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PERSPECTIVES

Anabolic Therapy for Osteoporosis: Calcilytics

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Abstract

Calcilytics are calcium receptor antagonists that stimulate the secretion of parathyroid hormone (PTH) from the parathyroid glands. Some calcilytics are orally bioavailable and can elicit a rapid and transient increase in circulating levels of endogenous PTH. Daily oral administration of a calcilytic compound to ovariectomized rats stimulates new bone formation and increases bone mineral density and biomechanical strength at cortical and cancellous sites. An orally-active calcilytic compound is currently being assessed for safety and efficacy in a Phase II clinical trial in postmenopausal women with osteoporosis. It can be anticipated that some of the safety and efficacy properties of exogenously administered PTH peptides will be shared with calcilytics, whereas others will be unique to these small molecule compounds. *IBMS BoneKEy*. 2008 June;5(6):196-208.

Introduction

Parathyroid hormone (PTH) is perhaps unique amongst the hormones that affect skeletal metabolism because it stimulates both osteoclastic bone resorption and osteoblastic formation of new bone; and it is largely the pharmacokinetics of PTH that determine the net effect on the skeleton. Thus, a sustained increase in PTH, as occurs in severe cases of primary or secondary hyperparathyroidism, results in net bone loss at both cortical and cancellous sites. In contrast, intermittent increases in circulating levels of PTH caused by the daily (or near daily) administration of exogenous hormone result in an anabolic effect, especially at sites of cancellous bone (1-4). Both anabolic and catabolic effects of PTH are mediated by a G protein-coupled receptor, the PTH-R1 (5). One approach to anabolic therapy for osteoporosis therefore involves the administration of ligands that activate this receptor. At present, the only ligands reported to do so are the native hormone and various N-terminal-intact peptide fragments of PTH or parathyroid hormone-related peptide (PTHrP). When administered daily by subcutaneous injection, PTH or PTH(1-34) (teriparatide) stimulates new bone formation, increases bone mineral density (BMD) and reduces the risk of fracture in postmenopausal women with osteoporosis (6;7). Other anabolic peptides in clinical development include PTHrP(1-36) (8), an N-terminal analog of PTHrP (BA058; (9)) and the cyclic peptide analog Ostabolin C™ (10).

While peptide agonists of the PTH-R1 are generally safe and effective anabolic therapies, their use is compromised by the systemic administration of a costly biological product. A number of alternative delivery systems are therefore being explored, of which the more common are nasal, pulmonary, and dermal (11). Although these routes of administration might increase patient compliance, they are unlikely to diminish the cost of goods inherent in biological products and their delivery devices.

An orally-active small molecule that directly or indirectly activates the PTH-R1 might be an alternative anabolic therapy for osteoporosis that would conceivably overcome these drawbacks of peptide ligands. However, potent and selective small molecule agonists of the PTH-R1 (and of Family B G protein-coupled receptors in general (12)) have proven difficult to design and discover. An indirect means of activating the PTH-R1 is by stimulating secretion of endogenous PTH. And the most effective way to do this is by blocking the

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activity of the parathyroid calcium receptor. Indeed, drugs acting on the calcium receptor might be the only way to achieve rapid and transient changes in the circulating levels of endogenous PTH. The ability of calcium receptor activators (calcimimetics) like cinacalcet to rapidly lower PTH levels in patients with hyperparathyroidism establishes the parathyroid calcium receptor as a tractable drug target. Calcium receptor antagonists (calcilytics) stimulate secretion of PTH and might provide a novel approach for anabolic therapy based on orally-active small molecules.

The purpose of this *Perspective* is partly to summarize the data currently obtained with calcilytics but mostly to anticipate some of the safety and efficacy issues relevant to this therapeutic approach to treating osteoporosis. Some of these issues will likely be similar to those that have been encountered with peptide agonists of the PTH-R1. Other aspects of safety and efficacy will be specific to calcilytics and, because there is no precedent for this kind of therapy, are more speculative.

Calcilytics and Their Effects on Parathyroid Cells

It was not always certain that a calcium receptor antagonist would stimulate PTH secretion because, unlike calcimimetics, there was no organic or inorganic substance known to block receptor activity (13). This ligand void has now been filled with several structurally distinct, small organic compounds that have been shown to act as calcilytics and stimulate the secretion of PTH (Figure 1).

