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REVIEW ARTICLE

PHARMACOLOGICAL MANIPULATION OF CALCIUM-SENSING RECEPTOR: PROSPECT AS ANABOLIC THERAPY FOR POSTMENOPAUSAL OSTEOPOROSIS

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ABSTRACT

The only anabolic therapy for osteoporosis that results in new bone formation involves daily injections of parathyroid hormone (PTH), marketed as Forteo®/Forsteo® (teriparatide) and Preotact®. An alternative strategy that might overcome costly systemic administration yet achieve a comparable anabolic effect on bone would be to increase the secretion of endogenous PTH from the parathyroid glands. This could be achieved by antagonizing the calcium-sensing receptor (CaSR) residing in the parathyroid gland. A calcimimetic CaSR agonist, cinacalcet hydrochloride, is already in clinical use. Marketed as Sensipar® and Mimpara®, it inhibits PTH secretion and is used for the treatment of various types of hyperparathyroidism. In contrast, CaSR antagonists or calcilytics decrease the sensitivity of the CaSR to calcium, thereby increasing PTH secretion. Sustained elevations of PTH, such as in hyperparathyroid states, will favor bone resorption. However, short bursts of PTH are anabolic, favoring new bone formation. The first reported calcilytic compound was NPS-2143, an orally active molecule that increased serum PTH levels by three- to fourfold over control. Oral administration of NPS-2143 in an ovariectomized rat model of osteopenia stimulated bone formation and resorption to a similar extent so that there was no net increase in bone mineral density (BMD). Optimization of the NPS-2143 pharmacophore has yielded ronacaleret hydrochloride, but the search is still on for novel and better calcilytics with shorter systemic half-lives. Presently, only a limited number of chemotypes for CaSR antagonists are available. Although more recently described calcilytics that are structurally different from NPS-2143 have the potential to treat osteoporosis, clinical evaluation and verification will be required.

INTRODUCTION

Extracellular (systemic) calcium concentrations are maintained within a narrow physiological range (1.1-1.3 mM) (1). Parathyroid hormone (PTH) acts as the "master" regulator of systemic calcium homeostasis due to its interaction with kidney and bone. The secretion of PTH from the parathyroid gland is tightly regulated by minute alterations in extracellular calcium signaling via the calcium-sensing receptor (CaSR) (2). CaSR is a G protein-coupled receptor (GPCR) that is coupled to G_q/G_{11} (3). The CaSR is highly promiscuous, as several di- and multivalent cations were found to activate the receptor (4).

The non-redundant role of the CaSR in systemic calcium homeostasis is best demonstrated by mutation of the CaSR gene (*CASR*) in humans (5, 6). For example, familial hypocalciuric hypercalcemia (FHH) is a condition caused by an inactivating mutation in a single allele of the *CASR* gene, leading to moderately elevated serum calcium and relative hypocalciuria. The abnormal biochemical parameters observed in FHH become lethally robust in neonatal severe hyperparathyroidism (NSHPT), which is caused by inactivating mutations in both alleles of the *CASR* gene.

Incontrovertible evidence for the nonredundant role of the CaSR was obtained from genetic manipulation of mice. Mice lacking a single or both alleles of the *Casrr* gene have biochemical parameters resembling FHH or NSHPT, respectively. In contrast to loss-of-function mutations, a gain-of-function mutation of the *Casrr* gene causes autosomal dominant hypoparathyroidism (ADH), with increased sensitivity to calcium levels, hypocalcemia, inappropriately low PTH levels and relative hypercalciuria (7).

As a GPCR, the CaSR is an attractive drug target. The receptor is coupled with the phospholipase C (PLC) pathway in many cell types, as well as in heterologous systems (i.e., HEK-293 or CHO cells trans-

fected with the CaSR) (4). Initially, bovine parathyroid cells were used to exploit the PLC coupling of CaSR as a homologous system, since there were no cell lines that could retain the appropriate parathyroid phenotype. Once the Ca^{2+} receptor was cloned, stably transfected cell lines were established and a high-throughput screening of small molecules could be performed in order to obtain putative receptor agonists and antagonists. The screening ultimately resulted in the identification of a phenylalkylamine, the small-molecule CaSR agonist NPS-R-568 (tecalcet hydrochloride; a calcimimetic compound), which acts as a positive allosteric modulator of the receptor (8). This allosteric modulation resulted in a more controlled and selective tuning of the receptor, because compared to the orthosteric ligand binding site allosteric modulation results in conformational changes that profoundly influence protein and hence GPCR function (9).

Calcimimetic compounds inhibit PTH secretion and hence lower the hypercalcemic burden. Cinacalcet hydrochloride, marketed as Sensipar® and Mimpara® in the U.S. and Europe, respectively, is used in patients with parathyroid carcinoma and in secondary hyperparathyroidism in patients receiving renal replacement therapy. Alternatively, compounds that block the action of the CaSR (CaSR antagonists or calcilytics) should theoretically lead to increased plasma PTH (10). Because increased PTH levels are associated with bone formation (11), CaSR antagonists could provide a novel approach to the treatment of osteoporosis.

