Modulation and action of the calcium-sensing receptor

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Abstract

The discovery and cloning of the calcium-sensing receptor (CaR) in 1993 has led to a better understanding of the regulation of calcium homoeostasis. Following activation by extracellular calcium ions, the CaR triggers a cascade of intracellular events. These events result in the release of secondary messengers, which have a number of biological effects, the most important of which is a reduction in parathyroid hormone (PTH) secretion. The way in which calcium acts on the CaR varies depending on the cell type. In the parathyroid gland cell, activation of the CaR by elevated serum levels of calcium leads to a decrease in PTH secretion. In the kidney, CaR activation is thought to have several different actions, leading to enhanced reabsorption of sodium chloride and increased calcium and magnesium excretion in the renal tubules. CaRs are also found in other tissues in the body that are not involved in calcium homoeostasis, suggesting that the CaR has actions that are not associated with calcium homoeostasis. In patients with end-stage renal disease, parathyroid gland hyperplasia is associated with down-regulation of the CaR. Discovery of the CaR has allowed the development of a group of drugs called calcimimetics, which mimic or potentiate the actions of extracellular calcium on the CaR. These compounds have considerable potential for the treatment of primary and secondary hyperparathyroidism.

Keywords: calcimimetics; calcium-sensing receptor; parathyroid gland; parathyroid hormone; secondary hyperparathyroidism

Introduction

Extracellular calcium is essential to a number of processes throughout the body, including blood coagulation, bone mineralization, enzymatic regulation and control of the permeability and excitability of plasma membranes. The regulation of extracellular ionized calcium (Ca\(^{2+}\)) is tightly controlled within a narrow range. Ca\(^{2+}\) concentrations are regulated largely by parathyroid hormone (PTH), which increases serum levels of calcium in order to maintain calcium homoeostasis. The past 10 years have seen significant progress in our understanding of this homoeostasis and, in particular, the identification and cloning of the extracellular calcium-sensing receptor (CaR) on the cells of the parathyroid glands. It has been shown subsequently that CaR expression in cells controlling systemic calcium homoeostasis is not confined to the parathyroid gland, and that the receptor is also expressed on many other cell types, including those of the kidney, thyroid C-cell, bone and intestines. CaR expression has also been observed in multiple tissues outside the calcium homoeostasis system, such as the brain, pituitary gland, stomach, breast and ovary, suggesting a possible role for CaR in processes that are not directly linked to calcium homoeostasis. Here, the role of the CaR in the regulation of extracellular calcium homoeostasis will be discussed.

Parathyroid and renal actions of the calcium-sensing receptor

The CaR is a member of the G-protein-coupled superfamily of receptors. It differs from most other G-protein-coupled receptors in that it possesses a large extracellular amino-terminal domain and a smaller intracellular carboxy-terminal domain. The CaR is not specific for Ca\(^{2+}\) as it also responds to a variety of divalent and trivalent cations, including magnesium, aluminium and gadolinium, as well as polycations, all of which have in common a net positive charge at physiological pH. A schematic diagram of the CaR on the parathyroid cell membrane is shown in Figure 1. As previously stated, the CaR is not specific for Ca\(^{2+}\), but Ca\(^{2+}\) has the highest affinity for the CaR compared with other cations.
Activation of CaR on the parathyroid gland cells by Ca\(^{2+}\) leads to the activation of a number of secondary messengers in a cascade of events eventually resulting in the inhibition of synthesis and secretion of PTH (Figure 1). The CaR, following activation by elevated serum levels of Ca\(^{2+}\), couples to phospholipase C (PLC) via a G-protein (probably G\(_{q}\) or G\(_{11}\)) and then indirectly to phospholipase A\(_2\) (PLA\(_2\)). PLA\(_2\) then acts on membrane phospholipids to release arachidonic acid, which in turn is converted to leukotriene metabolites that inhibit PTH secretion. Kifor et al. have also studied the effects of parathyroid gland CaRs on the mitogen-activated protein kinase (MAPK) pathway using bovine parathyroid gland cells [5]. Activation of the CaR results in G\(_{q}/G_{11}\)-mediated activation of phosphatidylinositol (PI)-PLC, which in turn mobilizes intracellular calcium leading to the inhibition of PTH secretion [6]. PI–PLC also activates protein kinase C (PKC), leading to PKC-mediated stimulation of the MAPK cascade. CaR activates MAPK via a pertussis toxin-sensitive G-protein, thought to be G\(_i\), leading to downstream activation of a tyrosine kinase-dependent process involving ras- and raf-dependent pathways. Activated MAPK phosphorylates and activates PLA\(_2\), releasing free arachidonic acid. Arachidonic acid is metabolized to active mediators, such as hydroxyperoxyecosatetraenoic acid or hydroxyecosatetraenoic acid (20-HETE), which can then decrease PTH secretion [7,8].

