RECEPTOR ANTAGONISTS

The search for calcium receptor antagonists (calcilytics)

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Abstract

The Ca^{2+} receptor on the surface of parathyroid cells is the primary molecular entity regulating secretion of parathyroid hormone (PTH). Because of this, it is a particularly appealing target for new drugs intended to increase or decrease circulating levels of PTH. Calcilytic compounds are Ca^{2+} receptor antagonists which increase the secretion of PTH. The first reported calcilytic compound was NPS 2143, an orally active molecule which elicits rapid, 3- to 4-fold increases in circulating levels of PTH. These rapid changes in plasma PTH levels are sufficient to increase bone turnover in ovariectomized, osteopenic rats. When administered together with an antiresorptive agent (estradiol), NPS 2143 causes an increase in trabecular bone volume and bone mineral density in osteopenic rats. The magnitude of these changes are far in excess of those caused by estradiol alone and are comparable with those achieved by daily administration of PTH or a peptide analog. These anabolic effects of NPS 2143 on bone are not associated with hyperplasia of the parathyroid glands. Calcilytic compounds can increase endogenous levels of circulating PTH to an extent that stimulates new bone formation. Such compounds could replace the use of exogenous PTH or its peptide fragments in treating osteoporosis.

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Introduction

Parathyroid hormone (PTH) is arguably the most important endocrine factor regulating systemic Ca^{2+} homeostasis. PTH acts on target cells in both the kidney and the skeleton to increase plasma levels of Ca^{2+} . Within the skeleton, PTH increases bone turnover but the resulting overall effect on bone is highly dependent on temporal changes in the circulating levels of PTH. Thus, sustained elevations in plasma PTH levels, as occur in primary or secondary hyperparathyroidism, have a net catabolic effect on the skeleton (Antonsen & Sherrard 1995). In contrast, temporary increases in plasma levels of PTH achieved by the daily (or near daily) injection of exogenous hormone, have a net anabolic effect on the skeleton (Dempster *et al.* 1993, Seeman & Delmas 2001). The profound stimulatory effect on bone formation has generated interest in the use of PTH or its biologically active

peptide analogs as first generation anabolic therapies for osteoporosis.

The need for such an anabolic therapy is underscored by the serious health problem posed by osteoporosis, the incidence of which is increasing as the general population ages. While currently available antiresorptive therapies, such as estrogens or bisphosphonates, prevent further bone loss, they cause relatively small increases in new bone formation. The ability to stimulate new bone formation and thereby replace bone already lost to disease would constitute a significant advance in the treatment of osteoporosis. A first step in this direction is teraparotide, a peptide fragment of PTH which has recently been recommended for approval in the US for treating severe osteoporosis (Fox 2002). Human PTH is in phase III clinical trials and other peptide fragments of PTH are in earlier stages of development. However, the therapeutic usefulness of all these

peptides is compromised by the need for systemic administration of a costly biological agent.

An alternative approach which might overcome these drawbacks, and yet achieve similar anabolic effects on bone, is based on the use of orally active compounds which block the activity of the parathyroid Ca^{2+} receptor and stimulate secretion of endogenous PTH (Nemeth 2002). Compounds with the appropriate pharmacokinetic profile would be expected to cause a marked but transient increase in circulating levels of PTH, sufficient to stimulate new bone formation. With this goal in mind, we initiated a drug discovery program in collaboration with SmithKline Beecham (now GlaxoSmithKline) in 1993. This Commentary is a snapshot of a work in progress which captures some of the challenges encountered in this novel approach to treating osteoporosis.

Trying to inhibit Ca^{2+} receptor activity

The Ca^{2+} receptor is a G protein-coupled receptor that shares structural features with the metabotropic glutamate receptors (mGluRs) and the γ -aminobutyric acid type B receptors (GABA_B Rs) (see Brown & MacLeod 2001). All these receptors possess a large extracellular domain which is believed to bind the cognate physiological ligand glutamate, γ -aminobutyric acid or extracellular Ca^{2+} (Hammerland *et al.* 1999). At present, only one gene coding for the Ca^{2+} receptor has been identified; no receptor subtypes have been found and splice variants of the Ca^{2+} receptor are few and of low abundance (Garrett *et al.* 1995). The parathyroid Ca^{2+} receptor couples through a G_i protein to adenylate cyclase and through a G_q/G_{11} protein to phospholipase C. Activation of the parathyroid cell Ca^{2+} receptor by increased levels of extracellular Ca^{2+} causes a slight decrease in cAMP levels, a profound increase in the concentration of cytoplasmic Ca^{2+} ($[\text{Ca}^{2+}]_i$) and a decrease in the secretion of PTH. In its structural and functional properties, the Ca^{2+} receptor is akin to other G protein-coupled receptors which transduce an extracellular signal into a functional cellular response. The unique difference is that the primary physiological ligand for the Ca^{2+} receptor is an inorganic ion rather than an organic molecule.

