Pharmacology of the calcium sensing receptor

Marcello Filopanti[1]
Sabrina Corbetta[2, 3]
Anna Maria Barbieri[2]
Anna Spada[1, 2]

[1] Endocrine and Diabetology Unit, Fondazione Istituto di Ricerca e Cura a Carattere Scientifico (IRCCS) Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[2] Department of Clinical and Community Sciences, University of Milan, Italy
[3] Endocrinology and Diabetology Unit, IRCCS Policlinico San Donato, San Donato Milanese, Italy

Address for correspondence:
Anna Spada, MD
Padiglione Granelli
Via F. Sforza 35
20122 Milan, Italy
Phone: +39 02 50320613
Fax: +39 02 503 20605
E-mail: anna.spada@unimi.it

Summary

Calcium sensing receptor (CASR) is a G-protein couple receptor which plays a key role in calcium homeostasis in vertebrates. Its extracellular domain is sensitive to divalent cations, aminoacids and polyamines. In parathyroid glands, CASR activation causes parathyroid hormone (PTH) reduction and subsequently a decrease in blood calcium concentration. In PTH-dependent disorders, e.g. primary and secondary hyperparathyroidism (HPT), the need for therapeutic options other than surgery led to the synthesis of various allosteric CASR agonists (calcimimetics), such as cinacalcet. Cinacalcet is the only calcimimetic approved for HPT secondary to chronic kidney disease (CDK), parathyroid carcinoma, and, in some countries, primary HPT. Clinical trials showed that cinacalcet reduced PTH and calcemia both in CDK and primary HPT, lowering the risk of bone fractures, surgery, and cardiovascular complications in the former patients. Long-term safety and pharmacoeconomics have to be fully tested yet. Few both *in vitro* and *in vivo* studies showed an association between Arg990Gly-CASR polymorphism and cinacalcet sensitivity, though in patients with severe CASR inactivating mutations the drug substantially retained its positive clinical effects.
Recently, a new class of allosteric antagonists of CASR, i.e. calcilytics, has been synthesized. Calcilytics are structurally similar to calcimimetics, but exert their effects acting on a different allosteric site. Infusion of calcilytics was followed by transient rise in PTH and calcium. One of these compounds, ronacaleret, was able to increase femur BMD in post menopausal women, but with induction of mild hyperparathyroidism. In the future, calcilytics may contribute to the osteoporosis treatment choice.

KEY WORDS: CASR; calcium sensing receptor; cinacalcet; calcium metabolism; ronacaleret.

Introduction

Calcium sensing receptor (CASR) is a G-protein coupled receptor (GPCR) involved in calcium homeostasis in vertebrates. In humans, extracellular calcium concentration regulation is mainly carried out by parathyroid glands, kidney, intestine, and bone, by mean of hormones, i.e. parathyroid hormone (PTH), calcitonin, and $1,25(OH)_2$-vitamin D (1). These hormones regulate calcium ion (Ca^{++}) flux through body compartments in order to maintain its blood concentration in a narrow range. As in other mineral homeostasis systems, a sensor able to detect ion concentration is essential for proper regulatory actions. In calcium homeostasis, this key role is played by CASR. This receptor belongs to the superfamily of GPCR, which is characterized by a common seven transmembrane domain structure, and it is included in the 3/C subfamily, together with metabotropic glutamate receptor and gamma-aminobutyric acid (GABA) B receptor (2). CASR maps on chromosome 3 q21.1 in a ~100 Kb locus. CASR transcripts include seven exons, the first of them is untraslated. An alternative splicing of exon 5 is reported to occur in keratinocytes, with an in frame deletion of 77 aminoacids of the extracellular domain (ECD) and consequent loss of function (3). To date, it is unknown if this variant may have a role in other tissues. The ECD is particularly large (612 aminoacids) and accounts approximately for half of the entire CASR protein (4). According to the three-dimensional model prediction, it is assumed that CASR ECD is divided in two subdomains, separated by a slot, resembling the two leafs of the trapping structure of the carnivorous plant Venus Flytrap (*Dionaea muscipula*) (5). Interestingly, cell membrane CASR expression occurs in dimers, whose components are linked by covalent disulfide bonds of cysteine residues in position 129 and 131 (5).
The calcium sensing action of CASR is probably performed by more than one binding site for each monomer (6) and one of these may be located in the slot between the two ECD lobes (6). Another Ca^{++} binding site locates at transmembrane domain (7), although evidence indicates that ECD Ca^{++} site is required for fully receptor activation, as shown by experiments with ECD deleted CASR constructs (8). In presence of calcium, the CASR complex undergoes to conformational changes that eventually cause the activation of the transduction pathways by mean of protein $G_{q/11,}$ which stimulates phospholipase C and subsequently induces intracellular Ca^{++} mobilization (9). Also protein G_i and $G_{12/13}$

