The calcium-sensing receptor: a key factor in the pathogenesis of secondary hyperparathyroidism

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Rodriguez, Mariano, Edward Nemeth, and David Martin. The calcium-sensing receptor: a key factor in the pathogenesis of secondary hyperparathyroidism. Am J Physiol Renal Physiol 288: F253–F264, 2005; doi:10.1152/ajprenal.00302.2004.—Serum calcium levels are regulated by the action of parathyroid hormone (PTH). Major drivers of PTH hypersecretion and parathyroid cell proliferation are the hypocalcemia and hyperphosphatemia that develop in chronic kidney disease patients with secondary hyperparathyroidism (SHPT) as a result of low calcium levels and decreased kidney function. Increased PTH production in response to systemic hypocalcemia is mediated by the calcium-sensing receptor (CaR). Furthermore, as SHPT progresses, reduced expression of CaRs and vitamin D receptors (VDRs) in hyperplastic parathyroid glands may limit the ability of calcium and calcitriol to regulate PTH secretion. Current treatment for SHPT includes the administration of vitamin D sterols and phosphate binders. Treatment with vitamin D is initially effective, but efficacy often wanes with further disease progression. The actions of vitamin D sterols are undermined by reduced expression of VDRs in the parathyroid gland. Furthermore, the calcemic and phosphatemic actions of vitamin D mean that it has the potential to exacerbate abnormal mineral metabolism, resulting in the formation of vascular calcifications. Effective new treatments for SHPT that have a positive impact on mineral metabolism are clearly needed. Recent research shows that drugs that selectively target the CaR, calcimimetics, have the potential to improve the management of SHPT and, more importantly, to reduce the mortality and morbidity associated with SHPT, with particular reference to the role of the parathyroid gland. SHPT develops as a result of impaired calcium homeostasis when renal failure disturbs the complex interactions among parathyroid hormone (PTH), calcium, phosphorus, and vitamin D. The elevated serum levels of PTH that are observed in patients with SHPT result directly in high-turnover bone disease (52, 97, 134) and, in conjunction with hyperphosphatemia and a high calcium–phosphorus product (Ca × P), can be a significant risk factor for cardiovascular morbidity and mortality (10, 39, 101). Elevated calcium may also be an independent risk factor for increased mortality in hemodialysis patients (9). Current treatment modalities, while correcting one aspect of mineral metabolism, also tend to have unwanted effects on others. This can add to the complexity of managing the disease (58). In fact, data from observational studies in Europe and the United States show that, at present, substantial proportions of dialysis patients do not meet the new targets for PTH, calcium, phosphorus, and Ca × P proposed by the National Kidney Foundation’s Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease (21, 32, 75, 147).

Given the complexity of SHPT, it is clear not only that a better understanding of its pathogenesis will improve management in the short term but also that knowledge of the physiological mechanisms underlying the condition will facilitate the search for appropriate treatment targets and, ultimately, novel therapeutic agents. In recent years, it has become apparent that the calcium-sensing receptor (CaR) plays a central role in regulating PTH levels and, subsequently, those of calcium and phosphorus (15). Thus agents targeted at the CaR have the potential to improve the management of SHPT and, more importantly, to reduce the mortality and morbidity associated with CKD. In this review, we reevaluate the pathogenesis of SHPT, with particular reference to the role of the parathyroid CaR.

CALCIUM HOMEOSTASIS: THE ROLE OF CaRs AND VITAMIN D RECEPTORS

Homeostatic systems work to ensure that the concentration of extracellular calcium is tightly controlled; under normal conditions, serum calcium fluctuates by a maximum of 2% either side of normal (15). This is achieved through the interactions between calciotropic hormones and their effector tissues in the kidney, intestine, and bone. Paramount within this system is the calcium-PTH axis. Extracellular calcium levels are the primary determinant of PTH secretion/production and, similarly, PTH is a key regulator of serum calcium levels. The principal actions of PTH are to release calcium and phosphorus from the bone, suppressing the synthesis and release of PTH, and to increase renal tubular reabsorption of calcium. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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from bone, reduce renal excretion of calcium, increase urinary excretion of phosphorus, and stimulate the production of the active form of vitamin D (1,25-dihydroxy vitamin D₃; calcitriol) in the kidneys. Vitamin D and the vitamin D receptors (VDRs), expressed within the nucleus of parathyroid cells, also play important roles in calcium homeostasis. However, a comprehensive discussion of this topic is beyond the scope of this review.