The search for Ca^{2+} receptor ligands began shortly after I convinced myself (and few others at

the time!) that such a receptor existed (Nemeth & Scarpa 1986). It soon became clear that this putative Ca^{2+} receptor was rather promiscuous and could be activated by a variety of di- and trivalent cations and many structurally diverse organic compounds which share the common property of possessing a net positive charge at physiological pH. Included in the category of molecular ligands are polyamines from mammals and the venoms of spiders, aminoglycoside antibiotics, and poly-basic amino acids. The response to extracellular Ca^{2+} and all the organic and inorganic polycations was potentiated by certain phenylalkylamines like verapamil or the trimethoxybenzoate derivative TMB-8. All these substances have now been shown to act directly on the Ca^{2+} receptor. Ligands which mimic or potentiate the actions of extracellular Ca^{2+} at the Ca^{2+} receptor have been termed calcimimetics (Nemeth *et al.* 1998). Calcimimetics can be either inorganic ions or organic molecules and they can act as agonists (type I) or allosteric activators (type II) of the receptor. The phenylalkylamines are examples of type II calcimimetics whereas all the inorganic and organic polycations are type I calcimimetic ligands. Calcimimetic compounds inhibit the secretion of PTH and lower circulating levels of PTH. A type II calcimimetic compound is now in phase III clinical trials for the treatment of hyperparathyroidism (Nemeth 2002).

All this pharmacology regarding agonists and allosteric activators was established before the Ca^{2+} receptor was cloned and structurally characterized (in February 1993, Brown *et al.* (1993)). Yet it is curious that during all this testing we never discovered any ligand capable of blocking Ca^{2+} receptor activity. This is unusual for G protein-coupled receptors because it has traditionally been much easier to find antagonists of these receptors rather than agonists or allosteric activators. The Ca^{2+} receptor is somehow different, and calcimimetic ligands appear to be rather plentiful. There is seemingly some intrinsic feature of the Ca^{2+} receptor, or of the manner in which it couples to effector systems, which makes its activity difficult to suppress. In any event, this feature of Ca^{2+} receptor biology has made the quest for antagonists considerably more laborious than the corresponding search for agonists.

In searching for antagonists of G protein-coupled receptors, one typically starts with structural modifications to the cognate physiological agonist

in the hopes that a high affinity ligand lacking intrinsic efficacy will be created. The physiological agonist of the Ca^{2+} receptor, however, does not lend itself to the usual tricks of the medicinal chemist. And all the structural modifications made to molecular calcimimetics like NPS R-568 simply yielded more calcimimetics. Molecular modeling of the receptor and its presumed binding pocket for molecular calcimimetics was likewise unproductive. Given this vacuum of structural information, we had to rely on random screening of compound libraries. Initially, bovine parathyroid cells were used since there are no cell lines which retain the appropriate parathyroid phenotype. Once the Ca^{2+} receptor was cloned, however, we were able to construct a stably transfected cell line and initiate high-throughput screening. Doing this for a number of years reinforced the original suspicion that it is difficult to block Ca^{2+} receptor activity. But it is not impossible.

Eventually, a compound that did block Ca^{2+} receptor activity was discovered. The initial compound detected by high-throughput screening was far from potent and lacked specificity. In fact, it was four orders of magnitude more potent on adrenergic receptors than on the Ca^{2+} receptor. Yet this initial compound was not pan active. It did not, for example, inhibit the activity of many other G protein-coupled receptors upon which it was tested. And it did have drug-like characteristics which attracted the attention of the medicinal chemists. Their persistence and expertise was rewarded with a series of compounds with greatly improved potency and selectivity at the Ca^{2+} receptor. The compounds emerging from this effort are the first substances, either atomic or molecular, shown to block Ca^{2+} receptor activity. Ligands which block the activity of the Ca^{2+} receptor have been termed calcilytics (Nemeth *et al.* 2001). Together with certain calcimimetic molecules, these compounds are valuable tools to determine the feasibility of developing drugs which target the Ca^{2+} receptor for various bone and mineral related disorders.