The way in which extracellular Ca\(^{2+}\) acts on the CaR varies between cell types, and this can be illustrated in the kidney, where hypercalcaemia can alter many aspects of renal function, including a reduction in glomerular filtration rate [9] and an increase in renal vasoconstriction [10]. In the thick ascending limb of Henle, hypercalcaemia reduces cAMP generation induced by various hormones at this site [11]. The CaR is expressed on the basolateral membrane. When Ca\(^{2+}\) activates the receptor, there is a resultant enhancement in the formation of arachidonic acid-derived 20-HETE, which can reversibly inhibit apical K\(^{+}\) channels [12], as well as directly inhibiting Na\(^{+}\), K\(^{+}\) and Cl\(^{-}\)/C\(_{0}\) activity [13,14]. The inhibition of cAMP formation by the action of Ca\(^{2+}\) on the CaR leads, through a number of intracellular pathways, to a decrease in Na\(^{+}\), K\(^{+}\) and Cl\(^{-}\)/C\(_{0}\) reabsorption via their common transporter in the thick ascending limb and a decrease in K\(^{+}\) transfer across a specific potassium channel. In addition, CaR-mediated inhibition of cAMP reduces the stimulatory action of vasopressin and PTH on the tubule epithelium, in this area of the nephron and further downstream, respectively. The Na\(^{+}\), K\(^{+}\), Cl\(^{-}\) co-transporter can transport NH\(_{4}\)\(^{+}\) instead of K\(^{+}\) and can therefore also regulate net urinary acid excretion, suggesting that CaR-mediated processes may also be responsible for maintaining a correct acid–base balance [15]. Activation of CaRs on the renal tubules produces several different effects. CaR activation decreases Cl\(^{-}\) resorption and K\(^{+}\) secretion through the apical channel in the thick ascending limb. This decreases the lumen-positive voltage in the tubular lumen leading to decreased Ca\(^{2+}\) and Mg\(^{2+}\) reabsorption and, consequently, their increased distal delivery. In the distal tubule, the CaR is expressed on the basolateral side of the epithelium. Stimulation of the CaR by calcium in the distal tubule leads to inhibition of calcium transport [16]. CaRs are also expressed on the luminal side of the medullary collecting duct epithelium of the kidney. CaRs at this site use the same vesicles as the...
vasopressin-activated aquaporin-2 water channels, whose reduced insertion in the apical cell membrane in response to a rise in luminal Ca\(^{2+}\) concentration above a certain level leads to a decrease in water reabsorption. This, in turn, could be considered to be an efficient mechanism against excessive calcium concentration and calcium crystal formation in the terminal urine [17]. Thus, the CaR is not only involved in PTH secretion, but is also involved in many different processes in the kidney, participating directly and indirectly in the maintenance of calcium homeostasis and in several other homoeostatic regulations.

**Modulation of the CaR**

Under physiological conditions, Ca\(^{2+}\) does not appear to be a major regulator of CaR expression, and studies suggest that other factors may play a role in CaR regulation [18]. CaR expression is known to be downregulated in primary and secondary hyperparathyroidism (SHPT) [19,20], but this downregulation has been shown to occur in experimental animals only after the onset of parathyroid hyperplasia, suggesting that the reduction of CaR is a consequence, rather than the cause, of SHPT [21].

In a more recent study, the role of phosphate in CaR expression in rats with SHPT was examined [22]. Uraemic rats with established SHPT initially were fed a high-phosphate diet for 4 weeks, or given a high-phosphate diet for 2 weeks followed by a low-phosphate diet for 2 weeks. When the levels of CaRs on the parathyroid glands were measured, CaR expression was shown to decrease after 2 weeks of being on the high-phosphate diet and then to decrease further at 4 weeks. In the rats that were switched to a low-phosphate diet after 2 weeks of the high-phosphate diet, however, the expression of the CaR increased during weeks 3 and 4 of the study. These changes in CaR expression were accompanied by changes in parathyroid cell proliferation. The proliferation rate was high when the rats were on the high-phosphate diet and was reduced to normal levels when they were switched to a low-phosphate diet, but only after a lag period of ~2 days. As upregulation of CaR expression occurred after a reduction in parathyroid cell proliferation, the authors concluded that this was a secondary event and does not play a role in correcting SHPT. Similar results have also been obtained by other researchers [23].