**The CaR**

The sigmoidal, inverse relationship between serum levels of PTH and calcium illustrates an exquisitely sensitive control mechanism in which only minute changes in serum calcium are required to induce large changes in PTH (76). Although the CaR can be found in many tissues, its ability to sensitively control serum calcium levels is largely dependent on its presence on the chief cells of the parathyroid gland (CaRs on the luminal side of the medullary collecting duct epithelium of the kidneys also participate in calcium regulation). The responsiveness of the parathyroid glands to changes in serum calcium caused researchers to speculate for many years about the presence of a cell-surface calcium receptor. Indirect evidence of such a receptor was provided by Akerstrom and colleagues (61, 87) in the late 1980s, but it was not until 1993 that Brown et al. (16) cloned the bovine CaR. The CaR is part of a superfamily of G protein-coupled transmembrane receptors. It has three major domains: an extracellular 612-amino acid ligand-binding portion, a hydrophobic 250-amino acid membrane-spanning section, and a cytosolic COOH-terminal tail of ~250 amino acids (Fig. 1).

Activation of the CaR by extracellular calcium ions engages the mitogen-activating protein kinase C cascade through both G protein-linked phospholipase C and a member of the Src tyrosine kinase family (62, 63). The pathway eventually leads to activation of phospholipase A₂ and the generation of arachidonic acid (62). Several authors have demonstrated a role for arachidonic acid and its metabolites in the suppression of PTH secretion (2, 12, 20, 63). Thus PTH secretion is inhibited via the CaR when it is activated by elevated concentrations of ionized calcium. Prolonged hypercalcemia eventually leads to reduced parathyroid cell proliferation, an effect that is also likely to be mediated by the CaR (91, 114). Conversely, low serum levels of ionized calcium reduce activation of the CaR, and PTH secretion increases. Results from animal studies show
that persistent hypocalcemia leads to a posttranscriptional increase in PTH mRNA (78), and hypocalcemia eventually leads to an increase in parathyroid cell proliferation (79). Again, there is a progressive increase in the homeostatic response to hypocalcemia: within minutes, intracellular degradation of PTH is reduced and secretion is increased. In the following hours, PTH gene transcription is increased due to increases in PTH mRNA and over subsequent days and weeks parathyroid cell proliferation is accelerated (15).

The pivotal role of the CaR in PTH regulation is illustrated in patients with inherited mutations of the CaR gene who display abnormal calcium sensing and an altered response to fluctuations in serum calcium levels. For example, patients with familial hypocalciuric hypercalcemia (FHH) have a chronic mild asymptomatic hypercalcemia in the presence of normal or slightly elevated PTH (95) and a lower rate of urinary excretion of calcium than would be expected for hypercalcemic patients. A more serious genetic defect occurs in the form of neonatal severe hyperparathyroidism (NSHPT). In this disorder, pronounced hypercalcemia and markedly elevated PTH present shortly after birth, resulting in bone disease, parathyroid hyperplasia and, possibly, death (95). The genetic basis of these conditions has been elucidated with the aid of a knockout mouse model. Animals heterozygous for inactivating mutations of the CaR gene mimicked FHH, whereas those homozygous for the same mutations displayed NSHPT-like symptoms (51). It is thought that in humans these conditions are caused by mutations that functionally inactivate the CaR. Several different inactivating mutations of the CaR gene have been identified in patients with FHH or NSHPT (28, 30, 57, 93, 95).

In addition to the evidence provided by genetic abnormalities, the development of calcimimetics, agents that act directly on the CaR, has allowed a better understanding of receptor function. The prototype calcimimetic is, of course, the calcium ion, but the CaR also responds to a variety of other di- and trivalent ions (82). Agents that directly stimulate the CaR by interacting with its extracellular domain are termed “type I calcimimetics.” In addition, there are agents that interact with the membrane-spanning region of the CaR, inducing a conformational change and increasing the sensitivity of the receptor to calcium (83, 85). These allosteric modulators of the CaR are termed “type II calcimimetics.” They have been the subject of most research to date, including investigations into their use in the treatment of secondary and also primary hyperparathyroidism (44).

