Calcium-sensing receptor and calcimimetic agents

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Calcium-sensing receptor and calcimimetic agents. Recognizing the role of the extracellular calcium-sensing receptor (CaR) in mineral metabolism greatly improves our understanding of calcium homeostasis. The biology of the low affinity, G-protein-coupled CaR and the effects of its activation in various tissues are reviewed. Physiological roles include regulation of parathyroid hormone (PTH) secretion by small changes in ionized calcium (Ca\(^{2+}\)) and control of urinary calcium excretion with small changes in blood Ca\(^{2+}\). The CaR also affects the renal handling of sodium, magnesium and water. Mutations affecting the CaR that make it either less or more sensitive to Ca\(^{2+}\) cause various clinical disorders; heterozygotes of mutations causing the CaR to be less sensitive to extracellular Ca\(^{2+}\) cause familial hypocalciuric hypercalcemia, while the homozygous form results in severe infantile hyperparathyroidism. Mutations causing increased sensitivity of the CaR to extracellular Ca\(^{2+}\) produce hereditary forms of hypoparathyroidism. Disorders, such as primary and secondary hyperparathyroidism, may exhibit acquired abnormalities of the CaR. Calcimimetic drugs, which amplify the sensitivity of the CaR to Ca\(^{2+}\), can suppress PTH levels, leading to a fall in blood Ca\(^{2+}\). Experiences with this agent in patients with secondary and primary hyperparathyroidism are summarized. In animals and humans with hyperparathyroidism, this agent produces a dose-dependent fall in PTH and blood Ca\(^{2+}\), with larger doses causing more sustained effects. The treatment has been short-term except for one patient followed for more than 600 days for parathyroid carcinoma; nonetheless the drug did not cause major side-effects and appears to be safe. Further long-term controlled studies are needed with calcimimetic agents of this type.

The discovery and cloning of the extracellular calcium-sensing receptor (CaR) and its molecular role in mineral metabolism represent a major scientific advance during the last decade [1, 2]. This low affinity, G-protein-coupled receptor is found in high concentrations on the surface of parathyroid cells, on calcitonin-secreting C-cells of the thyroid, at various sites along the nephron, in certain areas of the brain, on bone cells, and in other tissues. The activation of this receptor by small changes in extracellular ionized Ca (Ca\(^{2+}\)) accounts for both the steep inverse relationship between parathyroid hormone (PTH) levels and small changes of blood Ca\(^{2+}\) [1] and the sharp rise in urinary Ca that occurs as serum Ca rises slightly above a “threshold” value. This provides the mechanism whereby the body controls calcium homeostasis and tightly regulates the blood Ca\(^{2+}\). Alterations of this receptor are responsible for certain disease states, including familial hypocalciuric hypercalcemia (FHH) [3], severe infantile hyperparathyroidism [4, 5], and hereditary forms of hypoparathyroidism [6, 7]. Acquired alterations in the expression of CaR may play a role in the pathogenesis of secondary and primary hyperparathyroidism. A “calcimimetic” compound that enhances the affinity of the CaR for Ca\(^{2+}\) and reduces PTH secretion [8] has been explored as potential therapy for primary and secondary hyperparathyroidism. This review describes the CaR, its characteristics and its presumed roles in various physiologic and pathophysiologic states. Several clinical disorders involving the CaR are reviewed, and early data on the clinical use of a calcimimetic agent are presented.

Characteristics of the CaR

The features of the CaR, a member of the superfamily of G-protein-coupled receptors, are reviewed in detail elsewhere [9–11]. It has a large extracellular domain comprised of approximately 700 amino acids, seven membrane-spanning segments, and a cytoplasmic carboxyl terminal segment consisting of approximately 200 amino acids. Unique in comparison to many hormone receptors, which are activated by nanomolar quantities of agonist, the CaR is sensitive to relatively small changes in Ca\(^{2+}\) in extracellular fluid having a very high concentration of extracellular Ca\(^{2+}\) (over 1 mM). The CaR is a G-protein coupled receptor that on activation stimulates phospholipase C (PLC), which results in increased levels of inositol 1,3,5-triphosphate (IP3), which in turn elevates the cytosolic Ca\(^{2+}\) activity by mobilizing Ca from various intracellular sites. Activation of the CaR also inhibits the accumulation of hormone-stimulated intracellular cAMP [1].

