Control of parathyroid cell growth by calcimimetics

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Abstract
Parathyroid cell hyperplasia is commonly observed in patients with chronic renal insufficiency and largely accounts for refractory secondary hyperparathyroidism. Calcimimetics are newly synthesized compounds that activate a calcium receptor on the parathyroid cell and can suppress parathyroid hormone secretion. The calcimimetic compound AMG 073 has been examined in clinical trials, and the data obtained so far demonstrate that the compound can lower the circulating levels of parathyroid hormone and calcium–phosphorus product in patients with secondary hyperparathyroidism. Furthermore, experimental evidence indicates that calcimimetics have the potential to inhibit parathyroid cell proliferation and block the progression of parathyroid hyperplasia. These beneficial effects, especially the potential to control parathyroid cell proliferation, would place calcimimetics among the essential therapeutic agents for treating secondary hyperparathyroidism.

Keywords: calcimimetics; calcium receptor; chronic renal insufficiency; hyperparathyroidism; parathyroid hormone; parathyroid hyperplasia

Introduction
Management of parathyroid hyperplasia in chronic renal insufficiency (CRI) is still a challenge [1–3]. Despite intensive use of calcitriol therapy, secondary hyperparathyroidism often progresses over the long term from diffuse (hyperplastic) to nodular (adenomatous) forms that are refractory to conventional medical therapy [4,5]. Small organic molecules named ‘calcimimetics’ that can enhance the sensitivity of the calcium receptor (CaR) to extracellular Ca²⁺ as its physiological ligand [6,7], and their efficacy and safety have been examined in clinical trials [8–11]. Calcimimetics can suppress parathyroid hormone (PTH) secretion, and these compounds would be expected to improve medical treatment of hyperparathyroidism secondary to CRI [8–11]. Moreover, experimental evidence indicates that calcimimetics have the potential to inhibit parathyroid cell proliferation and block the progression of parathyroid hyperplasia [12–15]. This review summarizes the pharmacological studies evaluating the inhibition of parathyroid cell proliferation by calcimimetics. These data demonstrate that the CaR plays an essential role in regulating parathyroid cell proliferation [16,17]. Also, this review examines the intracellular signalling pathways linking the CaR and cell proliferation. The finding that calcimimetics can suppress parathyroid cell proliferation is important in understanding the pathogenesis of parathyroid hyperplasia and is of significant therapeutic relevance.

Calcium receptor and the control of PTH secretion
The parathyroid glands play an important role in maintaining the plasma Ca²⁺ concentration within a narrow physiological range [18,19]. Brown et al. identified a G-protein-coupled receptor (GPCR) that functions as a key element in the parathyroid Ca²⁺-sensing mechanism, called the calcium-sensing receptor or calcium receptor because it recognizes Ca²⁺ as its physiological ligand [20]. The CaR consists of an extracellular domain N-terminal of ~600 amino acids, a central core of ~250 amino acids with seven transmembrane domains, and an intracellular C-terminal tail of ~200 amino acids [19,20]. The CaR is coupled with the G-proteins, G11, Gq and probably one or more isoforms of Gi, which participate in the activation of phosphatidylinositol-specific phospholipase C (PLC) and the inhibition of adenylate cyclase [19,21].

The primary role of the CaR is the continuous control of PTH secretion in response to Ca²⁺ concentration on a minute-to-minute basis, but the mechanism underlying this process has not yet been fully elucidated [18,19]. Activation of the CaR by Ca²⁺ increases the activity of PLC, thereby producing inositol 1,4,5-trisphosphate and diacylglycerol, which
mobilize intracellular Ca\(^{2+}\) [6,18]. The increases in diacylglycerol and intracellular Ca\(^{2+}\) concentration cause the activation of protein kinase C (PKC) [19]. Kifor et al. reported that cytosolic phospholipase A\(_2\) (cPLA\(_2\)) is phosphorylated by mitogen-activated protein kinase (MAPK), at least in part, in a PKC-dependent manner [22]. Thus, the PKC–cPLA\(_2\) pathway may be involved in the regulation of PTH secretion, because arachidonic acid, produced from phospholipids by the action of cPLA\(_2\), and its further metabolites, e.g. hydroxyperoxyeicosatetraenoic acids, can suppress PTH secretion [23]. However, the effect of Ca\(^{2+}\)o on PTH secretion is relatively unaffected by inhibitors of PKC, or when PKC activity has been down-regulated, indicating a trivial role for PKC in the control of PTH secretion [6]. Furthermore, little is known about the mechanism of these changes in the process of PTH exocytosis [19].