The role of the CaR in regulating PTH has been demonstrated in in vitro studies with calcimimetic agents (41). In an early study, Hammerland et al. (46) expressed bovine and human CaRs in *Xenopus laevis* oocytes. They showed that increasing the extracellular concentration of calcium led to an increase in the chloride ion current across the cell membrane. The early type II calcimimetics, NPS R-467 and NPS R-568, potentiates the effects of extracellular calcium and other type I agents. Neither calcimimetic had any effect on the chloride current in the absence of extracellular calcium. Further studies from the same group showed that NPS R-467 and NPS R-568 increased the sensitivity of bovine parathyroid cells to extracellular calcium (85). R-enantiomers of both compounds in nanomolar concentrations inhibited PTH release and shifted the calcium-PTH response curve to the left. In vivo experiments performed in rats showed that when NPS R-568 was given by gavage to normal rats, there was a rapid decrease in serum PTH (36). Minimum PTH levels were reached within 15 min of dosing and were followed by a decrease in serum calcium, which was expected after a marked reduction in PTH.

**EARLY SHPT**

Calcium and phosphorus homeostasis are disturbed relatively early in the course of CKD. This, together with a decrease in calcitriol production, leads to the elevated PTH levels that typify SHPT. While some renal function remains, the kidneys can respond to PTH to a certain extent by reabsorbing calcium, excreting phosphorus, and increasing production of calcitriol (104, 128). However, as kidney failure progresses toward ESRD, higher PTH concentrations are required to maintain calcium homeostasis (104, 128).

A study by Martinez et al. (74) in CKD patients not receiving calcium supplements, phosphate binders, or vitamin D sterols found that PTH began to rise above normal levels when creatinine clearance fell below 70 ml/min. There are probably dual triggers for this increase: first, an increase in phosphorus burden due to a decrease in the glomerular filtration rate leads to accumulation of phosphorus; and, second, the remaining kidney mass fails to produce sufficient quantities of calcitriol (94, 125). Both the accumulation of phosphorus and the calcitriol deficiency act to decrease serum calcium. The decreased serum calcium is detected by the parathyroid CaR and is the stimulus for PTH secretion and production by the parathyroid glands (Fig. 2). Independent of their effects on lowering serum calcium, the accumulation of phosphorus stimulates parathy-
roid cell function directly and the decrease in calcitriol production reduces suppression of PTH synthesis, resulting in increased PTH secretion, synthesis, and cell proliferation (28).

The involvement of calcium, vitamin D, and phosphorus in the development of SHPT is reviewed in more detail in the following sections.

Role of Calcium

The CaR on parathyroid cells is the primary mechanism regulating secretion of PTH (1, 81). The extracellular calcium concentration may also regulate the level of PTH mRNA (144). Moallem et al. (78) showed that low calcium induces a post-transcriptional increase in PTH mRNA. This effect is mediated through binding of the regulatory protein AUF to the 3'-untranslated region of PTH mRNA, which confers stability to the PTH mRNA transcript (65, 112). In another set of studies, it was shown that rats maintained on a low-calcium diet had fivefold increases in PTH mRNA, whereas mRNA levels in rats receiving a high-phosphorus diet increased by a factor of 1.25. Similarly, the number of proliferating cells in the low-calcium group was significantly higher than in rats receiving a high-phosphorus diet, which in turn was increased compared with controls (Fig. 3) (79), suggesting that hypocalcemia is associated with enhanced parathyroid proliferation. A study in hypocalcemic rats showed increased concentrations of calreticulin, a calcium-binding protein, in the nuclear fraction of their parathyroid glands but not in other tissues. Calreticulin prevented binding of VDR to its response element (VDRE), thereby preventing calcitriol from reducing PTH synthesis (113). As previously mentioned, calcium has also been shown in rat models to regulate VDR expression by parathyroid cells independently of calcitriol (40). VDR mRNA and VDR protein levels were lower in hypocalcemic rats than in normocalcemic rats. Thus serum calcium levels may also regulate PTH levels indirectly through the feedback effect of calcitriol on the parathyroids.