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Another feature of the CaR is its lack of specificity; thus, it is stimulated by other divalent cations, such as magnesium, by the trivalent elements, gadolinium and lanthanum, and by polycationic compounds, such as neomycin and spermine. It is likely that the affinity of magnesium for the CaR is responsible for the effects of hypermagnesemia to suppress PTH secretion [12]. Also, many effects of elevated serum magnesium levels on the renal handling of calcium, sodium and chloride [13, 14] probably arise because of the activation of the renal tubular CaR by Mg$^{2+}$. The activation of the CaR by the polyvalent cations, neomycin and gentamicin, may account for certain effects of these agents on the kidney, including the development of nonoliguric renal failure, perhaps due to impairment of the vasopressin-sensitive renal concentrating mechanism via activation of the CaR of the collecting duct. Certain physiological roles and clinical implications of the CaR are listed in Table 1.

**Role of CaR in parathyroid cells**

The secretory response of the parathyroid cell to changes in blood Ca$^{2+}$ occurs within seconds, an observation suggesting that Ca$^{2+}$ acts directly on the plasma membrane [1]. The presence of CaR in very high concentrations on the parathyroid cell membrane provides the extracellular Ca$^{2+}$-sensing mechanism for these cells. The extracellular domain of the CaR contains several clusters of acidic amino acids that are involved in Ca$^{2+}$ binding. This provides a mechanism whereby the CaR regulates the secretion of PTH in response to small changes in extracellular Ca$^{2+}$ concentration [11, 15].

**Role of CaR in the kidney**

Within the kidney, transcripts of the CaR have been found in the juxtaglomerular apparatus, along the luminal proximal convoluted tubule, the basolateral surface of the cortical thick ascending limb (CTAL), and the apical as well as luminal membrane of the inner medullary collecting duct (IMCD) [10, 16]. The CaR localization in the CTAL may account for the effects of increased extracellular concentrations of Ca$^{2+}$ and Mg$^{2+}$ to inhibit the reabsorption of calcium, magnesium, sodium and chloride at this tubular site. Normally, Ca and Mg reabsorption occurs through the intercellular space and is driven by the high transtubular voltage gradient that is generated by the luminal Na$^+$/K$^+$/2Cl$^-$ cotransporter. Activation of the apical CaR stimulates PLC which releases arachidonic acid that is, in turn, metabolized by cytochrome P450; the active metabolites inhibit the apical K$^+$ channel and may also directly inhibit the Na$^+$/K$^+$/2Cl$^-$ cotransporter. These effects result in a marked reduction in transsluminal voltage, leading to reduced paracellular transport of Ca and Mg. Activation of the CaR also inhibits the PTH stimulated adenylate cyclase, reducing the cAMP-mediated transport of Ca and Mg.

In the IMCD, increased Ca$^{2+}$ inhibits the generation of cAMP by vasopressin through the effect of Ca$^{2+}$ on the CaR at the basolateral surface, a factor leading to less concentrated urine and polyuria. Also, increased tubular fluid Ca$^{2+}$ activates the luminal CaR which specifically reduces ADH-stimulated osmotic H$_2$O permeability via the aquaporin channels and lowers the urinary concentrating ability [17]. The action of increased ECF Ca$^{2+}$ to inhibit the Na$^+$/K$^+$/2Cl$^-$ cotransporter located in the thick ascending limb reduces the medullary hypertonicity and therefore the effectiveness of the countercurrent mechanism and reduces further the maximum urinary concentrating ability.