The current review discusses the potential therapeutic use of calcilytics in postmenopausal osteoporosis. Since daily injection of PTH is used to attain new bone formation in severe osteoporosis, orally active calcilytics that can transiently increase PTH may prove useful in the treatment of osteoporosis.

PTH AS THE ONLY APPROVED BONE ANABOLIC THERAPY

Chronic elevation of circulating PTH in response to hypocalcemia causes bone loss by stimulating the production of receptor activator of nuclear kappa B ligand (RANKL) from osteoblasts. RANKL stimulates all aspects of osteoclast function, resulting in increased bone resorption. Surprisingly, a once-daily bolus injection of PTH given to postmenopausal women resulted in new bone formation (11, 12). This discrepancy has been exploited therapeutically, with intermittent bolus doses of recombinant PTH (1-34) (teriparatide) increasing bone mass and decreasing fracture rates in osteoporosis (13). Recently, recombinant full-length PTH (1-84) was launched and constitutes a welcome addition as a therapeutic option for patients with severe osteoporosis (14, 15), although it appears to have a higher incidence of hypercalcemia, hypercalciuria and nausea than teriparatide. Since there are no comparative studies of the two types of PTH, at present no clear-cut pronouncements can be made about the potential differences in efficacy and safety. The differences attributed to PTH (1-84) may be ascribed to an actual difference in side effects as experienced by patients (14). PTH treatment is recommended only once in a lifetime for a maximum of 2 years and the therapy is not readily affordable for patients requiring bone anabolic therapy. In addition, daily injection is cumbersome and may negatively influence adherence to treatment. Intermittent administration of a calcilytic could mirror the cyclical pattern of injectable and endogenous PTH, promoting anabolic over catabolic actions.

BIOLOGY OF CaSR IN BONE

Bone cells (osteoblasts and osteoclasts) are exposed to qualitatively and quantitatively different fluctuations in extracellular free ionized calcium ($[Ca^{2+}]_e$), depending on their position and function (16). Short-term (minutes) pronounced elevations in the levels of $[Ca^{2+}]_e$ occur at the quiescent surfaces as an acute mechanism of calcium buffering, taking up or releasing calcium to correct hypo- or hypercalcemia (17), whereas chronic (hours to days) modest variations in $[Ca^{2+}]_e$ occur in the remodeling microenvironments, which participate in long-term calcium homeostasis and bone calcium recycling (18). Osteoblasts are capable of responding to both types of $[Ca^{2+}]_e$ stimuli (19). Freshly isolated fetal rat calvarial osteoblasts respond to acute stimulation with pronounced elevations in $[Ca^{2+}]_e$ up to 5.0 mM by activating intracellular signaling events such as mitogen-activated protein kinase ERK-1/2. These same cells respond to chronic (days) stimulation with a moderate increase in $[Ca^{2+}]_e$ from 1.2 mM to 1.8 mM by inducing their proliferation, differentiation and mineral production (20). Therefore, calcium sensing in bone cells is dependent on the quantity and duration of the stimuli in order to reflect the microenvironment at either remodeling or quiescent bone surfaces.

The molecular nature of CaSR in osteoblasts is a contentious issue. Several groups have reported the expression of parathyroid CaSR in rat, mouse and human osteoblasts (21). A study by Shalhoub et al. to determine whether the calcimimetic cinacalcet hydrochloride (AMG-073) potentiated the effect of calcium via the CaSR or via some other receptor mechanism showed that the calcium-induced increase in osteoblastic cell number involved a mechanism or receptor other than the CaSR (22). This calcimimetic agent also did not potentiate the effect of calcium in normal adult human bone cells in vitro. Congruently, reports from another group suggest that the orphan receptor GPCR6A may be the actual calcium sensor in osteoblasts (23). Setting aside the debate on the molecular nature of the receptor in osteoblasts, most CaSR agonists and antagonists generally modulate osteoblast functions (24).

Osteoclasts, the bone-resorbing cells, have been shown to express a CaSR similar to that cloned from parathyroid gland or kidney (25). Whereas activation of the CaSR in osteoblasts results in osteoblast proliferation and differentiation (19), activating the receptor in osteoclasts induces osteoclast apoptosis (26). Thus, the CaSR appears to serve as a dual-action molecule in bone, promoting osteoblast function while inhibiting osteoclast function.

