abnormalities in bone and mineral metabolism. Although less is known about the physiologic importance of calcium sensing by other receptors, it is likely that information will continue to emerge about the role of extracellular calcium ions as a key modifier of signal transduction and cellular function.

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