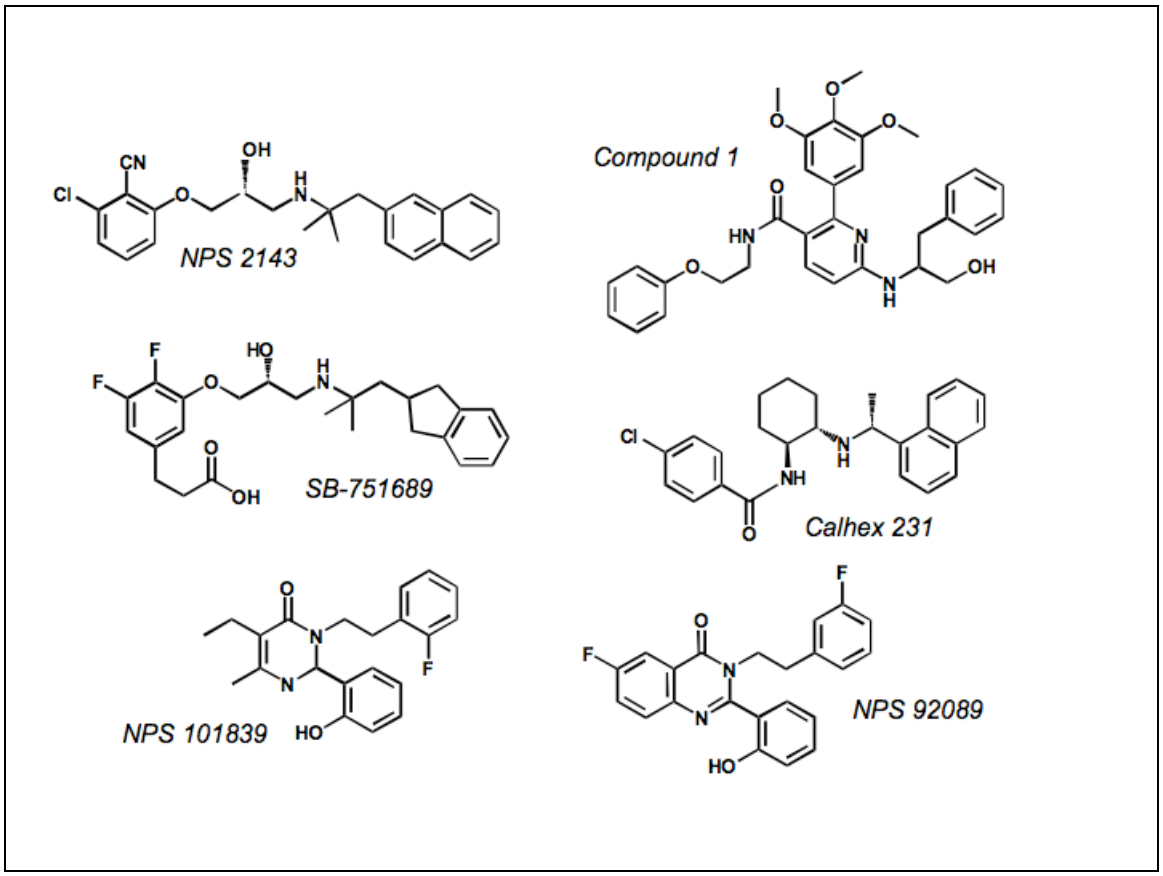

Figure 1. Structures of compounds reported to act as calcilytics. Most medicinal chemists would classify these compounds into four distinct chemotypes.

These compounds were discovered either by high-throughput screening or they were generated by progressive modifications to the amino alcohol structure of NPS 2143. All

of these compounds inhibit extracellular calcium-induced increases in inositol phosphates or cytoplasmic Ca^{2+} in heterologous expression systems like HEK 293 cells; in these *in vitro* assays, the compounds have potencies (IC_{50}) <100 nM (14-19). Most of the compounds shown in Figure 1 are believed to act as negative allosteric modulators of the calcium receptor; they shift the concentration-response curve for extracellular calcium to the right without affecting maximal or minimal responses (20). Radioligand binding studies reveal several distinct binding sites for these compounds within the transmembrane domain of the receptor (17;21;22). With the exception of Calhex 231, all of these compounds have been shown to elicit a rapid increase in plasma levels of PTH in rats when injected intravenously.

The parathyroid calcium receptor regulates a number of distinct responses including the synthesis and secretion of PTH and cellular proliferation. Activating the calcium receptor with a calcimimetic compound like cinacalcet inhibits all these cellular responses (23), so it might be expected that calcilytic compounds would affect these same responses but conversely. Calcilytics do stimulate the secretion of PTH, and they additionally stimulate the synthesis of PTH, in part by stabilizing the mRNA for prepro-PTH (24). This latter effect might ensure that daily dosing with a calcilytic will not deplete the supply of glandular PTH available for release.

One of the initial concerns in the calcilytic approach for treating osteoporosis was that repetitive antagonism of the calcium receptor could be perceived by the parathyroid gland as hypocalcemia and this, in turn, could elicit parathyroid gland hyperplasia. The long-term consequence could be sustained increases in serum levels of PTH – essentially a treatment-induced secondary hyperparathyroidism that would be expected to cause bone loss rather than gain. So it was reassuring to find that daily dosing of normal or ovariectomized (OVX) rats with NPS 2143 for five weeks did not stimulate parathyroid cell proliferation (25).

The failure of NPS 2143 to stimulate parathyroid hyperplasia does not necessarily conflict with the finding that calcimimetics completely block parathyroid cell proliferation (26). Parathyroid cells have very low rates of mitosis but undergo rapid and massive proliferative responses in animal models of end-stage renal disease. Calcimimetics are very effective in blocking this *induced* proliferative response and will halt ongoing proliferation even in animals with profound hyperphosphatemia and negligible levels of 1,25-$(OH)_2D_3$ (27). The failure of calcilytics to induce parathyroid cell proliferation in animals with normal kidney function suggests that hypocalcemia alone is not sufficient to trigger parathyroid cell proliferation; concomitant increases in phosphate and/or decreases in 1,25$(OH)_2D_3$ (or some other factor(s)) might be required. Together, the results obtained with calcimimetics and calcilytics suggest that activation of the calcium receptor is sufficient to block parathyroid cell proliferation but inhibition of the receptor is insufficient to stimulate proliferation.

It is not known if prolonged treatment with a calcilytic compound will decrease expression of the parathyroid calcium receptor. Calcimimetics have been shown to increase calcium receptor expression in hyperplastic parathyroid glands of rats with chronic renal failure (28). In HEK 293 cells engineered to overexpress the calcium receptor, incubation with the calcimimetic NPS R-568 increased whereas NPS 2143 decreased receptor expression (29). There is a rather direct relationship between calcium receptor number and functional responses to calcimimetics and calcilytics in this heterologous expression system but it might differ from that in cells that normally express the calcium receptor. In parathyroid cells, there appears to be a large receptor reserve and calcium receptor expression can be profoundly decreased without altering the magnitude of response to or the potency of calcimimetic compounds (30). Moreover, patients with primary or secondary hyperparathyroidism have reduced expression of the calcium receptor yet respond with rapid decreases in serum PTH levels when administered cinacalcet and do not show tolerance to the effects of

this drug (31;32). Analogously, rats treated daily with NPS 2143 for eight weeks or with SB-423557 for twelve weeks still show a rapid increase in serum PTH levels when challenged with the calcilytic compound near the end of the dosing regimen (25;33). So even if there is a reduction of parathyroid calcium receptors following repeated dosing with a calcilytic, it does not manifest itself as a diminished secretory response, at least within the time frames studied so far.