Role of Vitamin D

The physiological sequelae of reduced vitamin D levels have been shown in various animal models of vitamin D deficiency. For example, Takahashi et al. (127) estimated that secretion of PTH in vitamin D-deficient rats was up to 10-fold higher than in rats fed a normal diet (127). Naveh-Many and Silver (80) also showed that PTH mRNA in parathyroid cells from vitamin D-deficient normocalcemic rats was much higher than in controls and that in vitamin D-deficient hypocalcemic rats the upregulation of PTH mRNA was even more pronounced. In a canine model, Hendy et al. (49) observed that vitamin D-deficient dogs were unable to defend against acute hypocalcemia despite the presence of abundant PTH. They concluded that, initially, increased serum PTH is primarily due to the augmentation of PTH synthesis; subsequently, it is due to parathyroid cell proliferation. Liach and Massry (69) showed that low calcitriol plays a key role in the genesis of SHPT in patients with moderate renal failure.

Many studies have been conducted to examine whether supplementation with vitamin D sterols can prevent or ameliorate SHPT in CKD. Szabo et al. (126) demonstrated that calcitriol administration was capable of preventing the development of SHPT in uremic rats when given at the very start of chronic renal failure. However, calcitriol failed to reduce parathyroid cell proliferation once secondary parathyroid hyperplasia was already established (126). In patients with early CKD, small doses of calcitriol controlled hyperparathyroidism (103). Following the demonstration by Slatopolsky et al. (120) that calcitriol administered intravenously reduced serum PTH levels in patients undergoing dialysis, a large number of clinical studies have also shown that calcitriol administration is effective in decreasing PTH levels in patients with ESRD. Whether it can also induce regression of parathyroid hyperplasia remains a matter of debate. Nevertheless, the use of calcitriol in CKD patients to control SHPT carries the risk of hypercalcemia and hyperphosphatemia (99).

Calcitriol deficiency may also have an effect on parathyroid CaR. Brown et al. (18) showed that calcitriol, but not calcium itself, regulated the expression of CaR mRNA. Recent work by Canaff and Hendy (19) revealed that administration of calcitriol produces upregulation of CaR mRNA through VDREs present in the promoters of the CaR gene. If calcitriol levels influence the expression of CaR, calcitriol deficiency may also indirectly affect the control of PTH secretion through abnormal extracellular calcium sensing by the parathyroid cell.

In addition to the effects of vitamin D deficiency, uremia itself may prevent vitamin D action. Once calcitriol is bound to its nuclear receptor VDR, the hormone-receptor complex interacts with specific DNA response elements (VDREs) located in the 5'-flanking regions of specific target genes. Experimental evidence indicates that uremic plasma contains substances that specifically inhibit this interaction (53–55, 92).

Role of Phosphorus

Accumulation of phosphorus due to the decreased glomerular filtration rate is another important factor in the pathogenesis of SHPT (25). The increased burden of phosphorus induces SHPT through indirect and direct mechanisms (106). High phosphorus levels inhibit calcitriol production (96, 128), which, in turn, causes hypocalcemia. Furthermore, high serum

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**Fig. 3.** Effects of 21-day high-phosphorus and low-calcium diets on parathyroid cell proliferation in weanling rats. PCNA, proliferation cell nuclear antigen. Adapted from Ref. 86.
phosphorus prevents the reduction in PTH induced by calcitriol administration (99, 110). In addition, high phosphorus levels impair the calcemic action of PTH, which is another cause of hypocalcemia in renal patients (35, 111, 121). In keeping with this, a high serum phosphorus level partially prevented the inhibition of PTH secretion by calcium in hemodialysis patients (23).

The direct effects of phosphorus have been demonstrated in animal studies and in vitro. In rats, diet-induced hypophosphatemia and hyperphosphatemia produced a respective decrease and increase in parathyroid PTH mRNA and serum PTH levels (50, 66). In dogs exposed to a calcium clamp, an acute increase in phosphorus levels produced a threefold increase in PTH secretion (33). Almaden et al. (4) demonstrated that, in vitro, a high phosphorus concentration stimulated PTH secretion by intact rat parathyroid glands. At an ionized calcium concentration of 1.25 mM, phosphorus concentrations of 3 and 4 mM increased PTH secretion two- and threefold, respectively, with 1 or 2 mM phosphorus. The in vitro evidence for a direct effect of phosphorus on PTH secretion was subsequently confirmed by others (86, 119). Interestingly, the in vitro effect of phosphorus on PTH secretion could be observed in intact parathyroid tissue preparations, but not in isolated, dispersed parathyroid cells (86). More recently, other researchers were able to show an effect of phosphorus on PTH secretion in confluent human hyperplastic parathyroid cell cultures (29). The in vitro effect of phosphorus on PTH secretion and PTH gene expression was also observed in parathyroid gland tissues obtained from patients who had advanced SHPT with diffuse or nodular secondary hyperplasia (6).

