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# THERAPEUTIC USE OF CALCIMIMETICS

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**Key Words** calcium-sensing receptor, calcilytics, parathyroid gland, parathyroid hormone, PTH, osteoporosis

■ **Abstract** It has long been recognized that the secretion of PTH by chief cells in the parathyroid gland is regulated by extracellular ionized calcium. The molecular mechanism by which extracellular $Ca^{2+}$ performs this feat was deduced by the cloning of the extracellular calcium-sensing receptor (CaSR) in 1993 in the laboratories of Brown and Hebert. The CaSR is a G protein–coupled cell surface receptor that belongs to family 3 of the GPCR superfamily. The CaSR senses the extracellular ionic activity of the divalent minerals $Ca^{2+}$ and $Mg^{2+}$ and translates this information, via a complex array of cellular signaling pathways, to modify cell and tissue function. Genetic studies have demonstrated that the activity of this receptor determines the steady-state plasma calcium concentration in humans by regulating key elements in the calcium homeostatic system. CaSR agonists (calcimimetics) and antagonists (calcilytics) have been identified and have provided both current and potential therapies for a variety of disorders. Calcimimetics can effectively reduce PTH secretion in all forms of hyperparathyroidism. They are likely to become a major therapy for secondary hyperparathyroidism associated with renal failure and for treatment of certain patients with primary hyperparathyroidism. On the therapeutic horizon are calcilytics that can transiently increase PTH and may prove useful in the treatment of osteoporosis.

## INTRODUCTION

The identification of ion sensors as "thermostats" that regulate ion metabolism in animal and plant cells (1–4) has begun to revolutionize our understanding of the roles of ions in diverse physiological processes (5–8). In the early 1990s, my colleague, E.M. Brown, and I cloned a heterotrimeric G protein–coupled receptor expressed on the surface of parathyroid chief cells that senses extracellular calcium and regulates the release of parathyroid hormone (PTH) (2). This extracellular calcium-sensing receptor (CaSR) is unique in the G protein–coupled receptor (GPCR) superfamily for three reasons:

1. Calcium and magnesium are the physiological ligands, establishing divalent mineral ions as first messengers.

2. The normal extracellular free ionized calcium concentration of 1.2 mM is the $EC_{50}$ for the CaSR, which is several orders of magnitude higher than for ligands of other GPCRs.
3. A very steep activation curve (Hill of 3–5) provides a mechanism for detecting small deviations from the normal 1.2 mM ionized calcium concentration.

The cloning of the CaSR set off a flurry of research that established the biological roles of this receptor in mineral ion homeostasis as well as in diverse cellular processes seemingly unrelated to mineral ion balance (5–8). CaSR agonists, termed calcimimetics, and antagonists, termed calcilytics, have been developed that can modify PTH secretion from parathyroid chief cells. This review focuses principally on the current clinical uses and potential clinical benefits of calcimimetics.



## THE CaSR AS A MULTIFUNCTIONAL SENSOR

### Type I and Type II Agonists

The CaSR is activated by $Ca^{2+}$ or $Mg^{2+}$ and certain other polycations [e.g., neomycin, $Gd^{3+}$, polyarginine (9–11)] in the absence of extracellular $Ca^{2+}$ and $Mg^{2+}$. These agonists are referred to as type I agonists because they can independently activate the receptor (Figure 1). The type I ligands activate one or more

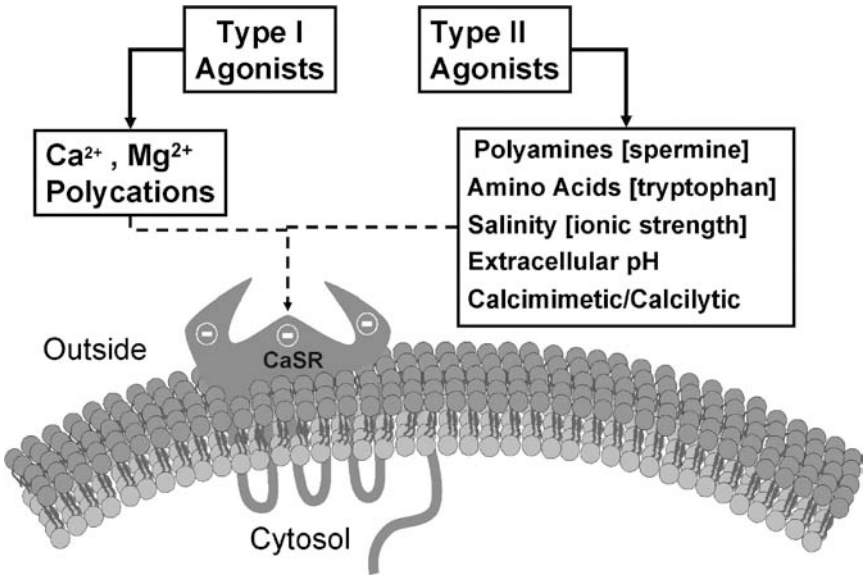


**Figure 1** Agonists of the calcium-sensing receptor. Type 1 agonists directly activate the receptor. Type 2 agonists function as allosteric modifiers of calcium sensitivity.