dependent pathways are triggered by CASR activation (9). In addition to Ca^{++}, other physiologic endogenous allosteric modulators are able to activate CASR. Both divalent (Mg^{++} and Sr^{++}) and trivalent (Gd^{3+}, La^{3+}) cations may induce CASR allosteric activation (10). Ca^{++} and these cations are called type 1 agonists, i.e. ligands able to directly activate the receptor with no need of other cofactors. Conversely, type 2 agonists like polyamines, e.g. spermine, putrescine (11), aminoacids (e.g. tryptophan, phenylalanine, histidine, glutamate, alanine) (12, 13), and glutathione (14), need Ca^{++} to act, even in small amounts. These modulators are also called allosteric ligands, to differentiate them from Ca^{++}, which is referred orthosteric ligand.

In parathyroids, CASR stimulation reduces PTH secretion and synthesis, as well as cell proliferation (1). CASR plays an important role also in kidney, where inhibits $1,25(OH)_2$-vitamin D production and Ca^{++} reabsorption by cortical thick ascending limb of the kidney tubule (1). In bone, CASR is expressed in almost all cell type involved in bone metabolism and morphogenesis and the net effect of receptor activation seems to be anabolic (reviewed in 15).

CASR allosteric drugs: calcimimetics

The search for pharmacological CASR agonists was driven by the need of a medical approach for parathyroid hormone (PTH) hypersecretion diseases, principally secondary hyperparathyroidism and parathyroid carcinoma, in addition to the surgery option. Unfortunately, organic compounds able to directly mimic the action of an inorganic ion are very difficult to synthesize. However, Ca^{++} is not a classic ligand for CASR since it activates the receptor by multisite steric interaction (6), a mechanism that may be exploited by artificial molecules.

The first members of the calcimimetic pharmacological class were the phenylalkylamines NPS568 and NPS467. These modulators showed a clear and specific activation of CASR, but only in presence of extracellular calcium (16). NPS568 was able to induce rise in intracellular Ca^{++} concentration in various models (HEK293 cell lines, Xenopus laevis oocytes, bovine parathyroid cells, human parathyroid adenoma cells) and to reduce PTH secretion in bovine and human cells (16, 17). NPS568 was also able to increase CASR expression (18). Moreover, these drugs did not showed any activity in thyroid parafollicolar C cells and kidney tubule, a phenomenon probably due to differences in transduction mechanisms or receptor expression in these tissues compared to parathyroids (19). However, doses ~30-fold higher than those administered for controlling PTH levels also induced rise in calcitonin levels (20). Further structure modifications led to a new generation of calcimimetics, which includes cinacalcet, the only calcimimetic presently on the market. Cinacalcet showed the best pharmacological profile and lesser interindividual variability in absorption and response. Cinacalcet binding site was found to be located near the external end of the seventh transmembrane helic (21), although the residues involved in this interaction have not yet been clearly identified. Interestingly, cinacalcet and structurally similar calcilytics (see below) share most of their binding site (22).