With sustained hyperphosphatemia, the rate of parathyroid cell proliferation increases (48, 141, 142). One group of researchers noted that hyperphosphatemia increased parathyroid cell proliferation within 2 days and that the rate of cell growth almost doubled within 2 wk in rats with renal failure (26). Other groups have also studied the effect of a high-phosphorus diet on parathyroid cell proliferation and CaR expression (31, 79, 115, 117, 119). Several studies have shown that in experimental animal models, a phosphorus-rich diet increases parathyroid gland function and volume. This effect was observed independently of changes in plasma calcium and calcitriol, suggesting a direct effect of phosphorus on cell proliferation (79, 116, 119). Moreover, in a low-phosphorus diet prevented parathyroid hyperplasia (79, 119, 146). The effect of phosphorus on parathyroid cell proliferation is also seen in advanced SHPT; hyperplastic glands from patients with hyperphosphatemia have been found to contain more proliferating cells than those with lower serum phosphorus levels (5).

Studies have provided information that is helping to determine the mechanisms by which phosphorus regulates parathyroid function. First, a specific sodium-phosphate cotransporter (Pit-1) has been cloned from rat parathyroid tissue (129). This transporter may detect changes in extracellular phosphate (77, 129). Second, the regulation of PTH mRNA by phosphorus has been found to occur posttranscriptionally through binding of parathyroid cytosolic proteins to the 3’-untranslated region and, in particular, to the terminal 60 nucleotides of PTH mRNA (78). Third, a high serum phosphorus concentration inhibits the production of arachidonic acid by the parathyroid cell. Activation of the intracellular phospholipase A2-arachidonic acid signaling pathway has been shown to mediate the inhibition of PTH secretion by high calcium (20), which may explain the increase in PTH secretion induced by high phosphorus concentrations (2, 3). Finally, the work by Dusso et al. (31) suggests that the cyclin-dependent kinase inhibitor p21WAF1 and transforming growth factor-α mediate dietary phosphorus regulation of parathyroid cell growth.

**Bone Resistance to PTH**

Even in the early stages of CKD, many patients exhibit skeletal resistance to the action of PTH; therefore, high PTH levels are required to mobilize bone calcium and to maintain normal bone turnover (134). Numerous different mechanisms have been identified as contributing to the development of skeletal resistance in uremic patients. The accumulation of phosphorus prevents PTH-induced calcium efflux from bone (13, 111, 121). Furthermore, a possible role for altered vitamin D metabolism in skeletal resistance is supported by the observation that administering calcitriol to rats with chronic renal failure partially corrects the blunted calcemic response to PTH (109, 123). In addition, markedly decreased expression of PTH receptor mRNA (PTH/PTHrP mRNA) has been observed in the bones of uremic rats (27, 135) and persistently high PTH levels may lead to a decrease in the sensitivity of the PTH/PTHrP receptor (38). More recently, it has been suggested that particularly high levels of PTH metabolites (e.g., PTH 7–84) in uremic patients may oppose the action of bioactive PTH (PTH 1–84) (118). Last, elevated levels of uremic toxins and metabolic acidosis have also been implicated in the development of the condition (60, 122). Experiments in rats with renal failure showed that despite normalization of calcitriol, calcium, phosphorus, and PTH, some degree of skeletal resistance persisted (8, 14). Thus uremia, per se, may also play a role in the skeletal resistance to the calcemic action of PTH.