Calcimimetics suppress PTH secretion and parathyroid cell proliferation

Ligands that mimic or potentiate the effects of Ca\(^{2+}\) at the CaR have been termed calcimimetics [6]. Fendiline, a phenylalkylamine derivative, was found to activate the CaR, and the modification of this prototype molecule resulted in the identification of the calcimimetics NPS R-467, NPS R-568, and AMG 073 [7]. These calcimimetics can potentiate the response of the CaR to Ca\(^{2+}\)o and suppress PTH secretion [7,11]. NPS R-568 suppresses PTH secretion in rats with or without secondary hyperparathyroidism [11,24,25] and in patients with primary or secondary hyperparathyroidism [8,9]. Another beneficial effect of calcimimetics is that PTH-related bone diseases such as osteitis fibrosa ameliorate as a long-term outcome of suppressed PTH levels in rats with CRI [26,27] and in ovariectomized rats [28]. However, clinical data indicate that NPS R-568 has limited bioavailability and undergoes metabolic clearance by the polymorphic cytochrome P450 enzyme, CYP2D6 [8,9]. To address these issues, a related but improved compound, AMG 073, was selected for further study [10,29]. The efficacy and safety of AMG 073 have been examined in clinical trials, and the results demonstrate that this calcimimetic can lower both serum PTH concentration and calcium–phosphorus product in patients with secondary hyperparathyroidism [10,29].

Growing experimental evidence indicates that calcimimetics can inhibit parathyroid cell growth in vitro and in vivo. In a short-term experiment, treatment with NPS R-568 inhibited parathyroid cell proliferation that had been accelerated in partially nephrectomized rats [12]. This result was confirmed by long-term experiments showing that the development of parathyroid hyperplasia is almost completely blocked in partially nephrectomized rats by treating them with NPS R-568 immediately, or 11 weeks after nephrectomy [13,15]. Apoptotic cell death was not observed in any parathyroid glands of rats treated with NPS R-568 or vehicle [12]. Moreover, it has been shown recently that AMG 073 also inhibits parathyroid cell proliferation and prevents parathyroid hyperplasia in rats with CRI [14]. The effects of both NPS R-568 and AMG 073 occur despite unchanged serum vitamin D and increased phosphorus concentrations (because of lowered PTH), indicating a direct role for the CaR in regulating parathyroid cell proliferation [12–15]. Finally, the direct in vitro antiproliferative effect of NPS R-467 on parathyroid cells has been demonstrated in human parathyroid cells maintained in long-term culture [30]. These findings support the proposal that calcimimetics can inhibit parathyroid cell proliferation and block the development of hyperplasia.

Role of calcium in parathyroid cell proliferation

In 1997, the antiproliferative effect of NPS R-568 was described [12], although the role of the CaR in regulating parathyroid cell proliferation was still speculative [1,3]. The link between the CaR and parathyroid cell proliferation was suggested because severe neonatal hyperparathyroidism and occasional cases of familial hypocalciuric hypercalcaemia, both of which are caused by inactivating mutations in the CaR, develop parathyroid hyperplasia [16]. Despite this compelling evidence, the role of the CaR remained unclear because the effects of Ca\(^{2+}\)o on parathyroid cell proliferation were debatable [2]. Recent advances, especially those obtained using calcimimetics, may help to reconcile any discrepancy among the results obtained by manipulating Ca\(^{2+}\)o.

The secretion of PTH is regulated reciprocally by the concentration of Ca\(^{2+}\)o, and this feedback loop is essential in maintaining systemic Ca\(^{2+}\) homeostasis [18,19]. The Ca\(^{2+}\)o–PTH feedback loop is thought to operate on two time scales, i.e. the regulation of PTH secretion and synthesis in the short term, and the control of cell proliferation in the long term [3]. It is reasoned that prolonged demand for PTH creates the compensatory need for a greater number of cells, and hypocalcaemia is the most likely proximate stimulator of parathyroid cell growth, as inferred by Marine in 1914 [31] and subsequently extended by many investigators [3]. This classical view has been questioned [2], because the in vitro effect of Ca\(^{2+}\)o on parathyroid cell proliferation is inconsistent (Table 1). Several studies have shown the inhibitory effect of Ca\(^{2+}\)o on cell proliferation [32–36], but this has not been a consistent finding [37–39]. However, these results may be discounted because dispersed parathyroid cells in the primary culture system lose their response to Ca\(^{2+}\)o, probably by the down-regulation of CaR expression [16,19]. Recently, Roussanne et al. reported that proliferation of cultured human parathyroid cells was stimulated by an increase in Ca\(^{2+}\)o concentration, whereas it was inhibited by the calcimimetic NPS R-467 [30]. In this case, the persistence of functionally
active CaR expression was observed. Given that Ca\(^{2+}\) has multiple CaR-independent effects, this result has relevance for the hypothesis that only the CaR-specific pathway primarily mediates the antiproliferative effect of Ca\(^{2+}\) on parathyroid cells.