Clinical utility of cinacalcet

In most countries cinacalcet may be administered in patients affected with stage 5 chronic kidney disease (CKD) hyperparathyroidism or parathyroid carcinoma, whereas the use in primary hyperparathyroidism due to parathyroid adenoma or hyperplasia is restricted to cases in which surgery is contraindicated or followed by disease recurrence.

In stage 5 CKD, cinacalcet is able to reduce PTH and calcemia, with a better clinical outcome (23). Various studies carried out on a overall group of more than one thousand patients, showed a reduction in risk of parathyroid surgery, fractures, and cardiovascular comorbidities, as well as an improvement in perceived quality of life and pain. Clinical trials carried out in CKD are fully reviewed in (24). In parathyroid carcinoma, cinacalcet is able to reduce calcemia in about one third of patient, with minor effects on PTH levels and survival (25).

Primary hyperparathyroidism may be treated with cinacalcet only in some European countries and restricted to patients in whom surgery is not suitable. In these patients, calcemia was well controlled by cinacalcet, whereas PTH levels were only mild reduced and frequently remained above the upper normal limit (26). Unlike the parathyroidectomy, cinacalcet treatment did not positively affect bone mineral density (27). Moreover, nausea and vomiting were common adverse effects during treatment, and may cause discontinuation of the therapy (26). No significant resistance or tachyphylaxis have been observed (27).

Some case reports and one trial in type 1 multiple endocrine neoplasia syndrome (MEN1) patients, showed the efficacy and the safety of cinacalcet in controlling calcium levels and PTH in these patients, at least in the short period (28-30). Cinacalcet was able to normalize calcemia in a cohort of 11 MEN1 patients treated for three months, with significant reduction of PTH, but normalization in only five patients (30). Similar results were observed in a control group of 20 patients with sporadic primary hyperparathyroidism, who showed a lower rate of PTH normalization, probably due to PTH basal levels higher in sporadic in comparison to MEN1 patients (30).

In primary hyperparathyroidism, some CASR polymorphisms were previously reported to affect receptor activity, since they associate with different serum calcium levels (31) and nephrolithiasis (32), although pharmacogenetics in cinacalcet treatment was not yes thoroughly investigated. Some evidences link the Arg990Gly polymorphism with a difference in cinacalcet sensitivity (33, 34), while other studies did not found significant association (30). Admittedly, the patients cohorts so far reported are inadequate to drew definitive conclusions.

As regards CASR mutations, sporadic reports in patients with familial hypocalciuric hypercalcemia (FHH), a genetic disorder due to inactivation of the receptor, showed a good clinical and biochemical response to cinacalcet administration (35). Clinical utility of cinacalcet in disorders unrelated to PTH hypersecretion (e.g. calciphylaxis, phosphate-wasting) are reviewed (36).

Finally, it should be highlighted that the use of cinacalcet in primary hyperparathyroidism gave rise to a number of pharmacoeconomics considerations. To date, cinacalcet treatment is still expensive and surgery, when suitable, is more cost effective than calcimimetics, especially considering the poor, if any, effect of cinacalcet on bone.

CASR allosteric drugs: calcilytics

To date, no physiological CASR orthosteric antagonists have been detected. In analogy with calcimimetics, the recently synthesized allosteric antagonists have been named calcilytics. The aim was to have compounds with anabolic effect on bone to be used for osteoporosis treatment. A *per os*

CASR antagonist with a well calibrated profile might induce transient blood PTH rises similar to those observed in daily injected recombinant PTH treatment (e.g. teriparatide), which is known to have positive effects on trabecular and compact bone (37). Moreover, these agents might also be used in genetic disorders due to CASR activation.