Elegant studies in mice lacking the *Casrr* gene suggest that the CaSR participates in hematopoietic stem cell (HSC) homing to bone marrow. During mammalian ontogeny, HSCs translocate from fetal liver to bone marrow, the site of hematopoiesis in adults. HSCs within the bone marrow cavity reside in close proximity to the endosteal surfaces of the bone, the so-called "stem cell niche" (27, 28). It has been shown that hematopoietic cells such as monocytes and macrophages (osteoclast precursors) express the CaSR and that its activation induces transmigration in vitro and in vivo (29). Consistent with the observation that transplanted HSCs migrate to the stem cell niche of bone marrow within hours of intravenous injection, antenatal mice lacking the *Casrr* gene showed primitive hematopoietic cells in the circulation and spleen, whereas only a few were found in bone marrow. It has been observed that HSCs lacking the

Casrr gene are unable to engage and remain within the niche. This defect could be due to the inability of CaSR-null cells to adhere to collagen I, the most abundant bone matrix protein produced by osteoblasts. It is therefore thought that CaSR may play an important role in stem cell behavior within the bone marrow and modulate osseus homeostasis (30).

Strontium ranelate (Protelos®) is marketed as an antiosteoporosis therapy for reducing fracture risk. The active molecule is strontium (Sr^{2+}), a divalent cation. Since the CaSR is activated by a variety of di- or multivalent cations, it was obviously exciting to see whether the activation of cell-surface CaSR mediates the effects of Sr^{2+} in osteoblasts and osteoclasts (31). Indeed, Sr^{2+} was found to activate the CaSR in osteoblasts and promoted osteoblast proliferation, likely through the involvement of the early oncogenes c-fos and early growth factor response protein 1 (EGR-1) (32). In osteoclasts, Sr^{2+}, via the activation of CaSR, induced osteoclast apoptosis via the PLC pathway (33, 34). Despite our current knowledge on the cellular effects of Sr^{2+}, its precise mechanism remains elusive. Some results suggest that Sr^{2+} acts partly through the CaSR and partly via a different divalent cation sensor, GPCR6A. Therefore, the activation of CaSR in bone cells could be dually beneficial (35), although there is no in vivo evidence to suggest that Protelos® acts on the CaSR to increase bone mineral density (BMD) and reduce fracture risk. How Protelos® produces its clinical effects is still under investigation. Studies suggest that as a bone-seeking element, Sr^{2+} deposits in the bone after administration either by absorbing on the bone apatite surface or substituting for calcium in bone crystals. A recent study in large animals suggested that Sr^{2+} administration for the prevention and treatment of osteoporosis might not change the bone crystal morphology and structure. Elemental analysis did not show any change in the content of crystal after Sr^{2+} treatment and only a limited amount of Sr^{2+} replaced calcium in hydroxyapatite crystals (36). To understand how Sr^{2+} produces its beneficial effects, new technology and insights into the determinants of bone quality will be invaluable for long-term monitoring of the safety and efficacy of this new compound. This may also help to elucidate the mechanism of its molecular effects on bone.

ANTAGONIZING CaSR FUNCTION FOR BONE ANABOLIC EFFECT

We have discussed how pharmacological manipulation of the CaSR is an effective way to control the levels of PTH secretion. According to this assumption, blocking the CaSR would result in increased PTH secretion. However, to be clinically useful for bone anabolic therapy, such compounds would have to provide only short-term elevation in PTH levels since chronically high PTH levels are known to lead to bone loss.

The first ligands that could act as CaSR antagonists were identified through rigorous high-throughput screening of libraries of small molecules (10). The active molecule that was discovered (NPS-2143) interacted with the GPCR with a high degree of selectivity and sensitivity and thus provided a basis for the pharmaceutical importance of this series.

Potency and selectivity of NPS-2143

In HEK-293 cells expressing the human CaSR increasing concentrations of extracellular calcium from 1.0 to 1.75 mM caused a rapid and transient increase in $[Ca^{2+}]_i$. Preincubation of these cells with NPS-2143 caused a concentration-dependent inhibition of the cytoplasmic Ca^{2+} levels in response to increasing levels of extracellular Ca^{2+}. Addition of NPS-2143 (300 nM) to cells during the prolonged phase of the increase in $[Ca^{2+}]_i$ resulted in an immediate decrease in $[Ca^{2+}]_i$ to baseline values. As calculated from the concentration–response curves, the IC_{50} for NPS-2143 was 43 ± 5 nM (10).

NPS-2143 did not affect the activity of several GPCRs of the class C family that are structurally similar to CaSR. NPS-2143 neither activated nor inhibited group I metabotropic glutamate (mGlu) receptors, which like the CaSR couple to PLC and mobilize the intracellular $[Ca^{2+}]_i$ response. In addition, NPS-2143 did not alter cytoplasmic Ca^{2+} responses to thapsigargin, suggesting that ATPase activity or capacitative Ca^{2+} influx is not affected by this compound. Altogether, these results suggest that NPS-2143 has reasonable selectivity for the CaSR.