Thus, the effect of hypercalcemia to lower GFR, to reduce the renal cortical synthesis of calcitriol [18], to cause increased urinary excretion of both Ca and Mg, and to lead to increased volumes of dilute urine may arise, in whole or in part, through activation of the CaR within various parts of the nephron.

**CaR in other tissues**

The roles of the CaR that are found in the intestine [19], parts of the brain [20], and the lungs remain to be
clarified. It is probable that Ca\(^{2+}\) activation of a Ca\(^{2+}\)-sensitive receptor acts to stimulate bone formation and to inhibit bone resorption; moreover, the G-protein-coupled CaR has been identified in bone cell precursors [21]. Alternatively, Ca\(^{2+}\)-sensing proteins of other types may play a role in regulating bone metabolism [22, 23].

It seems apparent that the very tight regulation of blood Ca\(^{2+}\), which is affected in a major way by PTH, calcitriol, and, to a lesser extent, by calcitonin, occurs due to the tight “fine tuning” that arises from activation of the CaR to modulate PTH secretion and to regulate the renal excretion of calcium.

**CLINICAL DISORDERS INVOLVING THE CaR**

Several inherited disorders and acquired conditions involving calcium metabolism may arise from aberrations in the structure or density of the CaR. The pathogenesis of the peculiar syndrome, benign familial hypocalciuric hypercalcaemia (FHH) may be explained by one or more mutations causing a partial loss of sensitivity of the CaR to Ca\(^{2+}\) [3, 4, 24]. This genetic defect is associated with enhanced renal tubular reabsorption of calcium leading to a rise in serum Ca with a consequent increase in the filtered load of Ca. The rise in serum Ca is not sufficient to suppress PTH secretion because of the reduced affinity of Ca\(^{2+}\) for the CaR on parathyroid cells. These patients are totally asymptomatic, but they have persistent mild hypercalcemia, low urinary Ca, and serum PTH levels that are not suppressed appropriately but are in the upper range of normal or even above normal. Of interest, these patients exhibit a normal urinary concentrating capacity, in contrast to findings in patients with primary hyperparathyroidism [25]. The condition, neonatal severe hyperparathyroidism, with severe hypercalcemia and multiple fractures that arise due to hyperparathyroid bone disease, represents the homozygotic inheritance of this mutation [19, 26]. In knockout mice lacking the CaR, modest and benign elevations of serum Ca and hypocalciuria are observed in heterozygotes, while homozygotes exhibit severe hypercalcaemia, markedly elevated PTH levels, skeletal abnormalities and premature death [27]. Mutations of other types that involve the CaR have been reported to cause somewhat less severe forms of neonatal hyperparathyroidism [3, 28]; these are beyond the scope of this review.

The syndrome of autosomal dominant hypocalcaemia with hypercalciuria is now known to arise from several mutations that result in increased sensitivity of the CaR to Ca\(^{2+}\) [6, 7, 29]. The affected individuals generally have severe hypocalcaemia that is often treated with calcium and vitamin D. Nephrocalcinosis, renal stones and impaired renal function are common [30]. Since it is often difficult to separate effects of the basic disorder from those produced by the treatment, early recognition of such cases is important for proper management.

There may be acquired disorders involving the CaR. Various data indicate that parathyroid glands obtained surgically from uremic patients with secondary hyperparathyroidism may exhibit reduced expression of the CaR on the surface of parathyroid cells [31, 32]. In patients with parathyroid adenomas or parathyroid carcinoma the data are somewhat inconsistent, although reduced staining is found in some glands. It is possible that changes in the density of CaR in parathyroid cells may account, in part, for the shift in “set point” of parathyroid cells (i.e., the calcium concentration required to suppress maximal PTH secretion by 50%). In experimental animals, data suggesting that there is regulation of the CaR are either negative or controversial. Two studies done in vitamin D-deficient rats have demonstrated no regulation of the mRNA for CaR by Ca\(^{2+}\) [33, 34]. One study reported no effect of calcitriol on the mRNA for the CaR, while another study found a 40% reduction of mRNA for CaR in vitamin D-deficient rats that was restored by calcitriol replacement [34]. Thus, some uncertainty exists about the role of altered regulation of the CaR being a major factor predisposing to the development of progressive secondary hyperparathyroidism in patients with progressive renal failure.