**PROGRESSION OF SHPT: REDUCED EXPRESSION OF CaRs AND VDRs**

**Parathyroid Gland Hyperplasia**

As previously detailed, in uremia, the parathyroid glands initially respond to functional demand by increasing PTH secretion and synthesis, but with constant stimulation the number of parathyroid cells begins to increase (91). Hyperplasia first manifests as an increase in the number of active secretory (chief) cells within the glands. This is followed by diffuse hyperplasia, in which there is a general increase in the total number of cells (133). Studies in uremic animals suggest that diffuse parathyroid hyperplasia is accompanied by a decrease in CaR and VDR expression (17, 102). When kidney failure is complete and dialysis is required, functional demand on the parathyroids is such that SHPT can become severe. In this setting, parathyroid glands become grossly enlarged and exhibit nodular hyperplasia (7, 133) (Fig. 4). In particularly advanced cases, nodules may fuse to form a single large tumor, although this is unusual. Analysis of parathyroid tissue from patients with advanced SHPT reveals that nodular hyperplasia is associated with increased cell proliferation and decreased CaR and VDR expression (37, 42, 64, 131–133, 143).

**Reduced Expression of CaRs and VDRs**

Reduced expression of CaRs in hyperplastic parathyroid glands has been demonstrated in a rat model (17), with the
same group of researchers subsequently showing that hyperplasia precedes downregulation of the receptor (102). Parathyroid hyperplasia was noted 2 days after nephrectomy in rats fed a high-phosphate diet, and the CaR content of cells began to decline 2 days later. In a study of human parathyroid glands, CaR expression was found to be reduced by ~60% in hyperplastic compared with normal parathyroid tissue (64). Gogusev et al. (42) differentiated between diffuse and nodular hyperplasia in their study, finding that CaR expression was reduced in both forms of cell growth, but that it was most marked in the latter (Fig. 5). When the expression of CaRs and VDRs was assessed, the presence of both receptors was markedly reduced in parathyroid glands from patients with SHPT compared with those from normal individuals (145).

Several authors have documented reduced VDR expression in hyperplastic human parathyroid glands (22, 37, 73, 130, 143). Fukuda et al. (37) examined VDR distribution in surgically excised parathyroid glands from chronic dialysis patients by immunohistochemistry. A lower density of VDR was observed in the parathyroids showing nodular hyperplasia than in those showing diffuse hyperplasia. The researchers also noted that potential nodule-forming areas in glands with diffuse hyperplasia were virtually devoid of staining for VDRs. Similar data relating to VDR expression were reported by Toku-moto et al. (130), who also examined VDR protein expression in normal parathyroid glands and tissues from patients with diffuse and nodular parathyroid hyperplasia. Using labeled VDR antibodies, they showed that VDR density was reduced in diffuse hyperplasia compared with controls, but not significantly so. VDR expression in nodular hyperplastic tissue was, however, significantly lower than in both diffuse hyperplasia and controls. Although an association between low VDR density and parathyroid cell proliferation has been shown, it is not yet clear whether the reduced VDR levels precede or follow development of hyperplasia.

"REFRACTORY" SHPT

The reduced CaR and VDR expression in hyperplasia causes the parathyroid glands to become increasingly resistant to regulation by calcium and calcitriol (67, 73, 105). Consequently, in patients with refractory SHPT, there is little restraint on PTH production and secretion. Very high levels of PTH may overcome skeletal resistance. Accordingly, a vicious cycle is set up in which PTH hypersecretion increases calcium and phosphorus levels, but as the parathyroids are now resistant to calcium regulation (70), PTH secretion continues unabated.

ABNORMAL CALCIUM SENSING IN SHPT

Patients with SHPT show a rightward shift of the PTH-calcium curve (increase in set point) and an increase in the minimum secretion rate of PTH, changes that are indicative of abnormal calcium sensing. These changes were documented by Malberti et al. (70), who compared the calcium set points of normal individuals, patients with normocalcemic SHPT, and patients with hypercalcemic SHPT. The PTH-calcium response curve moved upward and to the right of normal in both groups of patients with SHPT, indicating that greater concentrations of extracellular calcium are required to suppress PTH levels and that basal PTH secretion is elevated in SHPT (Fig. 6). The shift in calcium set point was of much greater magnitude in patients with hypercalcemia.