Structurally, first generation calcilytics were similar to calcimimetics. Their isolation was in fact obtained by screening various molecules based on the same phenylalkylamine structure (38). NPS2143 was the first calcilytic synthesized. In rats, NPS2143 induced a strong PTH increase, followed by hypercalcemia (39). Its pharmacokinetics, however, was not adequate to induce bone anabolism, due to its prolonged action. Further modifications of the NPS2143 led to compounds with better profile, such as SB423557, which demonstrated bone anabolic effects in ovariectomized rats (40). During this screening process, the structures of calcilytics differed significantly in comparison to first generation molecules, so that the second generation-calcilytic was found to act through a different site in CASR ECD (41). A theoretically possible adverse effect, i.e. parathyroid hyperplasia, was not detected in preliminary studies carried out in animals.

The most promising calcilytic, i.e. ronacaleret, was recently evaluated in postmenopausal women in a phase 3 clinical trial by comparing this agent with teriparatide, alendronate, and placebo. Ronacaleret was able to significantly increase volumetric bone mass density (vBMD). The effect was half of that observed in women treated with teriparatide (42) and similar to that obtained with alendronate. The main issue was the presence of mild vBMD decrease in proximal femur and in other cortical bone sites (42), that was attributed to a mild hyperparathyroidism, due to more prolonged PTH elevations induced by ronacaleret in comparison to teriparatide (42). A phase 2 study in humans with SB423557 evidenced a better *in vivo* profile (43), and this calcilytic might be tested for osteoporosis treatment in the near future.

Conclusions

Nowadays a number of allosteric agents acting on calcium sensing receptor has been synthesized and tested. The search for positive allosteric modificators (i.e. calcimimetics) arose from the need to have a medical treatment to flank surgery in patients with PTH hypersecretion disorders. These expectations was met by the first on market calcimimetic cinacalcet. Patients with stage 5 CHD secondary/tertiary hyperparathyroidism had greater benefit with calcimimetic treatment. In particular, this drug was able to reduce risk of parathyroid surgery, comorbidities, and quality of life in these patients. Good results was observed also in patients affected with primary hyperparathyroidism, although cost analysis is still favourable to surgery. The use of cinacalcet in other conditions like calciphylaxis, phosphate-wasting disorders, lithium induced hyperparathyroidism, and familial hypocalciuric hypercalcemia was proposed, although to date the same economic considerations should apply to these conditions.

By chemical variation of the calcimimetic structure, the pharmacological research obtained a new class of compounds with negative allosteric regulation of CASR, i.e. calcilytics. This orally active drugs are able to transiently increase PTH and they were thought to mimic the profile of recombinant PTH, without the need of daily injection. Calcilytics were tested in animal models of menopause with good results. A phase 3 trial in a cohort of postmenopausal women showed that the calcilytic ronacaleret improved femur BMD, but less than those observed with teriparatide, probably due to induction of mild hyperparathyroidism. Calcilytics with better profile are in evaluation.