Efficacy in the Rat Model of Osteoporosis

To be most useful as a therapeutic, a calcilytic compound must possess some challenging pharmacokinetic properties. The compound must be orally bioavailable and it must rapidly reach a maximal concentration (C_{max}) in blood yet have a short plasma half-life ($t_{1/2}$). Additionally, there can be no active metabolites that linger in the circulation. These are the pharmacokinetic properties that will facilitate patient compliance and achieve a rapid and transient increase in plasma levels of PTH.

Compound 1 (Figure 1) has some of the desired pharmacokinetic properties. It is orally bioavailable in rats and reaches a C_{max} of about 2 μM at 1 hour following a single oral 30 mg/kg dose (its IC_{50} *in vitro* is 64 nM). At this dose, there was a 2- to 3-fold increase in plasma levels of PTH that coincided with the C_{max} of the compound and returned to baseline levels within 3 hours (17). The effects of Compound 1 in an animal model of osteoporosis have yet to be reported but the magnitude and time-course of the change in plasma PTH levels are appropriate to achieve an increase in BMD.

The first calcilytic compound examined in detail, NPS 2143, was orally-active but had a very long $t_{1/2}$ due mostly to a large volume of distribution; it caused a sustained increase in serum PTH levels that remained elevated for at least four hours (but returned to baseline by 24 hours; (25)). Despite this less than ideal pharmacokinetic profile, the compound was useful for determining whether a calcilytic could release enough endogenous PTH to affect bone turnover in the OVX rat. Daily oral administration of NPS 2143 for five weeks increased circulating levels of PTH and increased bone turnover in osteopenic rats (25). It did not, however, achieve a net anabolic effect in the OVX rat because it increased both formation and resorption to a similar extent so there was no net increase (or decrease) in BMD. When resorption was depressed by the co-administration of 17β-estradiol, then formation predominated and there was an increase in BMD. Although the compound by itself did not result in an anabolic effect, it did demonstrate that PTH could be released from the parathyroid glands in amounts sufficient to stimulate bone turnover. As noted above, these studies additionally showed that prolonged daily dosing with a calcilytic did not cause hyperplasia of the parathyroid glands and tolerance to the compound did not develop. In the aggregate, the results obtained with NPS 2143 established the preclinical proof-of-concept for the calcilytic approach to anabolic therapy.

These initial findings justified a medicinal chemistry effort aimed at improving the pharmacokinetic properties of the amino alcohol chemotype. It was a long and frustrating task because upgrades in one pharmacokinetic parameter were often accompanied by downgrades in another. And of course each structural modification can jeopardize potency and/or selectivity. A brute force approach finally did yield a series of compounds that were promising in all respects save one: their oral bioavailability was too low. To circumvent this caveat, esters of the amino alcohol chemotype were synthesized. This yields a pro-drug: the ester has much higher oral bioavailability and, once absorbed, is rapidly cleaved to the free acid that then enters the systemic circulation (19).

One ester/acid pair is SB-423557/SB-423562 (Figure 2). The pro-drug is SB-423557 and is rapidly converted to SB-423562 following oral administration. The oral administration of SB-423557 caused a dose-dependent, transient increase in plasma levels of PTH in rats, dogs and monkeys (33). The efficacy of this pro-drug approach was assessed in OVX rats. Six-

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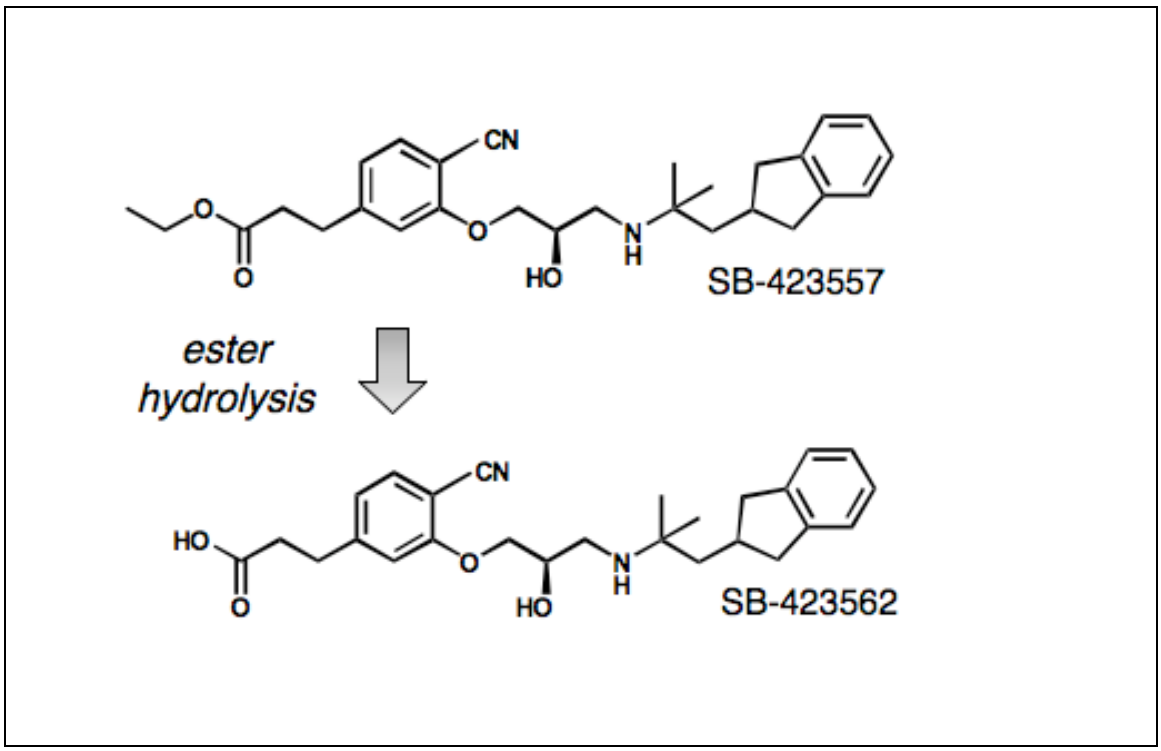