One of the early calcilytic compounds studied in some detail is NPS 2143 (Fig. 1). The structure of this compound illustrates some basic features of the first generation calcilytic pharmacophore. The stereochemistry of the hydroxyl group is critical and *R*-enantiomers are more potent – a fortunate outcome since potency on adrenergic receptors

tracks *S*-enantiomers. Replacement of the *gem*-dimethyl with hydrogen or methyl results in a loss of potency. The alkyl spacing between these moieties and the right and left hand aromatic portions of the molecule are also important. The left and right hand aromatic regions of the molecule are somewhat more tolerant of structural diversity but loss of the cyano group greatly diminishes potency.

As detailed below, NPS 2143 possesses sufficient potency and selectivity to be used as a tool compound. While not perfect, it served the purpose of testing some of our early concerns regarding the technical feasibility of blocking the Ca^{2+} receptor to stimulate PTH secretion and new bone formation.

Proof of concept with a prototype calcilytic compound

From the outset, there were a number of uncertainties which challenged the calcilytic approach to treating osteoporosis. Indeed, the fundamental hypothesis supporting this approach to building bone was suspect; because there was no ligand which inhibited the Ca^{2+} receptor, there was no reason to suppose that blocking the activity of the Ca^{2+} receptor would, in fact, stimulate PTH secretion. Although hypocalcemia stimulates PTH secretion, this is not necessarily equivalent to blocking Ca^{2+} receptor activity in the setting of normocalcemia. More worrisome was the possibility that PTH could not be secreted from the parathyroid glands in sufficient quantities to stimulate new bone formation. And finally, would blocking the Ca^{2+} receptor on a daily basis be perceived by the parathyroid glands as hypocalcemia, and possibly trigger cellular hyperplasia leading to chronically elevated levels of PTH (and net bone loss!)?

NPS 2143 was selected as a tool compound to address these issues. This compound inhibits extracellular Ca^{2+} -evoked increases in $[\text{Ca}^{2+}]_i$ in HEK 293 cells expressing the human Ca^{2+} receptor ($\text{IC}_{50}=43$ nM) and stimulates PTH secretion from bovine parathyroid cells *in vitro* ($\text{EC}_{50}=41$ nM; Fig. 1). The inhibitory effects of NPS 2143 are not dependent on the nature of the ligand used to activate the Ca^{2+} receptor and this compound has similar potencies on responses

NPS 2143

Figure 1. Structure of the calcilytic compound NPS 2143. Left panel: NPS 2143 stimulates PTH secretion from bovine parathyroid cells *in vitro*. Right panel: parathyroid cells were incubated in the presence (●) or absence (○) of 300 nM NPS 2143 and the indicated concentration of extracellular Ca²⁺. (Fig. 7 in Nemeth *et al.* 2001.)

elicited by type I or type II calcimimetics. Calcilytic compounds with this pharmacophore appear to act by an allosteric mechanism which shifts the concentration–response curve for extracellular Ca²⁺ to the right without affecting maximal or minimal responses (Fig. 1). In this respect, these compounds act like type II calcimimetics, only they decrease, rather increase, the sensitivity of the Ca²⁺ receptor to activation by extracellular Ca²⁺. This mechanism of action suggests that the maximal effect of this compound *in vivo* will occur under normocalcemic conditions.

When administered to rats either orally or by i.v. infusion, NPS 2143 causes a prompt increase in circulating levels of PTH, the magnitude of which is dose-dependent (Fig. 2). These findings were comforting because, as noted above, it was never a certainty that a calcilytic compounds would in fact stimulate PTH secretion. Moreover, the magnitude of the increase in circulating levels of endogenous

PTH is similar to that achieved by exogenous peptide when administered at doses which stimulate new bone formation (Fox *et al.* 1997).

NPS 2143 possesses reasonable selectivity at concentrations below 1 μM. It does not, for example, affect the activity of receptors which are most homologous to the Ca²⁺ receptor such as various mGluRs or the two GABA_BRs (Nemeth *et al.* 2001). When studied in rats, the compound stimulated tubular reabsorption of Ca²⁺, an effect that might result from a direct action on renal Ca²⁺ receptors or be indirectly mediated by an increase in plasma PTH levels. This compound is not, however, without its blemishes, and its pharmacokinetic profile is less than ideal. To achieve an anabolic effect on bone, the increase in circulating levels of PTH must be transient. Although the boundaries of ‘transient’ have not been rigorously explored, it is generally accepted that a 3- to 4-fold increase in plasma PTH levels lasting 1–2 h would