The role of vitamin D in CaR expression remains controversial. Although one study has shown that vitamin D regulates CaR expression [24], another study failed to confirm this [25].

With regard to the modulation of CaR under pathophysiological conditions, there is substantial evidence to show that the receptor is linked to the regulation of PTH secretion. Its activation represents the key physiological mechanism inhibiting PTH secretion [26]. There is also evidence indicating that parathyroid gland hyperplasia is associated with CaR downregulation [19,20]. As both excessive secretion of PTH and parathyroid gland hyperplasia are features of SHPT, it is important to understand precisely how a decrease in CaR expression in these conditions contributes to parathyroid gland over-function.

There are a few naturally occurring, genetically transmitted diseases that result from mutations in the CaR gene. Data from the three distinct phenotypes add to the body of evidence supporting the key role of the CaR in the regulation of serum calcium homeostasis.

Familial hypocalciferous hypercalcaemia (FHH) is caused by mutations that reduce the activity of the CaR. The condition is characterized by moderately elevated serum Ca\(^{2+}\) levels, lower than expected calciumuria and increased PTH levels [27,28]. Individuals with FHH are generally asymptomatic and show none of the morbidity associated with hypercalcaemia. In contrast, neonatal severe hyperparathyroidism (NSHPT), the homozygous form of FHH [29], is characterized by severe hypercalcaemia, skeletal demineralization, parathyroid gland hyperplasia with severe hyperparathyroidism, respiratory distress and hypertonia [30, 31]. The condition is usually fatal unless total parathyroidectomy is performed at an early stage.

A different type of mutation in the CaR gives rise to the rare condition of autosomal dominant hypocalcaemia or familial hypocalcaemia. The CaR of patients with this condition has an increased affinity for calcium, thus enhancing its activity at normal or even low serum calcium levels [32]. Mutations on the CaR gene are all characterized by an abnormal response to Ca\(^{2+}\) from the parathyroid gland and the kidneys. As previously discussed, however, the CaR gene is expressed in many different tissues throughout the body, and it is not clear what contribution these other tissues make to the signs and symptoms of FHH and NSHPT, or which abnormalities are a direct result of CaR mutations and which are secondary to hypercalcaemia and hyperparathyroidism. Recent work conducted by Kos et al. studied CaR-deficient mice in which the PTH gene was also eliminated in order to separate the effects of hypercalcaemia and hyperparathyroidism from the direct effects of CaR deficiency [3]. They demonstrated that the CaR itself played an important role in the regulation of serum Ca\(^{2+}\), and this was also true for urine calcium excretion. Interestingly, when the authors examined the parathyroid glands of these animals, there was a marked increase in the size of the glands, compared with the controls, indicating that the CaR can regulate hyperplasia of the parathyroid tissue, even in the absence of PTH.

**Effect of calcimimetics on PTH secretion and parathyroid gland cell proliferation**

The recognition of the role of the CaR and the effect of calcium on the parathyroid gland has provided an ideal target for the development of new drugs that may be able to control circulating levels of PTH. Calcimimetics modulate the CaR, making it more...
sensitive to the effects of extracellular calcium, and lead to the inhibition of PTH secretion. These compounds could, therefore, potentially be useful in the treatment of SHPT. The onset of SHPT in patients with chronic renal failure occurs long before the start of dialysis. Together with evidence from animal models of hyperparathyroidism and from human parathyroid gland cell cultures, recently published data show that treatment with cinacalcet produced a significant benefit in the control of serum PTH levels in pre-dialysis patients [33].

In vitro studies

An in vitro model using cultured human parathyroid gland cells derived from uraemic patients was used by our group to examine whether calcium and the calcimimetic drug NPS R-467, a precursor of cinacalcet, exerted a direct effect on PTH secretion and parathyroid cell proliferation [34]. When the cells were exposed to a medium containing a high calcium concentration, the secretion of PTH was suppressed. When the calcimimetic was added to the medium in conjunction with a low calcium concentration, which by itself stimulated PTH release, PTH secretion was again suppressed, as was expected.