References

1. Brown EM. Clinical lessons from the calcium sensing receptor. Nat Clin Pract Endocriniol Metab 2007;3:122-33.
2. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev 2001;81:239-297. Review.
3. Oda Y, Tu CL, Pillai S, et al. The calcium sensing receptor and its alternatively spliced form in keratinocyte differentiation. J Biol Chem 1998;273(36):23344-52.
4. Garrett JE, Capuano IV, Hammerland LG, et al. Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. J Biol Chem 1995;270(21):12919-25.
5. Hu J, Spiegel AM. Structure and function of the human calcium-sensing receptor: insights from natural and engineered mutations and allosteric modulators. J Cell Mol Med 2007;11(5):908-22. Review.
6. Huang Y, Zhou Y, Yang W, et al. Identification and dissection of Ca(2+)-binding sites in the extracellular domain of Ca(2+)-sensing receptor. J Biol Chem 2007;282(26):19000-10.
7. Ray K, Northup J. Evidence for distinct cation and calcimimetic compound (NPS 568) recognition domains in the transmembrane regions of the human Ca2+ receptor. J Biol Chem 2002;277(21):18908-13.
8. Hammerland LG, Krapcho KJ, Garrett JE, et al. Domains determining ligand specificity for Ca2+ receptors. Mol Pharmacol 1999;55:642-648.
9. Huang C, Miller RT. Novel Ca receptor signaling pathways for control of renal ion transport. Curr Opin Nephrol Hypertens 2010;19(1):106-12. Review.
10. Brauner-Osborne H, Jensen AA, et al. The agonist-binding domain of the calcium-sensing receptor is located at the amino-terminal domain.J Biol Chem2 1999;74:18382-18386.
11. Quinn SJ, Ye CP, Diaz R, et al. The Ca2+-sensing receptor: a target for polyamines. Am J Physiol Cell Physiol 1997;273:C1315-C1323.
12. Feng J, Petersen CD, Coy DH, et al. Calcium-sensing receptor is a physiologic multimodal chemosensor regulating gastric G-cell growth and gastrin secretion. Proc Natl Acad Sci U S A. 2010;107(41):17791-6.
13. Conigrave AD, Mun HC, Lok HC. Aromatic L-amino acids activate the calcium-sensing receptor. J Nutr 2007;137(6 Suppl 1):1524S-1527S.
14. Broadhead GK, Mun HC, Avlani VA, et al. Allosteric modulation of the calcium-sensing receptor by gamma-glutamyl peptides: inhibition of PTH secretion, suppression of intracellular cAMP levels, and a common mechanism of action with L-amino acids. J Biol Chem 2011;286(11):8786-97.
15. Theman TA, Collins MT. The role of the calcium-sensing receptor in bone biology and pathophysiology. Curr Pharm Biotechnol 2009;10(3):289-301.Review.
16. Nemeth EF, Steffey ME, Hammerland LG, et al. Calcimimetics with potent and selective activity on the parathyroid calcium receptor. Proc Natl Acad Sci U S A 1998;95(7):4040-5.
17. Hammerland LG, Garrett JE, Hung BC, et al. Allosteric activation of the Ca2+ receptor expressed in xenopus laevis oocytes by NPS 467 or NPS 568. Mol Pharmacol 1998;53:1083-1088.
18. Cavanaugh A, Huang Y, Breitwieser GE. Behind the curtain: cellular mechanisms for allosteric modulation of calcium-sensing receptors. Br J Pharmacol 2012;165(6):1670-7. Review.
19. Nemeth EF and Fox J. Calcimimetic compounds: a direct approach to control-ling plasma levels of parathyroid hormone in hyperparathyroidism.Trends Endocrinol Metab 1999;10:66-71.
20. Nemeth EF, Heaton WH, Miller M, et al. Pharmacodynamics of the type II calcimimetic compound cinacalcet HCl. J Pharmacol Exp Ther 2004;308:627-635.
21. Hu J, Reyes-Cruz G, et al. AM. Identification of acidic residues in the extracellular loops of the seven-transmembrane domain of the human Ca2+ receptor critical for response to Ca2+ and a positive allosteric modulator. J Biol Chem 2002;277(48):46622-31.
22. Petrel C, Kessler A, Dauban P, et al. Positive and negative allosteric mod-