Figure 2. The pro-drug approach to overcoming limited bioavailability of amino alcohol calcilytics.

month-old female rats underwent ovariectomy and osteopenia was allowed to develop for 6 weeks before starting 12 weeks of daily oral dosing with 50 mg/kg SB-423557 or vehicle control. Teriparatide (5 μg/kg s.c.) was included as a comparator. A single oral dose of SB-423557 (50 mg/kg) caused a 2- to 3-fold increase in plasma PTH levels that peaked within 20 minutes and returned to baseline levels by 2 hours. The changes in plasma levels of PTH induced by SB-423557 were similar at the start and at the end of the study. These changes in endogenous levels of PTH were associated with a number of skeletal parameters indicative of a net anabolic effect. The OVX-induced decrease in BMD occurring during the three-month treatment period was completely (lumbar spine) or partially (proximal tibia) prevented by daily treatment with SB-423557. There were improvements in a number of biomechanical properties at the lumbar spine and femoral diaphysis, compared to vehicle-treated controls. Significantly, there was a 72% increase in cortical area and a more than 2-fold increase in endosteal bone formation rate in the distal tibia that was not accompanied by any change in eroded perimeter. The magnitude of the anabolic effect of SB-423557 was similar to that achieved by teriparatide and neither effect required the co-administration of an anti-resorptive (33). These findings in the OVX rat showed that daily oral dosing with a calcilytic compound can, by itself, increase BMD and biomechanical estimates of bone strength when administered orally.

Clinical Studies

The preclinical studies with SB-423557 prompted clinical studies with this pro-drug and its corresponding acid. In the first study, SB-423562 (the acid) was infused intravenously over ten minutes in healthy adult men (34). There was a rapid, dose-dependent increase in plasma levels of PTH that peaked at 15 min. after infusion and, depending on the dose, returned to baseline levels within one hour. The higher doses (1.25 to 5 mg) elicited 3- to 5.5-fold increases in plasma PTH levels that were accompanied by dose-dependent increases in total and ionized levels of serum calcium.
In the second study, SB-423557 was administered orally to healthy adult men (35). As anticipated, blood levels of SB-

423557 were typically undetectable; instead, levels of SB-423562 increased with increasing doses of SB-423557. There were dose-dependent increases in plasma levels of PTH that tracked the time course of SB-423562 concentrations and that peaked (T_{max}) at 1 to 2 hours after dosing. Doses of 50 mg SB-423557 or higher elicited 1.8- to 4.2-fold increases in plasma levels of PTH (at T_{max}) when compared to placebo. Elevations in plasma PTH, defined as twice the pre-dose levels, rarely exceeded eight hours. In this study, there were no significant increases in serum levels of calcium. And in both studies, the calcilytic compounds were generally safe and well-tolerated at all doses and no serious adverse events were reported.

Moving forward in the clinic is SB-751689 (ronacaleret), another orally-active amino alcohol chemotype. A multiple dose, proof-of-concept study in postmenopausal women has been completed and this compound is currently in a Phase II study in postmenopausal women with osteoporosis.

Comparison with Anabolic PTH Peptides

It's tempting but risky to draw comparisons between calcilytics and exogenously administered PTH peptides, mostly because the clinical experience with the former is so nascent. Moreover, not all anabolic PTH peptides are clinically equivalent: they differ in their pharmacokinetic profiles and, although all act on the PTH-R1, they have subtle pharmacodynamic differences even when tested at bioequivalent doses (36). Finally, calcilytic compounds stimulate secretion from parathyroid cells so all vesicular contents (in addition to PTH) will be released. We don't know everything that's in the parathyroid secretory vesicle but besides the intact hormone there are C-terminal fragments derived from it by proteolysis, cathepsin-like enzymes, and perhaps as much chromogranin A as in chromaffin cells. The impact of these additional constituents on the efficacy and safety profile of a calcilytic, if any, is not known. So there are some reasons to suppose that a calcilytic will not simply be an oral version of any of the anabolic PTH peptides either on the market or in clinical development. Nonetheless, it would not be prudent to ignore the data that have been obtained with the anabolic PTH peptides.

There are a number of unresolved issues concerning the efficacy of the anabolic PTH peptides. Many of them are related to the dosing regimen and include how long to treat, how fast bone will be lost after treatment ceases, and whether daily and alternate day dosing are equivalent. One surprising finding was the decrease in efficacy when teriparatide or PTH was co-administered with alendronate (37;38). The diminished efficacy of combination therapy is dependent on the type of anti-resorptive and efficacy is not impaired when teriparatide or PTH is co-administered with estrogen (39;40). In the OVX rat, co-administration of the long-acting calcilytic NPS 2143 and estrogen uncovered an anabolic effect that could not be obtained by treating with this calcilytic alone. It is not known how alendronate or other bisphosphonates will affect the efficacy of the newer calcilytic compounds that are by themselves anabolic. Nor has the optimal duration and frequency of dosing with a calcilytic compound been established.

The safety concerns with anabolic PTH and PTHrP peptides that have received the most attention are osteosarcoma and hypercalcemia (3;41;42). An increased incidence of osteosarcoma in rats was observed following daily treatment for two years with high doses of PTH (43) or teriparatide (44;45). As yet, there is no evidence linking these PTH peptides with osteosarcoma when used therapeutically in humans. These peptides are not approved for use in children or in any patient with an increased risk for cancer or with a high turnover bone disease (like Paget's disease). The increased incidence of osteosarcoma seen in the rodent studies might be a result of daily, essentially life-long treatment with a supra-therapeutic dose of peptide. It is not at all certain that such high levels of endogenous PTH, similar to those that are produced by administration of the exogenous peptide, can be achieved with a calcilytic compound.