of several G proteins (e.g., $G\alpha_{q/11}$, $G\alpha_i$), which generate a complex of cellular second messengers [↑ $IP_3$ and $Ca^{2+}$, ↓ cAMP, ↑ arachidonic acid and 20-HETE, ERK1/2, etc. (5)]. How this second messenger "soup" and intracellular signaling pathways integrate to modulate cellular functions such as PTH secretion or proliferation-differentiation by parathyroid chief cells has not been completely worked out.

The CaSR can also be activated by certain other substances or conditions that function by modifying the $EC_{50}$ for extracellular $Ca^{2+}$. These receptor modifier agents are differentiated from type I agonists by the necessity for the presence of extracellular $Ca^{2+}$ and are referred to as type II agonists (Figure 1). They function as allosteric modifiers of the calcium sensitivity of the receptor. Calcimimetics and calcilytics belong to this type II group of agonists. Physiologically relevant type II agonists include polyamines (e.g., spermine), L-amino acids [particularly aromatic amino acids such as phenylalanine and tryptophan (12)], extracellular pH (13), and physiologically relevant changes in extracellular ionic strength (3). Increases in polyamine (9) or amino acid concentrations (12, 14), or isoosmotic reductions in ionic strength (3, 15), reduce the $EC_{50}$ (increase the affinity) for extracellular $Ca^{2+}$ and can give rise to CaSR activation in the absence of any change in extracellular ionized $Ca^{2+}$. Thus, the CaSR senses divalent minerals in the context of a complex extracellular milieu of nutrients, pH, and ionic strength.

The ability of the CaSR to be modulated by such a wide variety of agonists and extracellular conditions probably accounts for its multifunctional nature, regulating divalent mineral balance as well as modulating cell function seemingly unrelated to mineral homeostasis. Examples of the latter include CaSR effects in regulating salt and water transport by the kidney (6) and gastrointestinal epithelia (16). The CaSR also provides differentiation signals to certain epithelial cells [e.g., skin keratinocytes, mammary gland cells, colonocytes (7)] and might provide one link between increased dietary calcium intake and a reduced risk for development of colon cancer (17–22). This latter effect of the CaSR in proliferation-differentiation processes has important implications in parathyroid hyperplasia in hyperparathyroidism (HPT), to be discussed below.



## Ligand Binding Sites for Type I and Type II Molecules

The CaSR belongs to family 3 of the GPCR superfamily, whose members exhibit a topology consisting of a large N-terminal extracellular domain (ECD) connected to the signature 7-transmembrane (7-TM) domain of GPCRs. The ECD of family 3 receptors forms a bi-lobed structure referred to as a "Venus flytrap" because the lobes close around the ligand upon binding (23, 24). Agonist binding results in a conformational change in the ECD; how this molecular motion is translated to the 7-TM domain and ultimately to G protein activation is being actively pursued (23, 24). In addition, the CaSR forms disulfide-linked dimers (and possibly higher-order multimers) on binding ligands (25, 26), and this may enhance downstream cellular signaling. Dimerization involves regions in both the ECD and 7-TM domains.

The ligand-binding sites on the CaSR have begun to be identified and are located on both the ECD and 7-TM domains. Removal of the ECD drastically reduces receptor activation by $Ca^{2+}$. This finding emphasizes the important effect of negatively charged acidic residues in the ECD on the binding of polyvalent cations (24, 27–29). However, acidic residues in the second and third extracellular loops in the 7-TM domain also participate in $Ca^{2+}$ interactions (30, 31). In contrast, response to calcimimetics is retained when the ECD is removed from the CaSR (27). Recent observations indicate that the critical sites for interaction of calcimimetics and calcilytics in the CaSR are located in the 7-TM domain, primarily the TM6–TM7 region (30–32).