ulators of the Ca2+-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. J Biol Chem 2004;279(18):18990-7.
23. Komaba H, Kakuta T, Fukagawa M. Diseases of the parathyroid gland in chronic kidney disease. Clin Exp Nephrol 2011;15(6):797-809.
24. Drüeke TB, Ritz E. Treatment of secondary hyperparathyroidism in CKD patients with cinacalcet and/or vitamin D derivatives. Clin J Am Soc Nephrol 2009;4(1):234-41.
25. Silverberg SJ, Rubin MR, Faiman C, et al. Cinacalcet hydrochloride reduces the serum calcium concentration in inoperable parathyroid carcinoma. J Clin Endocrinol Metab 2007;92(10):3803-8.
26. Peacock M, Bolognese MA, Borofsky M, et al. Cinacalcet treatment of primary hyperparathyroidism: biochemical and bone densitometric outcomes in a five-year study. J Clin Endocrinol Metab 2009;94(12):4860-7.
27. Peacock M, Bilezikian JP, Bolognese MA, et al. Cinacalcet HCl reduces hypercalcemia in primary hyperparathyroidism across a wide spectrum of disease severity. J Clin Endocrinol Metab 2011;96(1):E9-18.
28. Falchetti A, Cilotti A, Vaggelli L, et al. A patient with MEN1-associated hyperparathyroidism, responsive to cinacalcet. Nat Clin Pract Endocrinol Metab 2008;4:351-7.
29. Moyes VJ, Monson JP, Chew SL, et al. Clinical use of cinacalcet in MEN1 hperparathyroidism. Int J Endocrinol 2010. doi:10.1155/2010/906163.
30. Filopanti M, Verga U, Ermetici F, et al. MEN1-related hyperparathyroidism: response to cinacalcet and its relationship with the calcium-sensing receptor gene variant Arg990Gly. Eur J Endocrinol 2012;167(2):157-64.
31. Yamauchi M, Sugimoto T, Yamaguchi T, et al. Association of polymorphic alleles of the calcium-sensing receptor gene with the clinical severity of primary hyperparathyroidism. Clin Endocrinol 2001;55:373-379.
32. Corbetta S, Eller-Vainicher C, Filopanti M, et al. R990G polymorphism of the calcium-sensing receptor and renal calcium excretion in patients with primary hyperparathyroidism. Eur J Endocrinol 2006;155:687-92.
33. Rothe HM, Shapiro WB, Sun WY, et al. Calcium-sensing receptorgene polymorphism R990G and its possible effect on response to Cinacalcet HCl. Pharmacogenetics and Genomics 2005;15:29-34.
34. Terranegra A, Ferraretto A, Dogliotti E, et al. Calcimimetic R-568 effects on activity of R990G polymorphism of calcium-sensing receptor. J Mol Endocrinol 2010;45(4):245-56.
35. Timmers HJ, Karperien M, Hamdy NA, et al. Normalization of serum calcium by cinacalcet in a patient with hypercalcaemia due to a de novo inactivating mutation of the calcium-sensing receptor. J Intern Med 2006;260(2):177-82.
36. Brown EM. Clinical utility of calcimimetics targeting the extracellular calcium-sensing receptor (CaSR). Biochem Pharmacol 2010;80(3):297-307. Review.
37. Han SL, Wan SL. Effect of teriparatide on bone mineral density and fracture in postmenopausal osteoporosis: meta-analysis of randomised controlled trials. Int J Clin Pract 2012;66(2):199-209. Review.
38. Nemeth EF. The search for calcium receptor antagonists (calcilytics).J Mol Endocrinol 2002;29:15-21.
39. Nemeth EF, Delmar EG, Heaton WL, et al. Calcilytic compounds: potent and selective Ca2+ receptor antagonists that stimulate secretion of parathyroid hormone. J Pharmacol Exp Ther 2001;299:323-331.
40. Kumar S, Matheny CJ, Hoffman SJ, et al. An orally active calcium-sensing receptor antagonist that transiently increases plasma concentrations of PTH and stimulates bone formation. Bone 2010;46:534-542.
41. Arey BJ, Seethala R, Ma Z, et al. A novel calcium-sensing receptor antagonist transiently stimulates parathyroid hormone secretion in vivo.Endocrinology 2005;146:2015-2022.
42. Fitzpatrick LA, Dabrowski CE, Cicconetti G, et al. Ronacaleret, a calcium-sensing receptor antagonist, increases trabecular but not cortical bone in postmenopausal women. J Bone Miner Res 2012;27(2):255-62.
43. Kumar S, Matheny CJ, Hoffman SJ, et al. An orally active calcium-sensing receptor antagonist that transiently increases plasma concentrations of PTH and stimulates bone formation. Bone 2010;46:534-542.