In contrast to the tentative relationship between PTH and osteosarcoma is the well-established relationship between circulating levels of PTH and calcium. Although there is no single pharmacokinetic parameter of the PTH response that is prognostic, serum levels of calcium appear to respond more to time above baseline, rather than to the C_{max} or area under the curve (AUC) of plasma PTH (46). The $t_{1/2}$ of calcilytics is therefore a pharmacokinetic property that impacts both efficacy (transient increase in PTH) and safety (no accompanying hypercalcemia). It's noteworthy, but not clear, why the intravenous injection of SB-423562 elicited a significant increase in serum levels of calcium whereas the oral administration of SB-423557 did not (34;35).

Known Knowns, Known Unknowns, and Unknown Unknowns

Decades before Donald Rumsfeld's seminal contribution to epistemology, I heard this nub of wisdom: "There are two mechanisms of action for every drug: those we know, and those we don't know…" (see footnote). Those we don't know are the off-target actions of a compound: in the case of calcilytics, actions that are exerted on molecules other than the calcium receptor. These are the unknown unknowns that can affect safety and/or efficacy and that, we hope, reveal themselves before the compound enters the clinic. The calcilytic currently moving forward in the clinic (ronacaleret; SB-751689) has already jumped a number of safety hurdles, but clinical experience with this compound is still at an early stage.

The mechanism of action we know is antagonism of the calcium receptor. And we know that a number of distinct cellular functions of the parathyroid cell are linked to the calcium receptor and some are affected by calcilytic compounds. Overall, antagonism of this receptor produces the desired responses (stimulation of PTH synthesis and secretion) and is not accompanied by effects that could compromise efficacy (development of tolerance) or safety (parathyroid hyperplasia). But calcium receptors are expressed throughout the body (47), and it is uncertain whether the activity of these receptors will be affected by therapeutic doses of calcilytics. And the extent to which such actions might contribute to the overall safety and efficacy of calcilytic compounds is unclear. These are the known unknowns.

While the functions of the calcium receptor in many tissues are uncertain, there are some tissues where the consequences of blocking receptor activity can be inferred. Most of these are the classic calciotropic tissues involved in systemic calcium homeostasis: the parathyroid gland, parafollicular cells (C-cells) of the thyroid, the kidney, the gastrointestinal tract, and the skeleton. The C-cell calcium receptor is linked to the regulation of calcitonin secretion and calcimimetic compounds like cinacalcet stimulate secretion of calcitonin (48); calcilytic compounds would be expected to inhibit secretion of calcitonin. In humans, this hormone is a minor player in systemic calcium homeostasis and it is unlikely that transiently lowering circulating levels of calcitonin will adversely affect the safety or efficacy of a calcilytic compound.

Functions of the calcium receptor in the gastrointestinal tract are thought to include the regulation of acid and gastrin secretion in the stomach and fluid excretion in the distal colon (49). Calcilytics might decrease all three of these functions. In the kidney, the calcium receptor is expressed along most of the nephron and many of its functions within discrete segments of the nephron are known (50). Blocking calcium receptor activity in the kidney should yield an overall effect not unlike that caused by increasing circulating levels of PTH: increased phosphate excretion and increased calcium reabsorption. So in humans with intact parathyroid glands, it will be difficult to distinguish a direct effect of a calcilytic on renal calcium receptors from one that is indirect and results from increases in serum levels of PTH.

In contrast to the aforementioned tissues is the exceedingly controversial role of the calcium receptor in skeletal tissue. In summarizing a session on skeletal calcium receptors several years ago, I stated that a compelling story has yet to emerge and

instead we have "a collection of chapters, each using the same characters (PCR, antibodies, pharmacological probes) but providing a different account, not unlike Kurosawa's *Rashomon*" (51). I regret that this *Perspective* is a re-run. One can still make a case that the calcium receptor is expressed in various types of bone cells (52;53) or that it is not (54) and instead a homologous G protein-coupled receptor (GPRC6A) mediates the effects of extracellular calcium in bone (55). Likewise, cellular responses *in vitro* have been shown to be affected by calcimimetic or calcilytic compounds in some studies (56;57) but not in others (25;58). And while a global knockout of the calcium receptor did not reveal any altered skeletal phenotype (59;60), more recent experiments using conditional expression or deletion of the receptor in bone cells do show profound changes in a number of skeletal parameters (61;62). I find this morass somewhat ironic because it was, after all, a bone cell (the osteoclast) that, together with the parathyroid cell, got people thinking about cell surface calcium receptors (63). And yet, two decades later, we haven't remotely neared a consensus on this topic.

The data already obtained with NPS 2143 and SB-423557 in the OVX animals are interesting in this context. Recall that these two compounds have markedly different effects on the skeleton: the latter is anabolic whereas the former is not. Yet the essential difference between these two compounds is their pharmacokinetics, not their pharmacodynamics. So it seems reasonable to look to pharmacokinetics to explain the difference and the most parsimonious explanation is that SB-423557 causes a transient increase in serum levels of PTH (and therefore is anabolic) whereas NPS 2143 causes a sustained increase (and therefore is not). We need not invoke any action on calcium receptors in bone to explain the differential effects of these two compounds in the OVX rat. This explanation does not deny some important role for skeletal calcium receptors during development and in adult animals. It implies only that these receptors might not contribute significantly to the efficacy of these particular calcilytic compounds.

Conclusion

The calcilytic approach to treating osteoporosis is conceptually simple. And this therapeutic approach arguably carries a lower technical risk than the typical drug development program, for two reasons. First, the parathyroid calcium receptor is a tractable drug target. There are now four years of clinical experience with the calcimimetic cinacalcet; the efficacy of this compound shows that targeting the calcium receptor is probably the best, if not the only way to rapidly alter circulating levels of PTH on a daily basis. Second, exogenously administered teriparatide and PTH are approved anabolic therapies. There is no reason to suppose that transient increases in levels of endogenous PTH will not likewise build new bone in patients with osteoporosis. And they certainly do so in the OVX rat model. A validated drug target and therapeutic approach tends to lower the risk of development – but the risk is still high.