## THE CaSR AND PARATHYROID FUNCTION

### CaSR in Calcium Homeostasis and Secretion of PTH

The CaSR is critical for maintaining the virtual constancy of plasma ionized $Ca^{2+}$. It accomplishes this task primarily by inhibiting PTH secretion and by promoting the excretion of $Ca^{2+}$ by the kidney in response to increases in extracellular calcium (5, 6, 33). Figure 2 shows the typical inverse relationship between PTH secretion and plasma $Ca^{2+}$ and the direct relationship between urinary $Ca^{2+}$ excretion and plasma $Ca^{2+}$. Both the $Ca^{2+}$ $EC_{50}$ of individual receptors and the total number of receptors expressed per chief cell influence the steady-state plasma ionized $Ca^{2+}$ concentration. Thus, mutations in the CaSR that lower $Ca^{2+}$ affinity or reductions in the number of normal receptors can cause a left shift in the $Ca^{2+}$-PTH secretion curve, resulting in hypercalcemia.

The fundamental role of the CaSR in divalent mineral homeostasis has been established not only by in vitro studies in cells that express the normal or mutant receptors (5, 34), but also by observation of inherited hyper- or hypocalcemia disorders that result from receptor mutations (33, 35, 36). Distinct loss-of-function mutations in the CaSR result in one of three types of hypercalcemic disorders: familial (and benign) hypocalciuric hypercalcemia (type 1 FHH), in which one allele has an inactivating mutation; neonatal hyperparathyroidism (NHPT), also heterozygous for inactivating mutations but with symptomatic hypercalcemia; and neonatal severe hypercalcemia (NSHPT), in which both alleles have inactivating mutations and infants exhibit severe hypercalcemia frequently necessitating total parathyroidectomy (37). The severity of phenotypic expression of hypercalcemia in these disorders is determined by whether one or both alleles are affected and by the dominant negative interaction of mutant receptors with the normal receptor from the unaffected allele. In addition, autoantibodies that inhibit the CaSR can cause an acquired syndrome that mimics FHH (38, 39).

Hetero- or homozygous activating mutations in the CaSR result in hypocalcemia due to downward resetting of the receptor $EC_{50}$ in both parathyroid and kidney [autosomal dominant hypocalcemia (40)]. Severe activating mutations in the CaSR have also been shown to cause type V Bartter's syndrome (41, 42), a renal salt

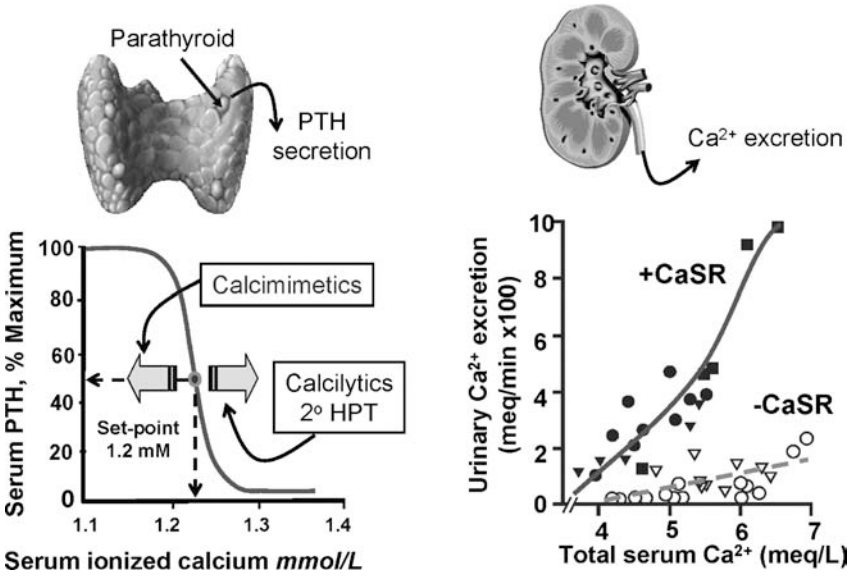


**Figure 2** Regulation of PTH secretion in the parathyroid gland and the urinary excretion of calcium in the kidney by the serum ionized calcium concentration. Note the steep relationship between serum ionized calcium and the physiological response in both the parathyroid gland and kidney. In both organs there is a remarkable ability to "sense" small changes in millimolar calcium. The serum calcium concentration is set by the integrated EC50 values of both organ responses.

wasting disease with hypercalciuria; this finding demonstrates the importance of this receptor in regulating the interplay between salt and calcium handling by the kidney (43). *Casr* gene polymorphisms have also been suggested to contribute to the normal variation in steady-state serum calcium concentration, at least in certain populations [(44, 45); see Reference 46 for issue regarding this possibility]. Autoantibodies that can activate the CaSR have also recently been identified in two patients with autoimmune hypoparathyroidism (47).