Figure 2 NPS 2143 rapidly increases circulating levels of PTH in normal rats. NPS 2143 was infused i.v. (0.1 $\mu\text{mol/kg}$ per min) (•) for 2 h. (Fig. 9 in Nemeth *et al.* 2001.)

be required to stimulate new bone formation without activating resorption resulting in a net anabolic effect on the skeleton (Hodsman *et al.* 2002). Thus, a short acting calcilytic is desired. The compound must be orally active and rapidly absorbed and the compound (and any active metabolite) must be rapidly cleared from the body once it is in the systemic circulation. NPS 2143 is not rapidly eliminated from the body following oral administration and it causes a sustained, rather than transient increase in circulating levels of PTH (Gowen *et al.* 2000). Nonetheless, this compound can still be used to determine if the magnitude of the increase in plasma levels of PTH is sufficient to stimulate new bone formation.

The effects of NPS 2143 on circulating levels of PTH and bone turnover were assessed in ovariectomized rats. Daily oral administration of NPS 2143 began 3 months after ovariectomy and continued for 5 weeks at which time static and dynamic parameters of bone histomorphometry were estimated. Plasma levels of PTH were elevated for at least 4 h following oral dosing with NPS 2143 but returned to baseline levels within 24 h. Morphometric parameters indicative of bone resorption or bone formation were increased by

NPS 2143 and their magnitudes were comparable so there was no net increase in bone mineral density (BMD) in the distal femur or the proximal tibia (Gowen *et al.* 2000). This is exactly what would be expected from the chronically elevated levels of PTH and it demonstrates that blocking parathyroid Ca^{2+} receptor activity with a small molecule will release PTH in amounts which are sufficient to increase bone turnover. It seemed reasonable to suppose that blocking the increased bone resorption induced by NPS 2143 might isolate the mechanisms underlying bone formation and result in a net anabolic effect. Indeed, when the animals received the combination of NPS 2143 and the antiresorptive agent 17β -estradiol, there were increases in trabecular area and bone formation rate accompanied by net increases in BMD (Fig. 3). Thus, a calcilytic compound can result in a net anabolic effect on bone when administered together with a conventional antiresorptive.

The long half-life of NPS 2143 in the body also affords a stringent test of whether the parathyroid cell will perceive a calcilytic compound as a hypocalcemic stimulus and thereby undergo glandular hyperplasia and/or hypertrophy (Wada

Figure 3. Treatment with NPS 2143 and estrogen increases BMD in the distal femur of ovariectomized rats. (Fig. 8 in Gowen *et al.* 2000.)

et al. 1997, Parfitt 2001). Cellular proliferation was assessed during the last week of dosing in the studies described above. When compared with sham or ovariectomized animals, NPS 2143 did not cause hyperplasia (as assessed by bromodeoxyuridine staining), whether given alone or in combination with 17β -estradiol (Gowen *et al.* 2000). Measurements of NPS 2143 in the plasma revealed that levels were still elevated 8 h after a single oral dose and this might be expected to provide a chronic hypocalcemic stimulus to the parathyroid glands, yet this did not result in cellular hyperplasia or hypotrophy. It is always possible that more prolonged treatment with a long-acting calcilytic compound might have triggered cellular proliferation in the parathyroid glands but the proliferative response to hypocalcemia occurs rapidly in the parathyroids (within a week). It seems that a short-acting calcilytic compound would be unlikely to stimulate parathyroid gland hyperplasia.

Conclusion

All the evidence obtained to date supports the view that the parathyroid Ca^{2+} receptor is a viable target for drugs which could have anabolic effects on bone. Although the prototype calcilytic compound NPS 2143 is not a clinical candidate, it has proven to be a valuable tool to explore the

feasibility of targeting the Ca^{2+} receptor with antagonist compounds to increase circulating levels of PTH and thereby stimulate new bone formation. There are still some basic issues to resolve, however, and it cannot be stated with certainty that all the effects of calcilytic compounds on bone result from an action solely at the parathyroid Ca^{2+} receptor. Ca^{2+} receptors might be expressed on certain cells of bone and these would be expected to be targets for calcilytic compounds. So far, however, we have not noted any effects of calcilytic compounds on osteoblastic or osteoclastic activity *in vitro*. Perhaps the most challenging aspect of this new approach to treating osteoporosis will be achieving the necessary pharmacokinetic features of rapid absorption and elimination to cause a transient increase in circulating levels of PTH. A calcilytic compound with an improved pharmacokinetic profile has recently entered phase I clinical trials so we will soon know if we can achieve this therapeutic goal, which we irreverently refer to as 'get in...get out...nobody gets hurt'.

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