Footnote: The quote in its entirety, delivered by Professor Jack Cooper during a lecture to us graduate students, is: "There are two mechanisms of action for every drug: those we know, and those we don't know…except ouabain."

Conflict of Interest: The author reports that he is a former employee of NPS Pharmaceuticals; owns NPS stock; and is an inventor on patents related to calcimimetics and calcilytics.

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References

1. Dempster DW, Cosman F, Parisien M, Shen V, Lindsay R. Anabolic actions of parathyroid hormone on bone. *Endocr Rev*. 1993 Dec;14(6):690-709.

2. Fox J. Developments in parathyroid hormone and related peptides as bone-formation agents. *Curr Opin Pharmacol*. 2002 Jun;2(3):338-44.

3. Hodsman AB, Bauer DC, Dempster DW, Dian L, Hanley DA, Harris ST, Kendler DL, McClung MR, Miller PD, Olszynski WP, Orwoll E, Yuen CK. Parathyroid

hormone and teriparatide for the treatment of osteoporosis: a review of the evidence and suggested guidelines for its use. *Endocr Rev*. 2005 Aug;26(5):688-703.

4. Canalis E, Giustina A, Bilezikian JP. Mechanisms of anabolic therapies for osteoporosis. *N Engl J Med*. 2007 Aug 30;357(9):905-16.

5. Gensure RC, Gardella TJ, Jüppner J. Parathyroid hormone and parathyroid hormone-related peptide, and their receptors. *Biochem Biophys Res Commun*. 2005 Mar 18;328(3):666-78.

6. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH. Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N Engl J Med*. 2001 May 10;344(19):1434-41.

7. Greenspan SL, Bone HG, Ettinger MP, Hanley DA, Lindsay R, Zanchetta JR, Blosch CM, Mathisen AL, Morris SA, Marriott TB; Treatment of Osteoporosis with Parathyroid Hormone Study Group. Effect of recombinant human parathyroid hormone (1-84) on vertebral fracture and bone mineral density in postmenopausal women with osteoporosis: a randomized trial. *Ann Intern Med*. 2007 Mar 6;146(5):326-39.

8. Horwitz MJ, Tedesco MB, Gundberg C, Garcia-Ocana A, Stewart AF. Short-term, high-dose parathyroid hormone-related protein as a skeletal anabolic agent for the treatment of postmenopausal osteoporosis. *J Clin Endocrinol Metab*. 2003 Feb;88(2):569-75.

9. O'Dea L, Lyttle CR, Rosen CJ. BA058, a novel analog of human parathyroid hormone-related peptide (PTHrP), induces evidence of bone formation without evidence of bone resorption over 7 days of exposure. *89th Annual Meeting of the Endocrine Society*. 2007; Abstract P2-137.

10. Whitfield JF, Morley P, Willick GE. Parathyroid hormone, its fragments and their analogs for the treatment of osteoporosis. *Treat Endocrinol*. 2002; 1(3):175-90.

11. Morley P. Delivery of parathyroid hormone for the treatment of osteoporosis. *Expert Opin Drug Deliv*. 2005 Nov;2(6):993-1002.

12. Hoare SR. Mechanisms of peptide and nonpeptide ligand binding to class B G-protein-coupled receptors. *Drug Discov Today*. 2005 Mar 15;10(6):417-27.

13. Nemeth EF. The search for calcium receptor antagonists (calcilytics). *J Mol Endocrinol*. 2002 Aug;29(1):15-21.

14. Kessler A, Faure H, Roussanne MC, Ferry S, Ruat M, Dauban P, Dodd RH. N(1)-Arylsulfonyl-N(2)-(1-(1-naphthyl) ethyl)-1,2-diaminocylcohexanes: a new class of calcilytic agents acting at the calcium-sensing receptor. *Chembiochem*. 2004 Aug 6;5(8):1131-6.

15. Shcherbakova I, Balandrin MF, Fox J, Ghatak A, Heaton WL, Conklin RL. 3H-Quinazolin-4-ones as a new calcilytic template for the potential treatment of osteoporosis. *Bioorg Med Chem Lett*. 2005 Mar 15;15(6):1557-60.

16. Shcherbakova I, Huang G, Geoffroy OJ, Nair SK, Swierczek K, Balandrin MF, Fox J, Heaton WL, Conklin RL. Design, new synthesis, and calcilytic activity of substituted 3H-pyrimidin-4-ones. *Bioorg Med Chem Lett*. 2005 May 16;15(10):2537-40.

17. Arey BJ, Seethala R, Ma Z, Fura A, Morin J, Swartz J, Vyas V, Yang W, Dickson JK Jr, Feyen JH. A novel calcium-sensing receptor antagonist transiently stimulates parathyroid hormone secretion in vivo. *Endocrinology*. 2005 Apr;146(4):2015-22.

18. Yang W, Wang Y, Roberge JY, Ma Z, Liu Y, Michael Lawrence R, Rotella DP, Seethala R, Feyen JH, Dickson JK Jr. Discovery and structure-activity relationships of 2-benzylpyrrolidine-substituted aryloxypropanols as calcium-sensing receptor anatagonists. *Bioorg Med Chem Lett*. 2005 Feb 15;15(4):1225-8.

19. Marquis RW. Antagonists of the calcium sensing receptor: parathyroid hormone secretagogues. *235th Annual Meeting of the American Chemical Society*. 2008; MEDI 10.

20. Nemeth EF, Delmar EG, Heaton WL, Miller MA, Lambert LD, Conklin RL, Gowen M, Gleason JG, Bhatnagar PK, Fox J. Calcilytic compounds: potent and selective Ca2+ receptor antagonists that stimulate secretion of parathyroid hormone. *J Pharmacol Exp Ther*. 2001 Oct;299(1):323-31.