Bovine parathyroid cells were used to test the suitability of this compound in the physiological setting. Parathyroid cells incubated with increasing concentrations of NPS-2143 responded with increased secretion of PTH in vitro in the absence of changes in the levels of extracellular Ca^{2+}. In vivo NPS-2143 treatment resulted in increased levels of circulating PTH in normocalcemic rats.

In vivo studies

Results from studies of NPS-2143 in an ovariectomized rat model of osteopenia lend support to the suggestion that calcilytic compounds might provide an alternative to systemic administration of PTH by increasing the circulating levels of endogenous PTH. Intravenous administration of NPS-2143 resulted in a rapid increase in plasma PTH levels, peaking between 15 and 30 min following the start of infusion. Comparison of the magnitude of the rate of change in plasma levels of PTH induced by i.v. infusion of NPS-2143 with that elicited by s.c. administration of PTH in rats or humans demonstrated that the four-fold increase in plasma PTH following infusion of NPS-2143 falls within the range of levels produced by doses of exogenous PTH that stimulate new bone formation and increase BMD in osteopenic rats and osteoporotic humans (10). The decrease in the circulating levels of PTH after the end of infusion is likewise comparable to that seen following administration of exogenous hormone. Daily oral administration of NPS-2143 for 5 weeks, 3 months after ovariectomy, increased circulating levels of PTH within 24 h. Morphometric parameters indicative of bone formation or resorption were increased by NPS-2143, with no net increase in BMD in the distal femur or the proximal tibia. NPS-2143 resulted in a net anabolic effect on bone when administered together with 17β-estradiol, a conventional antiresorptive agent. The compound increased bone turnover, BMD and trabecular bone volume in osteopenic ovariectomized rats (37).

Desirable versus undesirable features of NPS-2143

As the first CaSR antagonist NPS-2143 has served the purpose of an ideal "lead" compound for developing a calcilytic agent that can potentially stimulate new bone formation via the stimulation of endogenous PTH secretion. In theory, calcilytics are perceived to

exert a hypocalcemic stimulus upon prolonged treatment, thereby giving rise to glandular hyperplasia and/or parathyroid hypertrophy. This attribute of calcilytic agents appears to go against their use as bone-forming agents in bone loss disorders such as osteoporosis. NPS-2143, however, demonstrated that such a concern is unfounded, as 5-week treatment of rats with NPS-2143 failed to elicit parathyroid hyperplasia or hypertrophy (10).

Nonetheless, NPS-2143 suffers from a range of pharmacological limitations, including inhibition of cytochrome P450 and a longer half-life than desired (38). The latter is due to the fact that NPS-2143 is not rapidly eliminated from the body following oral administration and causes a sustained rather than transient increase in circulating PTH levels. The ideal calcilytic for bone anabolic action should be orally active and have a shorter half-life.

Ronacaleret

In view of the limitations of NPS-2143, a putatively better version of NPS-2143 was synthesized and tested. Known as ronacaleret, this next-generation calcilytic is structurally very similar to NPS-2143. The addition of a carboxylic acid moiety to NPS-2143 contributed to improvement in the off-target effects associated with NPS-2143, as well as modulation of its half-life (Table I).

Ronacaleret was tested in a clinical trial in 81 postmenopausal women who were divided into three dose cohorts of 75, 175 and 475 mg, as well as a placebo group (39). As expected, ronacaleret treatment caused a transient and dose-dependent spike in plasma PTH. At the highest dose osteoblastic markers including osteocalcin, type I collagen and bone-specific alkaline phosphatase were 63%, 79% and 35% higher, respectively, than baseline values. At the same time, carboxy-terminal collagen crosslinks, a marker of bone resorption, did not change significantly. These results provide evidence for the stimulation of new bone formation by ronacaleret. Ronacaleret is presently undergoing phase II development by GlaxoSmithKline for the treatment of osteoporosis and related bone and mineral disorders.

Recently, Balan et al. synthesized more potent calcilytics (38) (Table I) starting from ronacaleret as the lead, with the aim of achieving improvements over NPS-2143 and ronacaleret in terms of efficacy and off-target pharmacology. When profiled for in vivo tolerance in rats, the key analogues exhibited no adverse effects at doses as high as 500 mg/kg during a 4-day period. The lead compound in this series is being evaluated further in preclinical studies.

Other calcilytics

A number of known chemotypes acting as CaSR antagonists, including N^1-arylsulfonyl-N^2-[1-(1-naphthyl)ethyl]-1,2-diaminocyclohexanes, 4-aryl-1*H*-quinazolin-2-ones and 2-benzylpyrrolidine-substituted aryloxypropanols, were found to be structurally similar to NPS-2143 (Table I). However, variations in the template of NPS-2143 (the 1,1-dimethyl-2-naphthalen-2-ylethylamine part of the molecule) have not resulted in more potent calcilytics. Novel calcilytics, particularly those that are structurally distinct from NPS-2143, are also being developed to increase PTH secretion. Some of the recently discovered calcilytics are shown in Table I.