The findings of Malberti et al. are in agreement with those of Goodman et al. (45), who showed that the inhibitory effect of calcium ions on PTH release was blunted in SHPT and that this
effect was more pronounced in those with increased parathyroid gland mass. Rodriguez et al. (108) observed that abnormal increases in the set point were associated with severe SHPT and a poor response to calcitriol treatment. In addition, these authors observed that sustained increases in serum calcium, as a result of calcitriol therapy, were associated with an increase in set point, and patients with the highest serum calcium levels had the greatest rightward shift of the PTH-calcium curve. A subsequent study by the same group of researchers showed that in dialysis patients with advanced SHPT with refractory hyperparathyroidism, an increase in serum ionized calcium to a concentration of 1.35–1.4 mM resulted in a reduction in PTH levels from 959 ± 80 to 314 ± 50 pg/ml (a 65% decrease); moreover, in patients who responded to calcitriol treatment, the same elevation in serum calcium concentration reduced PTH from 586 ± 51 to 206 ± 32 pg/ml (a 67% decrease) (107). If decreases in CaR levels lead to an increase in set point, these decreases do not appear to prevent a marked reduction in PTH in response to hypercalcemia. These findings have been observed repeatedly in other work published by the same group (34, 112). In fact, according to Pahl et al. (90), an increase in the set point may not be associated with a lack of inhibitory effect by hypercalcemia. Furthermore, Ramirez et al. (100) evaluated PTH response to calcium in dialysis patients with a mean PTH level of 480 ± 238 pg/ml. These authors did not see a significant increase in set point compared with healthy controls. An increase in serum calcium from 1.2 to 1.35 mM reduced the PTH level by 70% in uremic patients compared with 80% in healthy controls (100). Thus different authors find that in dialysis patients there is a significant response to hypercalcemia despite a presumed decrease in CaR expression. These studies illustrate that although the decrease in CaR expression in advanced SHPT may result in decreased sensitivity to calcium, calcium concentrations above the set point appear to reduce PTH levels (100).

THE CaR AND MANAGEMENT OF SHPT

As a result of pathological changes that occur during parathyroid gland hyperplasia, the utility of current SHPT treatment modalities can be compromised. In severe or refractory SHPT, when parathyroid glands are grossly enlarged or exhibit monoclonal growth patterns, patients can become resistant to vitamin D therapy (24, 89, 107). This may be the result of a significant reduction in the expression of VDRs that negates the actions of vitamin D sterols.

Hypercalcemia is a common feature of severe or refractory SHPT and adds to the risk of vascular calcification that is already present in these patients because of high serum phosphorus levels. Calcitriol facilitates the absorption of calcium and phosphorus from the gastrointestinal tract, thus adding to the calcium and phosphorus load and increasing the risk of vascular calcifications still further (56, 59, 99). Hypercalcemia also limits the potential use of calcium-based phosphate binders for reducing hyperphosphatemia in SHPT.

There is, therefore, a need for new SHPT treatments that will allow PTH levels to be managed without undesirable effects on calcium and phosphorus levels. Given its central role in calcium homeostasis and, consequently, in the pathogenesis of SHPT, the CaR represents an attractive therapeutic target. Ligands that mimic or potentiate the effects of extracellular Ca\(^{2+}\) at the CaR have been termed calcimimetics, of which there are two mechanistically distinct types. Type I calcimimetics are agonists and include inorganic and organic polycations (85). Furthermore, type I calcimimetics directly stimulate the CaR by interacting with its extracellular domain. Type II calcimimetics are allosteric modulators of the CaR and are thought to interact with the membrane-spanning segments of the CaR, thereby increasing the receptor sensitivity to calcium. They induce a conformational change in the receptor, thus enhancing signal transduction processes, which include L-amino acids and phenylalkylamines (85, 47). Type II calcimimetic agents represent a new class of therapeutic agents that increase the sensitivity of the parathyroid gland CaR to extracellular calcium, thereby inhibiting PTH secretion and rapidly decreasing PTH levels. Typical examples of these phenylalkylamine derivatives are cinacalcet HCl, NPS R-568, and NPS R-467. Type II calcimimetics have been investigated as novel treatments for hyperparathyroidism.