Mouse models of both gain of function and loss of function in the CaSR have been developed (48–50). Mice that lack the CaSR die soon after birth due to severe HPT and hypercalcemia (49). This lethal $Casr^{-/-}$ phenotype in mice can be rescued by ablation of the PTH receptor (51) or the parathyroid gland (50), supporting the importance of PTH in causing the severe hypercalcemia. However, serum $Ca^{2+}$ concentrations and renal $Ca^{2+}$ excretion show more variation in these "rescued" models, confirming that the CaSR is involved in fine tuning calcium homeostasis even in the absence of PTH (50, 51). In addition, a mouse with an activating mutation in the CaSR displays features similar to those in patients with autosomal dominant hypocalcemia (48).

## CaSR in Hyperparathyroidism

As discussed above (see Figure 1), the set point for calcium in the PTH-$Ca^{2+}$ response curve is shifted to the right in pathological HPT (Figure 2) (5, 33, 52). Unlike in FHH, the shift in set point for extracellular $Ca^{2+}$ in HPT is not due to inactivating mutations in the CaSR (53, 54). Instead, both CaSR messenger RNA and receptor protein expression are significantly decreased in hyperplastic parathyroid glands in primary and secondary HPT (55–61) and in parathyroid carcinoma (62). The decrease in CaSR expression appears to be associated with the parathyroid proliferation (60). In fact, the downregulation of CaSR is in proportion to the cell proliferation index assessed by Ki-67 staining (62).

The reduction in CaSR expression has been reproduced in the rat 5/6 nephrectomy model of secondary HPT, and both gland hyperplasia and the change in CaSR expression and function are abrogated by a low-phosphate diet (63, 64). The relationships among the reduced CaSR expression, hyperplasia, and the altered $Ca^{2+}$ set point have been investigated in both rat and rabbit nephrectomy models. In the rabbit, there is a significant negative correlation between CaSR mRNA expression and both glandular weight and PTH secretion (65). However, gland proliferation has been shown to precede downregulation of the CaSR in the 5/6 nephrectomy model (66). Nevertheless, parathyroid gland hyperplasia is seen in the *Casr*$^{-/-}$ mouse, which demonstrates that absence of the receptor can indeed influence chief cell proliferation in this non-uremic animal (64). Interestingly, Lewin and coworkers (67) found that the elevated PTH level in 5/6 nephrectomy rats reversed after an isogenic kidney transplant without upregulation of CaSR mRNA. Thus, the interplay among serum PTH, CaSR expression, and gland hyperplasia is complex and can be influenced by the uremic environment and abnormal mineral metabolism in chronic renal failure.

# CALCIMIMETICS

## Effects on PTH Secretion in Hyperparathyroidism

Calcimimetics are small, orally active phenylalkylamine derivatives that left-shift the $EC_{50}$ for ionized $Ca^{2+}$ on PTH secretion without altering the minimal or maximal responses (Figure 2). Thus, calcimimetics are type II agonists that modify the $EC_{50}$ of the receptor for ionized calcium (Figure 1). Over the past decade, calcimimetics have been developed for use in treating HPT, especially HPT secondary to chronic renal failure, and have been the subject of numerous reviews (e.g., 52, 68). The first-generation calcimimetic NPS R-568, although effective in reducing PTH secretion in vitro and in vivo (69–71), was discontinued for human use due to suboptimal pharmacodynamics. A second-generation calcimimetic, cinacalcet HCl (AMG 073, KRN 1493, NPS 12493; Cinacalcet™ HCl; Sensipar® in the United States), had much better bioavailability and pharmacodynamics (72, 73).

Calcimimetics cause a dose-dependent decrease in serum PTH and ionized $Ca^{2+}$ in rats with 5/6 nephrectomy and in animals with normal renal function (72, 74, 75). In addition, calcimimetics reduce serum $Ca^{2+}$ (72, 74, 75) and cause a transient increase in calcitonin secretion from thyroidal C cells (72, 76). This calcimimetic-induced reduction in serum $Ca^{2+}$ is not due to increased renal excretion of $Ca^{2+}$ as it is observed in nephrectomized rats (76). Moreover, the calcimimetic dose that suppresses PTH secretion is at least tenfold lower than that required to alter calcitonin (73).