ADDITIONAL THERAPEUTIC PROSPECTS FOR CALCILYTICS

CaSR is expressed in a variety of benign and malignant tumors. Both breast and prostate cancers metastasize to bone, as bone represents a fertile ground for cancer cells to flourish and thrive (40). CaSR has been shown to play an important role in humoral hypercalcemia of malignancy (HHM) as a mediator of the malignancy-associated feed-forward loop between tumor and bone resulting in osteolysis. CaSR accomplishes this function by stimulating the production of PTH-related peptide (PTHrP) (41, 42). PTHrP binds to the same receptor as PTH, thereby activating bone turnover, including the formation and activity of osteoclasts. In breast and prostate cancer cells PTHrP release is increased by activation of the CaSR (43). It is therefore conceivable that antagonizing the function of CaSR could reduce skeletal metastases of breast and prostate cancer cells. Whether antagonizing the CaSR with calcilytics could have therapeutic potential awaits in vivo documentation of the tumor-promoting role of the CaSR in HHM. Another important issue is the requirement of a calcilytic with a very different pharmacokinetic profile than those that are being developed for osteoporosis treatment. Whereas rapid clearance of the calcilytic is desirable for new bone formation, a calcilytic with a longer half-life must be developed for effectively inhibiting cancer metastasis to bone.

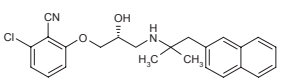
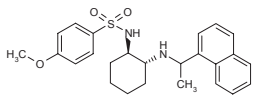
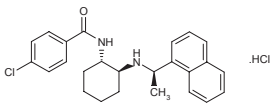
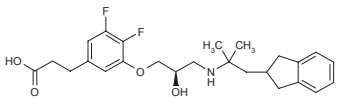
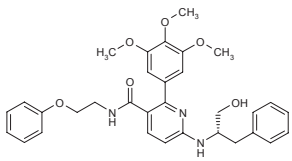
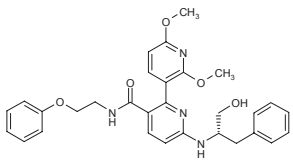
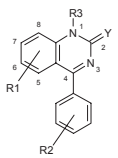
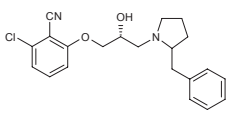
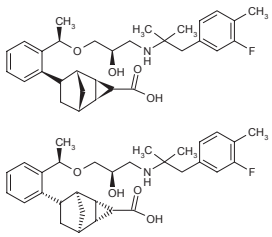
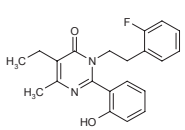
DELETERIOUS CONSEQUENCES OF CONTINUOUSLY ANTAGONIZING THE CaSR

Repeated antagonism of the CaSR could theoretically lead to parathyroid gland hyperplasia. Whether chronic stimulation of the parathyroid gland by dietary calcium deficiency increases parathyroid cell proliferation is controversial. Increased proliferation has been seen in weanling but not older rats fed a low-calcium diet (44, 45). Similarly, secondary and tertiary hyperparathyroidism is seen in patients with hypophosphatemic osteomalacia and X-linked dominant hypophosphatemic rickets, respectively, on long-term oral phosphate therapy, which may or may not involve the CaSR. Studies of longer duration will be necessary to evaluate the safety and efficacy of antagonizing the CaSR.

SUMMARY AND CONCLUSIONS

The function of the CaSR in bone biology has been actively investigated. Activation of the CaSR has been suggested to promote osteoblast function and inhibit osteoclast function. These effects indicate dual beneficial actions of the CaSR on bone that could be therapeutically exploited for postmenopausal osteoporosis. However, the lack of convincing data from animal studies precludes using calcimimetics (CaSR agonists) for osteoporosis treatment. However, calcilytics (CaSR antagonists) have been proposed for osteoporosis therapy, as they provide the means to achieve secretion of endogenous PTH, leading to new bone formation. The first-generation calcilytic NPS-2143 stimulates PTH secretion by three- to four-fold, but PTH remains elevated for several hours. In animal studies, NPS-2143 treatment resulted in an increase in bone turnover, but not BMD, in osteopenic rats. Therefore, the need for much shorter-acting calcilytics became apparent. Newer molecules have been synthesized that induce a much shorter stimulatory pulse of PTH secretion in rats. However, their role in new bone formation in vivo remains to be determined. The future use of calcilytics in osteoporosis is an entic-