Increasing the calcium concentration of the medium, however, caused an increase, rather than an expected decrease, in parathyroid cell proliferation (Figure 3). In contrast, in the presence of NPS R-467, we found that there was a decrease in the cell proliferation rate in vitro (Figure 4). It is known that a low-calcium diet in humans and animals leads to an increase in parathyroid cell proliferation, and other studies have suggested that parathyroid cell proliferation is inhibited in response to increased extracellular calcium concentrations. In order to explain these apparently contradictory observations, we proposed that the effect of calcium on parathyroid cell proliferation may occur through two separate pathways [34]. Under normal conditions, calcium acts via the CaR to inhibit cell proliferation in parathyroid tissue, but there may be a second pathway through which calcium stimulates cell proliferation, for instance via other calcium-sensing receptors or calcium channels. The latter pathway may have been dominant in the uraemic parathyroid cell culture used in our study. When a calcimimetic is given, this may have the effect of restoring to dominance the first pathway of suppression of proliferation. The tissue samples used in our study were taken from uraemic patients with long-term renal failure and severe SHPT, and it is known that such tissues generally exhibit decreased CaR expression [19,20]. It is possible that the number of CaRs expressed in this experimental model may have been high enough to exhibit a response to increased calcium levels (inhibition of PTH secretion), but insufficient to inhibit cell proliferation. Thus, inhibition of cell proliferation in response to extracellular calcium might predominate in tissues in which there is high CaR expression or activation, such as occurs in the normal parathyroid gland, and stimulation of cell proliferation in response to calcium might predominate when CaR expression is low, such as in chronic kidney disease (SHPT).

In vivo studies

Wada and co-workers demonstrated that the calcimimetic NPS R-568 administered orally to subtotally nephrectomized rats reduced PTH levels and inhibited the acute parathyroid gland cell proliferative response compared with control rats [35]. In a later
study [36], the same group confirmed that both daily oral doses and continuous subcutaneous infusions of NPS R-568 administered to rats with uraemia continued to inhibit parathyroid gland cell hyperplasia and glandular enlargement throughout an 8 week study period. They also demonstrated that although increased average cell volume (hypertrophy) also contributed to the enlargement of the parathyroid gland, the greater contributor was in fact an increase in cell number (hyperplasia) (Figure 5). In a recent study by Martin et al. [37], cinacalcet decreased parathyroid hyperplasia in nephrectomized rats. Cinacalcet caused significant reductions in proliferating cell nuclear antigen (PCNA)-positive cells and significant decreases in parathyroid weight compared with vehicle controls. These studies demonstrated that cinacalcet reduced parathyroid proliferation and could attenuate established parathyroid hyperplasia in a rodent model of chronic renal insufficiency accompanied by SHPT. Another recent study, by Silver et al. [38], found that the calcimimetic NPS R-568 markedly decreased PTH mRNA levels in rats with SHPT due to experimental uraemia. PTH mRNA levels were increased 3-fold in rats with induced renal failure compared with controls. However, when these rats were administered NPS R-568, a marked and dose-dependent decrease in PTH mRNA levels was observed. NPS R-568 regulates the parathyroid not only at the levels of parathyroid cell proliferation and PTH secretion but also at the level of PTH gene expression. Thus, in vivo, calcimimetics clearly prevent the parathyroid glands from increasing in size in uraemic rats as a result of an anti-proliferative action.

Results from phase III studies have demonstrated that cinacalcet significantly reduces mean intact PTH (iPTH) compared with those on placebo (control; \( P < 0.001 \)) [39–41]. A significantly greater proportion of patients receiving cinacalcet achieved the target iPTH level of \( \leq 250 \) pg/ml compared with control patients \( (P < 0.001) \). Furthermore, reductions in iPTH were accompanied by significant reductions in serum Ca \( (P < 0.001) \), phosphorus \( (P < 0.001) \) and Ca \( \times \) P \( (P < 0.001) \) [40,41].

**Conclusion**

The CaR is a member of the superfamily of receptors coupled with guanine nucleotide regulatory G-protein and is extremely sensitive to physiological changes in extracellular calcium. It is characterized by a large extracellular domain and a smaller intracellular domain. CaR is expressed on the surface of parathyroid gland cells and other cells throughout the body,
which may or may not be involved in the regulation of calcium homoeostasis. Activation of the receptor by Ca\(^{2+}\) initiates a cascade of intracellular events that results in the decreased synthesis and secretion of PTH. Expression of the receptor is modulated under physiological and pathological conditions. In renal failure, expression of the CaR is reduced and plays a key role in SHPT pathogenesis. Activation of the CaR by calcimimetic compounds provides a novel and effective means of reducing PTH secretion. The calcimimetics NPS R-568, NPS R-467 and cinacalcet have been shown to decrease PTH levels and have allowed the study of CaR function in vivo, demonstrating the importance of the CaR in the control of parathyroid gland cell proliferation. Cinacalcet has proven to be safe and effective in the large phase III studies completed to date, and it may provide a new and exciting treatment option in the management of SHPT.

Conflict of interest statement. None declared.

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