Experimental data indicate there are increases in the density of the CaR on the parathyroid cells of rats of increasing age but with decreased response; in the kidney, no alterations in the CaR were observed [35]. If a similar process exists in man, it could account for the changes in PTH levels with age and the propensity for the development of hyperparathyroidism with advancing years. There is evidence that the CaR expression in renal tissue is modestly reduced in experimentally induced renal failure in rats; this might contribute to hypocalciuria noted with renal insufficiency [36].

**EXPERIMENTAL AND CLINICAL USE OF CALCIMIMETIC AGENTS**

For the clinician, the development calcimimetic compounds that modulate the CaR making it more sensitive to Ca\(^{2+}\) and that can suppress PTH secretion may provide a means for the medical treatment for both primary and secondary hyperparathyroidism. The compound, R-568, which was developed by scientists at NPS Pharmaceuticals [37, 38], has undergone the most extensive testing.

In rats with experimental uremia, the proliferation of parathyroid cells, which is increased in this model of secondary hyperparathyroidism, was measured by in vivo labelling with 5-bromodeoxyuridine (BrdU). Groups of uremic rats that were treated with two different doses of R-568 for 4 days were compared to those receiving vehicle. The degree of proliferation of the parathyroid
cells, as assessed by BrdU labelling, was reduced by 20 and 50% by the low and high doses of R-568, respectively. There were no effects found in other proliferating tissues. Parathyroid cell volume was reduced only with the high dose, although increased apoptosis was not found [39]. The effect of R-568 on osteitis fibrosa was evaluated in another study of uremic rats [40]. Two different doses of the calcimimetic were given daily for 30 days; there was a dose-related reduction of PTH levels, and there was marked improvement in the degree of osteitis fibrosa. The reductions of cortical bone mineral density and of bone stiffness that developed with secondary hyperparathyroidism were largely reversed by treatment with R-568.

Several reports have documented the effectiveness of single doses or of two daily doses of R-568 to reduce PTH levels in patients with primary and secondary hyperparathyroidism [41, 42; abstract; J Am Soc Nephrol 9:516A, 1998]. The initial clinical experiences with the calcimimetic agent, R-568, in patients are summarized in Table 2. In primary and secondary hyperparathyroidism, a single initial oral dose of R-568 produced a maximum suppression of PTH levels, that was dose-dependent, at one to two hours after administration. The decrease in blood Ca\(^{2+}\) was significant only with the larger dose and occurred only after PTH levels fell. The percentage reductions of PTH from pretreatment values were remarkably similar following 100–200 mg doses of R-568, with the average PTH reductions between 63% and 73% in two reported studies [41, 42] and two preliminary reports (abstract; J Am Soc Nephrol 9:516A, 1998; abstract; J Am Soc Nephrol 10:619A, 1999); this suppression was independent of the pretreatment PTH, which averaged 77 pg/ml in patients with primary hyperparathyroidism and varied from 218 to 1287 pg/ml in the groups with secondary hyperparathyroidism.

In women with mild primary hyperparathyroidism, the maximal decrease in PTH occurred by one hour after the lowest doses of R-568, while it occurred at two hours after larger doses [41]. The duration of effect increased with the dose; the PTH levels had returned to baseline by four hours after doses of 80 mg or less but returned to baseline only at 8 hr after the 160 mg dose. A small but significant reduction of blood Ca\(^{2+}\) occurred only with the largest dose, 160 mg. After this dose, urinary calcium, expressed as the calcium/creatinine ratio, rose by four hours but returned close to baseline by 8 hr.