Another cellular response that complicates understanding of the mechanism of Ca\(^{2+}\) in parathyroid cell proliferation is that hypertrophy, not hyperplasia, mainly accounts for the increase in parathyroid cell mass in animals with normal renal function fed either a calcium-deficient or phosphate-rich diet [1,2]. The alternative view holds that hypocalcaemia does not stimulate parathyroid cell proliferation and does not account for the parathyroid hyperplasia observed in CRI [2]. However, this view is at odds with the findings that the number of proliferating cell nuclear antigen (PCNA)- or Ki67-positive cells significantly increases in animals with hypocalcaemia [40–42]. An important finding is that the calcimimetic NPS R-568 inhibits parathyroid cell proliferation in rats with CRI when given as either an intermittent (once-daily bolus) or a persistent regimen (twice-daily bolus or continuous infusion) [12–15], whereas parathyroid cell hypertrophy is only suppressed when NPS R-568 is administered persistently [12,13,15]. This provides circumstantial evidence suggesting that the duration (and possibly magnitude) of CaR activation determines whether the control of cell size or of proliferation will be the outcome, and the former possibly needs longer activation of the CaR. Depending on the severity of hypocalcaemia, therefore, it is likely that parathyroid hypertrophy will predominate, but this does not necessarily contradict the role of CaR in regulating parathyroid cell proliferation.

**Signalling pathway linking the calcium receptor and cell proliferation**

The proliferation of several cell types is stimulated by activation of the CaR [2,19]. Among multiple intracellular signalling pathways, extracellular signal-regulated kinases (ERKs) or MAPKs mediate the proliferative effects of the GPCR [43]. Diverse GPCR ligands can activate p42 and p44 MAPK (known as ERK2 and ERK1, respectively) in many cellular systems [19,43]. The first indication of a role for tyrosine kinases in the pathway linking the GPCR to Ras–MAPK was the ability of tyrosine kinase inhibitors to reduce the activation of ERK by Gi-coupled GPCR. In this signalling cascade, rapid tyrosine phosphorylation of Shc (Src homologue and collagen) following GPCR stimulation leads to the formation of Shc–Grb2 (growth factor receptor-bound 2) complexes [43]. The CaR also uses this tyrosine kinase–Ras pathway to activate ERK in some cell types, because it has been found that the loss-of-function mutation of the CaR results in diminished Src activation in CaR-transfected Rat-1 fibroblast cells [44].

In parathyroid cells also, the activation of tyrosine kinases partially contributes to ERK activation through the Gi and G\(_{\beta\gamma}\) protein pathway of the CaR [22]. However, the CaR uses multiple signalling cascades to activate MAPK where at least PKC and phosphatidylinositol 3-kinase (PI3kinase) play a key role in parathyroid cells [22,45]. The activation of ERK1/2 elicited by the increase in Ca\(^{2+}\) concentration or the addition of a calcimimetic compound is almost completely blocked by PKC inhibitors in a PTX-sensitive manner, indicating that the Gi11–PLC–PKC cascade plays a major role in bovine [22] and human parathyroid cells [45]. Human adenomatous parathyroid cells, even in a resting state, have constitutively high ERK activity that occurs in a PKC-dependent manner [45]. These results indicate that PKC is an important mediator to activate ERK downstream of the CaR. Furthermore, apart from the PKC cascade, PI3kinase, possibly activated by G\(_{\beta\gamma}\), also contributes to the activation of ERK in human parathyroid cells [45]. The downstream cascade of PI3kinase is not defined in parathyroid cells, but it is suggested that PI3kinase acts upstream of the Ras–Raf pathway in ovarian epithelial cells [46]. All available data thus indicate that activation of the CaR stimulates the activation of ERK through multiple signalling cascades even in parathyroid cells [21,22,45]. Kifor et al. have reported an important role for caveolin that functions as a scaffolding protein and coordinates the CaR and other signalling molecules to activate ERK in parathyroid cells [21].