21. Petrel C, Kessler A, Dauban P, Dodd RH, 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 Apr 30;279(18):18990-7.

22. Hu J, Jiang J, Costanzi S, Thomas C, Yang W, Feyen JH, Jacobson KA, Spiegel AM. A missense mutation in the seven-transmembrane domain of the human Ca2+ receptor converts a negative allosteric modulator into a positive allosteric modulator. *J Biol Chem*. 2006 Jul 28;281(30):21558-65.

23. Rodriguez M, Nemeth E, Martin D. The calcium-sensing receptor: a key factor in the pathogenesis of secondary hyperparathyroidism. *Am J Physiol Renal Physiol*. 2005 Feb;288(2):F253-64.

24. Brinton BT, Chan M, Li J, Le-Capling T, Fantaske R, Nemeth EF. Calcilytics block the suppressive effect of calcimimetics on PTH mRNA levels in bovine parathyroid cells. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S176-77.

25. Gowen M, Stroup GB, Dodds RA, James IE, Votta BJ, Smith BR, Bhatnagar PK, Lago AM, Callahan JF, DelMar EG, Miller MA, Nemeth EF, Fox J. Antagonizing the parathyroid calcium receptor stimulates parathyroid hormone secretion and bone formation in osteopenic rats. *J Clin Invest*. 2000 Jun;105(11):1595-604.

26. Wada M, Nagano N. Control of parathyroid cell growth by calcimimetics. *Nephrol Dial Transplant*. 2003 Jun;18(Suppl 3):iii13-7.

27. Chin J, Miller SC, Wada M, Nagano N, Nemeth EF, Fox J. Activation of the calcium receptor by a calcimimetic compound halts the progression of secondary hyperparathyroidism in uremic rats. *J Am Soc Nephrol*. 2000 May;11(5):903-11.

28. Mizobuchi M, Hatamura I, Ogata H, Saji F, Uda S, Shiizaki K, Sakaguchi T, Negi S, Kinugasa E, Koshikawa S, Akizawa T. Calcimimetic compound upregulates decreased calcium-sensing receptor expression level in parathyroid glands of rats with chronic renal insufficiency. *J Am Soc Nephrol*. 2004 Oct;15(10):2579-87.

29. Huang Y, Breitwieser GE. Rescue of calcium-sensing receptor mutants by allosteric modulators reveals a conformational checkpoint in receptor biogenesis. *J Biol Chem*. 2007 Mar 30;282(13):9517-25.

30. Fox J, Conklin RL, Lambert LD, Nemeth EF. The potency of the calcimimetic NPS R-568 is unaffected in rats with severe secondary hyperparathyroidism. *J Am Soc Nephrol*. 2000;11:575A.

31. Goodman WG. Calcimimetics: a remedy for all problems of excess parathyroid hormone activity in chronic kidney disease? *Curr Opin Nephrol Hypertens*. 2005 Jul;14(4):355-60.

32. Gal-Moscovici A, Sprague SM. The role of calcimimetics in chronic kidney disease. *Kidney Int*. 2006;70:S68-S72.

33. Kumar S, Liang X, Vasko JA, Stroup GB, Hoffman SJ, Vaden VR, Haley H, Fox J, Nemeth EF, Lago AM, Callahan JF, Bhatnagar P, Huffman WF, Gowen M. Oral treatment with the calcium receptor antagonist SB-423557 causes PTH release in multiple species and positive bone forming effects in the rat. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S123.

34. Ethgen D, Danoff T, Schultz M, Tenero D, Anderson A, Stroup GB, Kumar S, Lago AM, Callahan JF, Bhatnagar P, Huffman WF, Gowen M, Fitzpatrick LA. Antagonism of calcium sensing receptor stimulates dose-related release of endogenous parathyroid hormone in normal volunteers: a proof of concept study. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S128.

35. Ethgen D, Phillips J, Badoo C, Matheny C, Roshak A, Marquis R, Stroup G, Kumar S, Bhatnagar P, Huffman WF, Kitchen VS, Gowen M, Fitzpatrick LA. Dose-dependent increases in endogenous parathyroid hormone concentrations after administration of a calcium sensing receptor antagonist to normal volunteers: potential for an oral bone forming agent. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S38.

36. Nemeth EF. The parathyroid polyhormone hypothesis revisited. *Kidney Int Suppl*. 2006 Jul;(102):S22-8.

37. Black DM, Greenspan SL, Ensrud KE, Palermo L, McGowan JA, Lang TF, Garnero P, Bouxsein ML, Bilezikain JP, Rosen CJ; PaTH Study Investigators. The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. *N Engl J Med*. 2003 Sep 25;349(13):1207-15.

38. Finkelstein JS, Hayes A, Hunzelman JL, Wyland JJ, Lee H, Neer RM. The effects of parathyroid hormone, alendronate, or both in men with osteoporosis. *N Engl J Med*. 2003 Sep 25;349(13):1216-26.

39. Reeve J, Bradbeer JN, Arlot M, Davies UM, Green JR, Hampton L, Edouard C, Hesp R, Hulme P, Ashby JP, Zanelli JM, Meunier PJ. hPTH 1-34 treatment of osteoporosis with added hormone replacement therapy: biochemical, kinetic and histological responses. *Osteoporos Int*. 1991 Jun;1(3):162-70.

40. Fogelman I, Fordham JN, Fraser WD, Spector TD, Christiansen C, Morris SA, Fox J. Parathyroid hormone(1-84) treatment of postmenopausal women with low bone mass receiving hormone replacement therapy. *Calcif Tiss Intl*.

41. Tashjian AH Jr, Gagel RF. Teriparatide [human PTH(1-34)]: 2.5 years of experience on the use and safety of the drug for the treatment of osteoporosis. *J Bone Miner Res*. 2006 Mar;21(3):354-65.

42. Miller PD, Bilezikian JP, Deal C, Harris ST, Pacifici R. Clinical use of teriparatide in the real world: initial insights. *Endocr Pract*. 2004 Mar-Apr;10(2):139-48.