Table I. *NPS-2143-based calcilytics and new calcilytic compounds.*

CaSR antagonists	IC_{50}	Structure	In vitro/in vivo effects	Ref.
NPS-2143	0.043 μM		Increases plasma PTH levels and bone formation when administered daily by oral gavage to estrogen-treated osteopenic rats for 5 weeks. The latter effect was sufficient to increase bone mineral density and trabecular bone volume.	10
N^1-Arylsulfonyl-N^2-[1-(1-naphthyl)ethyl]-1,2-diamino-cyclohexanes (17)	10 μM		Derivative 17 activates the phospholipase C (PLC) casrcade, which is an established downstream event following CaSR activation in CaSR-transfected CHO cells, but not in wild-type CHO cells (lacking endogenous CaSR expression).	46
4-Chlorobenzoyl-N^2-[1-(1-naphthyl)ethyl]-1,2-diamino-cyclohexanes (calhex-231)	0.39 μM		In CaSR-transfected HEK-293 cells, calhex-231 elicited the production of inositol phosphates, suggesting activation of the PLC pathway.	47
Ronacaleret hydrochloride	150 μM			39
Compound 1 (48), compound 18c	64 nM, 0.076 μM		In rats compounds robustly increased plasma PTH levels following i.v. and p.o. administration. Plasma concentrations after a single oral dose of 30 mg/kg attained maximal levels of ~ 2 μM within 1 h after administration, with an oral half-life of 2 h.	48, 52
Compund 18d	0.14 μM			52
4-Aryl-1*H*-quinazolin-2-ones	10 nM-50 μM		Antagonist activity at the human parathyroid CaSR was tested in functional assays measuring inhibition of calcium-induced inositol phosphate formation in stably transfected CCL39 fibroblasts.	49, 50
2-Benzylpyrrolidine-substituted aryloxypropanols (compound (*S*)-3h)	0.35 μM		The active compound showed inhibition in the fluorescence-based intracellular Ca^{2+} mobilization assay in HEK cells overexpressing the human CaSR, with comparable potency to NPS-2143, but reduced hERG-blocking activity.	51
Benzyloxy analogues	50 nM		Benzyloxy analogues elicit a robust PTH response at a dose of 10 mg/kg in rats, comparable to that of other calcilytics. Assessment of regulatory pharmacological parameters showed no adverse effects at doses as high as 500 mg/kg administered over a 4-day period.	38
3*H*-Quinazolin-4-ones (compound 1)	0.097 μM		Administration of 1 or 3 μmol/kg i.v. to rats resulted in maximal plasma PTH levels at 1 min, followed by a decline to predose level by 10 min.	53

ing prospect, as they could substitute the injectable PTH therapy that is currently used to promote new bone formation. Another potential use of calcilytics could be the medical management of HHM, which causes skeletal metastases and osteolysis. However, the pharmacokinetic and pharmacodynamic profiles of putative calcilytics for the management of HHM must be very different from those being developed for the treatment of osteoporosis.

DISCLOSURE

The authors have nothing to disclose.