**Table 1. Effect of extracellular Ca\(^{2+}\) on parathyroid cell proliferation in vitro**

<table>
<thead>
<tr>
<th>Effect</th>
<th>Species (cell line)</th>
<th>Culture system</th>
<th>Indices</th>
<th>Sources (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppress</td>
<td>Chicken</td>
<td>Organ</td>
<td>Mitosis</td>
<td>Raisz [32]</td>
</tr>
<tr>
<td>Suppress</td>
<td>Rat</td>
<td>Organ</td>
<td>(^{3})H incorporation</td>
<td>Lee and Roth [33]</td>
</tr>
<tr>
<td>Suppress</td>
<td>Bovine</td>
<td>Long term</td>
<td>(^{3})H incorporation, cell number</td>
<td>Brandi [34]</td>
</tr>
<tr>
<td>Suppress</td>
<td>(PT-r)(^{a})</td>
<td></td>
<td>(^{3})H incorporation, cell number</td>
<td>Sakaguchi [35]</td>
</tr>
<tr>
<td>Suppress</td>
<td>Human</td>
<td>First passage</td>
<td>(^{3})H incorporation</td>
<td>Liu [36]</td>
</tr>
<tr>
<td>No effect</td>
<td>Bovine</td>
<td>Primary</td>
<td>Cell number</td>
<td>LeBoff [37]</td>
</tr>
<tr>
<td>No effect</td>
<td>Bovine</td>
<td>Primary</td>
<td>(^{3})H incorporation, cell number</td>
<td>Kremer [38]</td>
</tr>
<tr>
<td>Stimulate</td>
<td>Human</td>
<td>Long term</td>
<td>(^{3})H incorporation</td>
<td>Ishimi [39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Roussanne [30]</td>
</tr>
</tbody>
</table>

\(^{a}\)Clonal rat parathyroid cell line that secretes PTHrP instead of PTH.

\(^{b}\)NPS R-467 suppressed cell proliferation.
Mode of action of calcimimetics

The growth control of parathyroid cells is a complex process, and whether the regulation by the CaR is direct or secondary to autocrine (or paracrine) mechanisms still remains unclear. Two lines of evidence tend to favour the latter possibility, although this is far from confirmed. First, the observations that activation of the CaR results both in the inhibition of parathyroid cell growth and in the activation of MAPK are apparently paradoxical and rather compatible with the indirect role of the CaR. Nonetheless, it should be noted that the activation of MAPK does not always stimulate cell proliferation but sometimes inhibits it [47,48]. For example, somatostatin receptor subtypes 1 and 2 activate MAPK, but inhibit cell proliferation by up-regulating the cyclin-dependent kinase inhibitor p21 [47,48].

Secondly, it has been hypothesized that a number of candidate molecules act as autocrine or paracrine regulators of parathyroid cell proliferation in responding to Ca\(^{2+}\), but their individual roles remain to be examined [2]. These candidate molecules include parathyroid hormone-related protein [49], endothelin [42], acidic fibroblast growth factor [35] and transforming growth factor-\(\alpha\) (TGF-\(\alpha\)) [50]. For example, in rats fed a calcium-deficient diet, parathyroid cell growth is blocked by an endothelin antagonist, which does not occur in animals fed a normal diet, inferring that endothelin is a mediator of hypocalcaemia-induced parathyroid cell growth [42]. Likewise, the proliferation of parathyroid cells and the up-regulation of TGF-\(\alpha\) are both suppressed in rats with CRI fed a calcium-rich diet, suggesting that TGF-\(\alpha\) may play a role in the pathogenesis of parathyroid hyperplasia in CRI, and the increased concentration of Ca\(^{2+}\) may reverse this process [50]. These results indicate that complex autocrine or paracrine mechanisms may underlie the control of parathyroid cell growth by the CaR, particularly in the setting of pathological hyperplasia. Therefore, at present, it is prudent to state that the effect of calcimimetics on parathyroid cell proliferation is direct through activating the CaR, but the full picture of subsequent molecular events remains to be clarified.

Conclusion

The results obtained so far indicate that the calcimimetics NPS R-467, NPS R-568 and AMG 073 can inhibit parathyroid cell proliferation by activating the CaR, which completely blocks the development of hyperplasia in rats with CRI. Given that parathyroid hyperplasia is difficult to control with conventional therapies, these findings are therapeutically important. There is still much to understand about the molecular mechanisms underlying the control of parathyroid cell growth by the CaR, however. Finally, the data from clinical trials demonstrate that calcimimetics have potential as therapeutic agents for treating the hyperparathyroidism resulting from CRI.

Note

The calcimimetic compound AMG 073 is also referred to as KRN1493 in Japan, and these are the same molecule (cinacalcet hydrochloride).

References

Parathyroid cell growth and calcimimetics


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