Two trials in dialysis patients with secondary hyperparathyroidism employed two separate doses of R-568, each given on two successive days [42, abstract; J Am Soc Nephrol 9:516A, 1998]. In seven patients with mild hyperparathyroidism [42], doses of 40 or 80 mg caused PTH levels to fall by more than 30% after the first dose in 5 of 7 patients and more than 60% after the second dose in 6 of 7 patients. With doses of 120 and 200 mg, PTH was reduced by more than 60% after the first dose in 6 of 7 patients. After 24 hr, the pretreatment PTH level was still 50% lower than the initial basal value; nonetheless, the PTH fell 50% or more after the second dose in 6 of 7 patients. Blood Ca\(^{2+}\) was not changed significantly after the low dose but fell significantly after the high dose (Table 2). Preliminary data from hemodialysis patients with severe secondary hyperparathyroidism (abstract; J Am Soc Nephrol 9:516A, 1998) revealed significant reductions after 100 mg and even greater reductions after 200 mg of R-568. The PTH levels had not returned to baseline after 24 hr, when the second dose caused a similar percentage reduction of PTH. Serum total Ca levels fell, with more marked reductions after the larger dose. Calcitonin levels did not change after either dose.

In a preliminary report (abstract; J Am Soc Nephrol 10:619A, 1999), the administration of R-568 in doses of 100 mg per day led to the sustained suppression of PTH levels that persisted for 15 days, the duration of treatment. Hypocalcemia led to interruption of the study in 3 patients as the blood Ca\(^{2+}\) fell below 1.0 mm.

All these short-term trials indicate that the calcimimetic, R-568, effectively reduces plasma PTH levels in patients with mild primary hyperparathyroidism and those with secondary hyperparathyroidism of varying severity. The duration of action following a dose was substantially longer in the patients with end-stage renal disease than in those with primary hyperparathyroidism and normal renal function. In each study, there was a reduction in blood Ca\(^{2+}\) or total serum Ca that occurred after the PTH levels were reduced. The marked and consistent degree of reduction of PTH levels produced by calcimimetic treatment of patients with widely different PTH levels suggests that a decrease in CaR density occurring in some parathyroid glands may be less important than was initially believed.

The calcimimetic agent R-568 was given to a patient with inoperable parathyroid carcinoma, who presented with hypercalcemia (blood Ca\(^{2+}\) 1.96 mm), high PTH levels (1128 pg/ml), and altered mental status; the hypercalcemia failed to respond to intravenous saline and furosemide, several doses of intravenous pamidronate and salmon calcitonin over 18 days [43]. The calcimimetic was initiated at 200 mg/day and subsequently increased to 400 mg/day. The patient’s symptoms improved after three days of R-568 treatment, and he was discharged home after 28 days of treatment with a blood Ca\(^{2+}\) of 1.53 mm and PTH level of 357 pg/ml. Treatment with the calcimimetic was continued and the dose was titrated up to 600 mg/day; this treatment maintained the total serum calcium between 2.75 and 3.0 mm despite the progressive increase in PTH levels to 2000–3500 pg/ml; this PTH increase probably occurred due to progression of the parathyroid carcinoma. The patient remained active,
### Table 2. Clinical experience with the use of the calcimimetic agent R-568