REFERENCES

1. Brown, E.M. *Extracellular Ca2+ sensing, regulation of parathyroid cell function, and role of Ca2+ and other ions as extracellular (first) messengers.* Physiol Rev 1991, 71(2): 371-411.
2. Brown, E.M., MacLeod, R.J. *Extracellular calcium sensing and extracellular calcium signaling.* Physiol Rev 2001, 81(1): 239-7.
3. Hawkins, D., Enyedi, P., Brown, E. *The effects of high extracellular Ca2+ and Mg2+ concentrations on the levels of inositol 1,3,4,5-tetrakisphosphate in bovine parathyroid cells*. Endocrinology 1989, 124(2): 838-44.
4. Brown, E.M., Gamba, G., Riccardi, D. et al. *Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid.* Nature 1993, 366(6455): 575-80.
5. Brown, E.M. *Clinical lessons from the calcium-sensing receptor.* Nat Clin Pract Endocrinol Metab 2007, 3(2): 122-33.
6. Hofer, A.M, Brown, E.M. *Extracellular calcium sensing and signalling*. Nat Rev Mol Cell Biol 2003, 4(7): 530-8.
7. Chattopadhyay, N., Brown, E.M. *Role of calcium-sensing receptor in mineral ion metabolism and inherited disorders of calcium-sensing.* Mol Genet Metab 2006, 89(3): 189-202.
8. Nemeth, E.F., Steffey, M.E., Hammerland, L.G., Hung, B.C., Van Wagenen, B.C., DelMar, E.G., Balandrin, M.F. *Calcimimetics with potent and selective activity on the parathyroid calcium receptor.* Proc Natl Acad Sci U S A 1998, 95(7): 4040-5.
9. Soudijn, W., Van Wijngaarden, I., Ijzerman, A.P. *Allosteric modulation of G protein-coupled receptors: Perspectives and recent developments.* Drug Discov Today 2004, 9(17): 752-8.
10. Nemeth, E.F., Delmar, E.G., Heaton, W.L. et al. *Calcilytic compounds: potent and selective Ca2+ receptor antagonists that stimulate secretion of parathyroid hormone.* J Pharmacol Exp Ther 2001, 299(1): 323-31.
11. Dempster, D.W., Cosman, F., Parisien, M., Shen, V., Lindsay, R. *Anabolic actions of parathyroid hormone on bone.* Endocr Rev 1993, 14(6): 690-709.
12. Trivedi, R., Mithal, A., Chattopadhyay, N. *Anabolics in osteoporosis: The emerging therapeutic tool.* Curr Mol Med 2009, Epud ahead of print.
13. Fox, J. *Developments in parathyroid hormone and related peptides as bone-formation agents.* Curr Opin Pharmacol 2002, 2(3): 338-44.
14. Verhaar, H.J., Lems, W.F. *PTH-analogs: Comparable or different?* Arch Gerontol Geriatr 2009, 49(2): e130-2.
15. Kimmel, D.B., Bozzato, R.P., Kronis, K.A., Coble, T., Sindrey, D., Kwong, P., Recker, R.R. *The effect of recombinant human (1-84) or synthetic human (1-34) parathyroid hormone on the skeleton of adult osteopenic ovariectomized rats.* Endocrinology 1993, 132(4): 1577-84.
16. Parfitt, A.M. *Bone and plasma calcium homeostasis.* Bone 1987, 8(Suppl. 1): S1-8.
17. Parfitt, A.M. *Plasma calcium control at quiescent bone surfaces: A new approach to the homeostatic function of bone lining cells.* Bone 1989, 10(2): 87-8.
18. Raisz, L.G. *Physiology and pathophysiology of bone remodeling.* Clin Chem 1999, 45(8, Pt. 2): 1353-8.
19. Dvorak, M.M., Siddiqua, A., Ward, D.T., Carter, D.H., Dallas, S.L., Nemeth, E.F., Riccardi, D. *Physiological changes in extracellular calcium concentration directly control osteoblast function in the absence of calciotropic hormones.* Proc Natl Acad Sci U S A 2004, 101(14): 5140-5.
20. Dvorak, M.M., Riccardi, D. *Ca2+ as an extracellular signal in bone.* Cell Calcium 2004, 35(3): 249-55.
21. Yamaguchi, T., Chattopadhyay, N., Kifor, O., Ye, C., Vassilev, P.M., Sanders, J.L., Brown, E.M. *Expression of extracellular calcium-sensing receptor in human osteoblastic MG-63 cell line.* Am J Physiol Cell Physiol 2001, 280(2): C382-93.
22. Shalhoub, V., Grisanti, M., Padagas, J. et al. *In vitro studies with the calcimimetic, cinacalcet HCl, on normal human adult osteoblastic and osteoclastic cells*. Crit Rev Eukaryot Gene Expr 2003, 13(2-4): 89-106.
23. Christiansen, B., Hansen, K.B., Wellendorph, P., Brauner-Osborne, H. *Pharmacological characterization of mouse GPRC6A, an L-alpha-amino-acid receptor modulated by divalent cations.* Br J Pharmacol 2007, 150(6): 798-807.
24. Trivedi, R., Mithal, A., Chattopadhyay, N. *Recent updates on the calcium-sensing receptor as a drug target.* Curr Med Chem 2008, 15(2): 178-86.
25. Kameda, T., Mano, H., Yamada, Y. et al. *Calcium-sensing receptor in mature osteoclasts, which are bone resorbing cells.* Biochem Biophys Res Commun 1998, 245(2): 419-22.
26. Lorget, F., Kamel, S., Mentaverri, R., Wattel, A., Naassila, M., Maamer, M., Brazier, M. *High extracellular calcium concentrations directly stimulate osteoclast apoptosis.* Biochem Biophys Res Commun 2000, 268(3): 899-903.
27. Lord, B.I., Testa, N.G., Hendry, J.H. *The relative spatial distributions of CFUs and CFUc in the normal mouse femur*. Blood 1975, 46(1): 65-72.
28. Gong, J.K. *Endosteal marrow: A rich source of hematopoietic stem cells*. Science 1978, 199(4336): 1443-5.
29. House, M.G., Kohlmeier, L., Chattopadhyay, N. et al. *Expression of an extracellular calcium-sensing receptor in human and mouse bone marrow cells.* J Bone Miner Res 1997, 12(12): 1959-70.
30. Sharan, K., Siddiqui, J.A., Swarnkar, G., Chattopadhyay, N. *Role of calcium-sensing receptor in bone biology.* Indian J Med Res 2008, 127(3): 274-86.
31. Marie, P.J. *Strontium ranelate: A novel mode of action optimizing bone formation and resorption.* Osteoporos Int 2005, 16(Suppl. 1): S7-10.
32. Chattopadhyay, N., Quinn, S.J., Kifor, O., Ye, C., Brown, E.M. *The calcium-sensing receptor (CaR) is involved in strontium ranelate-induced osteoblast proliferation.* Biochem Pharmacol 2007, 74(3): 438-47.
33. Mentaverri, R., Yano, S:, Chattopadhyay, N. et al. *The calcium sensing receptor is directly involved in both osteoclast differentiation and apoptosis*. FASEB J 2006, 20(14): 2562-4.
34. Hofbauer, L.C., Heufelder, A.E. *Role of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin in bone cell biology*. J Mol Med 2001, 79(5-6): 243-53.
35. Bonnelye, E., Chabadel, A., Saltel, F., Jurdic, P. *Dual effect of strontium ranelate: Stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro.* Bone 2008, 42(1): 129-38.
36. Li, Z., Lu, W.W., Deng, L. et al. *The morphology and lattice structure of bone crystal after strontium treatment in goats*. J Bone Miner Metab 2009, Epub ahead of print.
37. Nemeth, E.F. *The search for calcium receptor antagonists (calcilytics).* J Mol Endocrinol 2002, 29(1): 15-21.
38. Balan, G., Bauman, J., Bhattacharya, S. et al. *The discovery of novel calcium sensing receptor negative allosteric modulators*. Bioorg Med Chem Lett 2009, 19(12): 3328-32.
39. Fitzpatrick L., Brennan, E., Kumar, S. et al. *Ronacaleret, a novel calcium-sensing receptor antagonist, demonstrates potential as an oral bone-forming therapy in healthy postmenopausal women.* J Bone Miner Res 2008, 23(Suppl. S): Abst 1174.