Calcimimetics have also been shown to reduce PTH secretion in primary HPT (77, 78), secondary HPT (69–71, 79–84), and parathyroid carcinoma (85). In primary HPT, cinacalcet HCl acutely lowers serum PTH and normalizes serum $Ca^{2+}$ without increasing urine $Ca^{2+}$ excretion (77). Activation of CaSR in kidney might be expected to increase urinary $Ca^{2+}$ excretion (6) and potentially increase risk of urinary obstruction by stones and nephrocalcinosis in primary HPT. Thus, the failure of cinacalcet to increase urine $Ca^{2+}$ excretion in primary HPT is interesting. It could be due to the fall in serum $Ca^{2+}$ and reduced glomerular filtered load of $Ca^{2+}$, reduced bioavailability of the drug at active sites in the kidney, or different sensitivity of the CaSR to cinacalcet in renal cells via parathyroid chief cells (76). However, like parathyroidectomy, the calcimimetic NPS R-467 prevents furosemide-induced nephrocalcinosis in rats, suggesting that the reduction in PTH is a factor in the capacity of calcimimetics to modulate renal $Ca^{2+}$ handling. Cinacalcet was subsequently shown to maintain normal serum $Ca^{2+}$ in a 52-week, randomized, double-blind, placebo-controlled trial (78), so this calcimimetic may constitute a rational nonsurgical approach for the management of primary HPT. Cinacalcet similarly lowers serum PTH and serum $Ca^{2+}$ in hemodialysis (79–84) and peritoneal dialysis (82) patients with secondary HPT. Based on these randomized, double-blind studies, cinacalcet HCl (Sensipar®) was recently approved by the U.S. FDA and in Europe for the treatment of secondary HPT.

## Ca × P Product and Cardiovascular Risk

An elevated serum calcium × phosphate product (Ca × P) predisposes patients with secondary HPT to vascular and tissue calcifications and increased cardiovascular mortality risk (86–88). Treatment of secondary HPT with vitamin D is generally initially effective in reducing serum PTH, but the calcemic and phosphatemic effects of vitamin D can enhance the abnormal mineral metabolism and exacerbate vascular calcifications. In contrast, cinacalcet generally lowers serum $Ca^{2+}$, serum phosphate, and the serum Ca × P product (80–82, 84). These findings suggest that this calcimimetic may lower morbidity and mortality due to cardiovascular complications. In the rat uremic model, treatment with NPS R-568 (a related calcimimetic) has been shown to diminish cardiovascular changes associated with chronic renal failure [cardiac interstitial fibrosis, capillary length density, and arteriolar wall thickness (89)]. This early animal study supports the potential for a beneficial effect of calcimimetics on cardiovascular risk in chronic renal failure,

but the possibility awaits clinical verification. Given that this is a very active area of investigation, look for a flurry of reports in both animals and humans over the next few years.

## Effects on Parathyroid Gland Hyperplasia

One of the important unresolved issues regarding the use of calcimimetics in secondary HPT is whether calcimimetic treatment will modify gland hyperplasia and reduce gland mass (90, 91). While we await data from clinical studies, results from cell culture and animal experiments have shown that calcimimetics can modify gland hyperplasia. In the rat 5/6 nephrectomy model of chronic renal failure, calcimimetics ameliorate the decreased CaSR expression (92), reduce parathyroid cell proliferation (93), prevent hyperplasia (75, 94), and reduce gland mass (75).

Dispersed parathyroid cells have been used to assess the mechanisms of CaSR activation on cell function. However, parathyroid cells in primary cultures generally exhibit a rapid decrease in CaSR expression, which limits their usefulness (95). Thus, it is not surprising that the effects of extracellular $Ca^{2+}$ on parathyroid cell proliferation in culture have been variable (see 90 for a review). However, in cultured parathyroid cells derived from uremic patients that continue to express the CaSR (96), the calcimimetic R-467 suppressed DNA synthesis by 35%, consistent with an antiproliferative effect (97). In these cells, raising extracellular $Ca^{2+}$ had a proliferative effect. Although it seems clear that calcimimetics have an antiproliferative effect on parathyroid cells and function via activation of the CaSR, the mechanisms for the disparate effects of extracellular $Ca^{2+}$ and calcimimetics remain to be defined (90, 91, 97). The downstream cell mediators of CaSR activation on parathyroid cell proliferation-differentiation, as in colon cancer (18), are actively being sought (33, 98, 99). The CaSR can couple via G proteins to mitogen-activated protein kinases (MAPKs; ERK1/2), but this pathway appears to be linked to the inhibition of PTH secretion by CaSR activation (99, 100).