Preclinical studies in rats provide some insights into the potential of calcimimetic treatment. The calcimimetic compound cinacalcet HCl was shown in a number of in vitro test systems to be a potent allosteric modulator of the CaR. Cinacalcet HCl increased the concentration of cytoplasmic calcium (EC\(_{50}\) 51 nM) in human embryonic kidney 293 cells expressing the human parathyroid receptor. Similarly, cinacalcet HCl (IC\(_{50}\) 28 nM) produced a concentration-dependent decrease in PTH secretion from cultured bovine parathyroid cells. The S-enantiomer of cinacalcet (SAMG 073) was at least 75-fold less active in these assay systems (84). In vivo studies in rats demonstrated that cinacalcet HCl is orally bioavailable and displays approximately linear pharmacokinetics over a dose range of 1–36 mg/kg (84). Furthermore, this compound suppressed serum PTH (Fig. 7) and blood ionized calcium levels and increased serum calcitonin levels in a dose-dependent manner (84). In a study in partially nephrectomized rats that were allowed to develop SHPT and osteitis fibrosa, subsequent administration of cinacalcet HCl was observed to ameliorate signs of bone disease. Histological examination of tibia and femurs showed that cinacalcet HCl significantly decreased fibrosis and cortical porosity compared with controls (137). These in vivo results are consistent with a previous study using...
the calcimimetic NPS R-568 (139). In addition, cinacalcet HCl was demonstrated to have beneficial effects on reducing parathyroid hyperplasia in subtotally nephrectomized rats (Fig. 8) (72). This is consistent with previous findings, showing that calcimimetic agents can lower PTH to levels similar to those in control animals and reduce parathyroid cell proliferation (138, 140).

More recent animal studies have shown possible benefits from the use of calcimimetics in reducing cardiovascular morbidity associated with SHPT and its treatment. For example, Ogata et al. (88) showed that NPS R-568 attenuated interstitial fibrosis and arteriolar wall thickening in the myocardium of nephrectomized rats. Furthermore, in a study in which cinacalcet HCl or calcitriol was administered to partially nephrectomized rats for 26 days, autopsy revealed aortic calcification in all animals receiving calcitriol (mean mineralization score, 3.25 vs. 0.00 for controls; \( P < 0.05 \)) compared with minimal calcification in one animal in the 1 mg/kg cinacalcet group and no mineralization in the 10 mg/kg cinacalcet group (\( n = 8 \) in each group) (71).

Calcimimetics have shown good efficacy in treating SHPT in humans. Data from small human studies involving NPS R-568 were encouraging, with the calcimimetic causing a rapid decrease in PTH levels (43). This initial promise was later fulfilled by the second-generation compound cinacalcet HCl (AMG 073) in larger trials (68, 98). Treatment with cinacalcet HCl was associated with significant sustained reductions in PTH, and also with meaningful reductions in Ca \( \times \) P, serum calcium, and serum phosphorus. In a publication by Block et al. (11), pooled results of two identical randomized, double-blind, placebo-controlled trials showed that cinacalcet was effective in significantly lowering PTH while concomitantly reducing Ca \( \times \) P, calcium, and phosphorus. The primary end point of these studies was the proportion of randomized patients who had a mean PTH level of 250 pg/ml (26.5 pmol/l) or less during the efficacy assessment phase. Forty-three percent of the cinacalcet group reached this end point, compared with 5% of the control group (\( P < 0.001 \)). Secondary end points included the proportion of patients with a reduction from baseline of at least 30% in mean PTH levels and the percentage change in the values for PTH, Ca \( \times \) P, calcium, and phosphorus. Overall, mean PTH values decreased by 43% in those patients receiving cinacalcet but increased by 9% in the control group (\( P < 0.001 \)). Similar proportions of patients achieved at least a 30% reduction in PTH levels during treatment with cinacalcet HCl, irrespective of baseline SHPT disease severity (defined by PTH values) (11). Furthermore, serum Ca \( \times \) P declined by 15% in the cinacalcet group and remained unchanged in the control group (\( P < 0.001 \)). Serum calcium and phosphorus levels were also reduced significantly compared with control patients.

CONCLUSIONS

In this review, we have endeavored to highlight the central role of serum calcium and the parathyroid CaR in the pathogenesis of SHPT. A new approach to SHPT management is required, and recent evidence demonstrates that the CaR is a key therapeutic target. In two phase 3 studies, cinacalcet HCl reduced PTH and corrected disturbances in mineral metabolism, suggesting that it will play an important role in the future treatment of SHPT.

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DISCLOSURES

Mariano Rodriguez has received industry-sponsored grants from Amgen, Inc., and has participated as a speaker at Amgen-sponsored events.
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