<table>
<thead>
<tr>
<th>Disorder: (reference) [no. of patients]</th>
<th>Treatment protocol: Duration of treatment</th>
<th>Dose R-568 (mg/dose)</th>
<th>PTH change [2 hr] % from baseline (P value)</th>
<th>Blood ionized Ca ++, mm (P value)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary hyperparathyroidism (41) [20 women]</td>
<td>Single doses: 4–13 patients received each dose; Followed for 36 hr</td>
<td>Placebo and 4, 10, 20, 80 and 160 mg</td>
<td>≤10 mg: no change 20 mg: −26% ( = 0.03) 80 mg: −42% ( = 0.01) 160 mg: −73% ( = 0.005)</td>
<td>≤40 mg: no change 80 mg: slight decrease (NS) 160 mg: 1.35 → 1.30 (0.03)</td>
<td>160 mg: Urine Ca/Creat: 0.27–0.63 mg/mg after 4 hr, then fell to 0.40 at 8 hr</td>
</tr>
<tr>
<td>Secondary hyperparathyroidism, mild; (42) [7 hemodialysis patients]</td>
<td>1 dose/day × 2d Low: 40 or 80 mg High (H) dose, N = 7</td>
<td>Day 1: −37%; Day 2: −52% Basal PTH, 186 ± 48 pg/ml Day 1: −63%; Day 2: −61%</td>
<td>Low: Basal PTH, 1.24→1.21, NS 1.40→1.22 at 24 hr→1.14 at 48 hr (both, 0.005)</td>
<td>High dose: PTH remained below baseline at 24 and 48 hr; Calcitonin levels rose after treatment</td>
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<tr>
<td>Secondary hyperparathyroidism, moderate to severe; (abstract, J Am Soc Nephrol 9:516A, 1998) [12 hemodialysis patients]</td>
<td>1 dose/day × 2 days; Low (L) dose, N = 6; High (H) dose, N = 6</td>
<td>Low: 100 mg/day High: 200 mg/day Day 1: −59%, High: Basal PTH, 1387 pg/ml; Mean, 2.6 → 2.3 High: 83% of patients &lt; 2.1 Mean, 2.6 → 1.98</td>
<td>Low: Basal PTH, 1030 pg/ml; High: Basal PTH, 218 ± 52 pg/ml Day 1: −73%, Day 2: Similar reductions Mean, 2.6 → 1.98</td>
<td>Mild symptoms of hypocalcemia disappeared without treatment</td>
<td></td>
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<td>Secondary hyperparathyroidism, moderate to severe; (abstract, J Am Soc Nephrol 10:619A, 1999) [21 hemodialysis patients]</td>
<td>Once daily dosing; Blinded, placebo-controlled: R-568, N = 16 Placebo, N = 5</td>
<td>100 mg/day for 15 days Basal PTH, 509 ± 112 pg/ml; After 1st dose: PTH was −66%, −70 &amp; −30% at 1, 4, &amp; 24 hr (P &lt; 0.01); Pre-Rx PTH on days 5, 8, 11, 12 &amp; 15 was 47–69% of baseline (each, P &lt; 0.05). PTH unchanged with placebo</td>
<td>Pre-Rx: 1.31 ± 0.02 → 1.13 ± 0.02, Day 2 → 1.23 ± 0.03, Day 3 3 patients with blood Ca++ &lt; 100 mg were withdrawn. Ca++ unchanged in placebo</td>
<td>The suppressive effect was sustained over 15 days; 5 patients had symptoms of hypocalcemia</td>
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<tr>
<td>Parathyroid carcinoma (43) [1 patient, treated 600 days]</td>
<td>Before R-568 treatment, TSCa not lowered by NaCl, pamidronate or calcitonin 200 up to 600 mg/d over approx 600 days (4 doses/day)</td>
<td>PreRx: 600–1050 pg/ml; Days 25–40: 250–600; Rose days 50–500 to 5000 pg/ml</td>
<td>TSCa, PreRx: 3.25; Days 20–30, &lt; 2.75; Days 250–650, 2.75–3.10</td>
<td>Hypercalcemic symptoms improved after 3 days of Rx; Urine Ca fell on days 20–40 with serum creatinine of 2.3 to 2.2 mg/dl</td>
<td></td>
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</table>

**Abbreviations are:** Rx, treatment; TSCa, total serum calcium; Basal, pre-treatment value; creat, creatinine; PTH, intact parathyroid hormone; N, number of patients; NS, not significant.
extracellular calcium-sensing receptor in human and mouse bone

REFERENCES