## Effects on Bone and Osteitis Fibrosa

Although several studies have suggested that osteoclasts, osteoblasts, and bone-related cells may express the CaSR (see 101, 102 for brief reviews), rescue of the severe skeletal phenotype in the CaSR knockout mouse by ablation of the parathyroid (50) shows that CaSR expression is not essential for bone formation/function. It should be noted, however, that osteoclasts do express another type of extracellular $Ca^{2+}$ sensor that regulates cells number and function, and there is an active search for the molecular identity of this sensor (101). Of interest in this regard, the calcimimetic cinacalcet HCl does not activate the $Ca^{2+}$ sensor in osteoclasts (103).

Renal osteodystrophy in uremic patients consists of a high bone turnover state, a low bone turnover state, or a mixture of both states (104). The severity of the high turnover disease, osteitis fibrosa, is directly proportional to the magnitude of the secondary HPT and the overproduction of PTH. In contrast, low bone turnover is associated with low PTH levels. In healthy individuals, secretion of PTH exhibits

complex fluctuations—a circadian rhythm on top of an ultradian rhythm comprising several PTH pulses per hour (see 105 for a review). These fluctuations of serum PTH correlate with serum $Ca^{2+}$, serum phosphate, and bone metabolism and are generally preserved in chronic renal failure (105, 106). Interestingly, treatment of secondary HPT with vitamin D analogues, which induce a chronic suppression of PTH secretion by inhibiting PTH gene transcription, reduce the ultradian and circadian rhythms of PTH (105). It has been suggested that this suppression of PTH rhythmicity may contribute to the low bone turnover disease of chronic renal failure.

A first-generation calcimimetic, NPS R-568, has been shown to both halt the progression of, and reverse the bone defects in, the characteristic osteitis fibrosis lesion in the rat 5/6 nephrectomy model of secondary HPT (107). This response of bone is consistent with the PTH-lowering effect of calcimimetics (72, 74, 75). In addition, daily intermittent NPS R-568, which induces an exaggerated circadian fluctuation in serum PTH, has an anabolic-like effect on the low bone turnover state in the rat uremic model, which manifests as stabilization of cortical and cancellous bone mass (108, 109). Consistent with this observation in experimental animals, a recent preliminary communication has suggested that cinacalcet may lower bone loss in the proximal femus in hemodialysis patients (109a). The anabolic-like effect of calcimimetics on uremic bone disease, however, is not mimicked in the rat ovariectomy model of bone loss (109). This issue is discussed further below.



## CALCILYTICS: POTENTIAL IN OSTEOPOROSIS

Whereas high and sustained elevations in serum PTH in HPT cause osteitis fibrosis, smaller and transient increases in PTH have a net anabolic effect on bone (110, 111). This latter stimulatory effect of PTH on new bone formation has suggested that intermittent PTH therapy may be beneficial in osteoporosis (110).

The circadian and ultradian rhythms appear to be suppressed in secondary HPT (105). Although calcimimetics induce a new circadian-type rhythm in serum PTH in secondary HPT, they do not increase bone mass in ovariectomized rats (109). Given the beneficial effects of intermittent PTH therapy, would it be possible to mimic parenteral PTH administration with an oral CaSR antagonist? To answer this question, NPS Pharmaceuticals identified an oral, fast-acting, first-generation CaSR antagonist or calcilytic, NPS 2143 (112). This calcilytic functions as an allosteric modifier of the CaSR and shifts the $Ca^{2+}$-PTH curve to the right without altering the minimal or maximal responses, and thus it functions as an allosteric inhibitor (Figure 2). Studies in cultured cells expressing the CaSR, freshly isolated bovine parathyroid cells, and acute infusions in normal rats have demonstrated that NPS 2143 stimulates PTH secretion (113). In the ovariectomized rat model of osteoporosis, NPS 2143 has been shown to increase bone turnover (114), whereas calcimimetics have no effect in this disease. Other novel compounds have also been shown to have calcilytic activity that similarly stimulates PTH secretion in

normal rats (115). Other calcilytic compounds with structures distinct from NPS 2143 have recently been developed that can also increase PTH secretion (115–117). The potential for calcilytics in the treatment of osteoporosis awaits clinical evaluation and verification of their utility.

## DISCLOSURE OF BIAS

I am one of the inventors on patents related to the calcium-sensing receptor and calcium receptor active compounds. I am now receiving royalties on calcimimetics.





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