40. Paget, S. *The distribution of secondary growths in cancer of the breast.* Cancer Metastasis Rev 1989, 8(2): 98-101.
41. Chattopadhyay, N. *Effects of calcium-sensing receptor on the secretion of parathyroid hormone-related peptide and its impact on humoral hypercalcemia of malignancy.* Am J Physiol Endocrinol Metab 2006, 290(5): E761-70.
42. Grill, V., Ho, P., Body, J.J. et al. *Parathyroid hormone-related protein: Elevated levels in both humoral hypercalcemia of malignancy and hypercalcemia complicating metastatic breast cancer.* J Clin Endocrinol Metab 1991, 73(6): 1309-15.
43. Brandt, D.W., Pandol, S.J., Deftos, L.J. *Calcium-stimulated parathyroid hormone-like protein secretion: Potentiation through a protein kinase-C pathway.* Endocrinology 1991, 128 (6): 2999-3004.
44. Naveh-Many, T., Rahamimov, R., Livni, N., Silver, J. *Parathyroid cell proliferation in normal and chronic renal failure rats. The effects of calcium, phosphate, and vitamin D.* J Clin Invest 1995, 96(4): 1786-93.
45. Wernerson, A., Widholm, S.M., Svensson, O., Reinholt, F.P. *Parathyroid cell number and size in hypocalcemic young rats.* APMID 1991, 99(12): 1096-102.
46. Kessler, A., Faure, H., Roussanne, M.C., Ferry, S., Ruat, M., Dauban, P., Dodd, R.H. *N'-Arylsulfonyl-N2-(1-(1-naphthyl)ethyl)-1,2-diaminocyclohexans: A new class of calcilytic agents acting on the calcium-sensing receptor.* ChemBioChem 2004, 5(8): 1131-6.
47. Petrel, C., Kessler, A., Dauban, P., Dodd, R.H., Rognan, D., Ruat, M. *Positive and negative allosteric modulators 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.
48. Arey, B.J., Seethala, R., Ma, Z. et al. *A novel calcium-sensing receptor antagonist transiently stimulates parathyroid hormone secretion in vivo.* Endocrinology 2005, 146(4): 2015-22.
49. Beerli, R., Tommasi, R.A., Weiler, S., Widler, L. Chem Abstr 2002, 138: 39293.
50. Altmann, E., Beerli, R., Gerspacher, M., Renaud, J., Weiler, S., Widler, L. (Novartis AG; Novartis Pharma GmbH). *Aryl-quinazoline/aryl-2aminophenyl methanone derivatives.* WO 2004056365.
51. Yang, W., Wang, Y., Roberge, J.Y. et al. *Discovery and structure-activity relationships of 2-benzylpyrrolidine-substituted aryloxypropanols as calcium-sensing receptor antagonists.* Bioorg Med Chem Lett 2005, 15(4): 1225-8.
52. Yang, W., Ruan, Z., Wang, Y. et al. *Discovery and structure-activity relationships of trisubstituted pyrimidines/pyridines as novel calcium-sensing receptor antagonists.* J Med Chem 2009, 52(4): 1204-8.
53. Shcherbakova, I., Balandrin, M.F., Fox, J., Ghatak, A., Heaton, W.L., Conklin, R.L. *3H-Quinazolin-4-ones as a new calcilytic template for the potential treatment of osteoporosis.* Bioorg Med Chem Lett 2005, 15(6): 1557-60.