43. Jolette J, Wilker CE, Smith SY, Doyle N, Hardisty JF, Metcalfe AJ, Marriott TB, Fox J, Wells DS. Defining a noncarcinogenic dose of recombinant human parathyroid hormone 1-84 in a 2-year study in Fischer 344 rats. *Toxicol Pathol*. 2006;34(7):929-40.

44. Vahle JL, Sato M, Long GG, Young JK, Francis PC, Engelhardt JA, Westmore MS, Linda Y, Nold JB. Skeletal changes in rats given daily subcutaneous injections of recombinant human parathyroid hormone (1-34) for 2 years and relevance to human safety. *Toxicol Pathol*. 2002 May-Jun;30(3):312-21.

45. Vahle JL, Long GG, Sandusky G, Westmore M, Ma YL, Sato M. Bone neoplasms in F344 rats given teriparatide [rhPTH(1-34)] are dependent on duration of treatment and

dose. *Toxicol Pathol*. 2004 Jul-Aug;32(4):426-38.

46. Frolik CA, Black EC, Cain RL, Satterwhite JH, Brown-Augsberger PL, Sato M, Hock JM. Anabolic and catabolic bone effects of human parathyroid hormone (1-34) are predicted by duration of hormone exposure. *Bone*. 2003 Sep;33(3):372-9.

47. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev*. 2001 Jan;81(1):239-97.

48. Nemeth EF, Heaton WH, Miller M, Fox J, Balandrin MF, Van Wagenen BC, Colloton M, Karbon W, Scherrer J, Shatzen E, Rishton G, Scully S, Qi M, Harris R, Lacey D, Martin D. Pharmacodynamics of the type II calcimimetic compound cinacalcet HCl. *J Pharmacol Exp Ther*. 2004 Feb;308(2):627-35.

49. Hebert SC, Cheng S, Geibel J. Functions and roles of the extracellular Ca2+-sensing receptor in the gastrointestinal tract. *Cell Calcium*. 2004 Mar;35(3):239-47.

50. Ba J, Friedman PA. Calcium-sensing receptor regulation of renal mineral ion transport. *Cell Calcium*. 2004 Mar;35(3):229-37.

51. Nemeth EF. Summary--calcium receptors: potential targets for novel treatments for skeletal disease. *J Musculoskelet Neuronal Interact*. 2004 Dec;4(4):416-7.

52. Kameda T, Mano H, Yamada Y, Takai H, Amizuka N, Kobori M, Izumi N, Kawashima H, Ozawa H, Ikeda K, Kameda A, Hakeda Y, Kumegawa M. Calcium-sensing receptor in mature osteoclasts, which are bone resorbing cells. *Biochem Biophys Res Commun*. 1998 Apr 17;245(2):419-22.

53. Yamaguchi T, Kifor O, Chattopadhyay N, Brown EM. Expression of extracellular calcium (Ca2 + o)-sensing receptor in the clonal osteoblast-like cell lines, UMR-106 and SAOS-2. *Biochem Biophys Res Commun*. 1998 Feb 24;243(3):753-7.

54. Pi M, Garner SC, Flannery P, Spurney RF, Quarles LD. Sensing of extracellular cations in CasR-deficient osteoblasts. Evidence for a novel cation-sensing mechanism. *J Biol Chem*. 2000 Feb 4;275(5):3256-63.

55. Pi M, Faber P, Ekema G, Jackson PD, Ting A, Wang N, Fontilla-Poole M, Mays RW, Brunden KR, Harrington JJ, Quarles LD. Identification of a novel extracellular cation-sensing G-protein-coupled receptor. *J Biol Chem*. 2005 Dec 2;280(48):40201-9.

56. Ye CP, Yamaguchi T, Chattopadhyay N, Sanders JL, Vassilev PM, Brown EM. Extracellular calcium-sensing-receptor (CaR)-mediated opening of an outward K(+) channel in murine MC3T3-E1 osteoblastic cells: evidence for expression of a functional CaR. *Bone*. 2000 Jul;27(1):21-7.

57. Dvorak MM, Siddiqua A, Ward DT, Carter DH, Dallas SL, Nemeth EF, 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 Apr 6;101(14):5140-5.

58. Shalhoub V, Grisanti M, Padagas J, Scully S, Rattan A, Qi M, Varnum B, Vezina C, Lacey D, Martin D. In vitro studies with the calcimimetic, cinacalcet HCl, on normal human adult osteoblastic and osteoclastic cells. *Crit Rev Eurkaryot Gene Expr*. 2003;13(2-4):89-106.

59. Kos CH, Karaplis AC, Peng JB, Hediger MA, Goltzman D, Mohammad KS, Guise TA, Pollak MR. The calcium-sensing receptor is required for normal calcium homeostasis independent of parathyroid hormone. *J Clin Invest*. 2003 Apr;111(7):1021-8.

60. Tu Q, Pi M, Karsenty G, Simpson L, Liu S, Quarles LD. Rescue of the skeletal phenotype in CasR-deficient mice by transfer onto the Gcm2 null background. *J Clin Invest*. 2003 Apr;111(7):1029-37.

61. Dvorak MM, Chen TH, Orwoll B, Garvey C, Chang W, Bikle DD, Shoback DM. Constitutive activity of the osteoblast Ca2+-sensing receptor promotes loss of cancellous bone. *Endocrinology*. 2007 Jul;148(7):3156-63.

62. Chang W, Tu C, Chen T, Liu B, Elalieh H, Dvorak M, Clemens T, Kream B, Halloran B, Bikle D, Shoback D. Conditional knockouts in early and mature osteoblasts reveal a critical role for Ca2+ receptors in bone development. *J Bone Miner Res*. 2007 Sep;22(Suppl 1):S79.

63. Nemeth EF, Carafoli E. The role of extracellular calcium in the regulation of intracellular calcium and cell function. *Cell Calcium*. 1990 May;11(5):319-21.