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PERSPECTIVES

Coupling PTH and Arrestins to Uncouple Bone Formation from Resorption: A New Road to Osteoporosis Anabolic Therapy?

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Abstract

The main physiological role of PTH is to maintain serum calcium homeostasis, which is achieved by increasing tubular calcium reabsorption and bone resorption. The latter supposes that increases in PTH levels induce activation of osteoclasts, which is mediated primarily by intracellular cAMP and increased RANKL over OPG expression in osteoblasts. Hence even intermittent (daily) administration of PTH for osteoporosis treatment is often accompanied by a transient increase in serum calcium levels and/or urinary calcium excretion. Stimulated bone resorption may ultimately limit the net anabolic effects of intermittent PTH on the skeleton, particularly through Haversian bone remodeling of the cortical compartment. Nevertheless, the PTH mechanisms leading to bone loss are normally regulated by coupling the activated PTH/PTHrP receptor with intracellular β-arrestins. In turn, the development of a "β-arrestin-biased" PTH ligand, (D-Trp^{12},Tyr^{34})-PTH(7-34), suggests that such hormone-derived analogs could retain bone-forming activities while having limited effects on bone resorption. *IBMS BoneKEy*. 2009 December;6(12):470-476.

Introduction: the PTH Paradox

Both intermittent and continuous exposure to PTH induce bone formation, however, the net bone mineral balance depends on the magnitude of the concomitant bone resorption. Hence intermittent (daily) administration of PTH(1-84) or the shorter bioactive peptide PTH(1-34) (teriparatide) increases bone mass, bone strength, and reduces fracture risk, and has therefore been approved for treatment of osteoporosis (1). In comparison, continuous exposure to PTH, as seen in primary hyperparathyroidism, may still improve cancellous, but not cortical bone volume (2). The paradox of concomitant PTH anabolism/catabolism has been particularly well-demonstrated by the targeted expression in osteoblasts of a (cAMP) constitutively active PTH/PTHrP receptor mutant (carrying the H223R mutation of Jansen's chondrometaphyseal dysplasia) (3). In these mice, trabecular bone volume was increased despite a prominent increase in osteoclast number, while cortices appeared thinner and porous. Furthermore, administration of a PTH-Fc fusion molecule characterized by a long circulating half-life and mean residency time, *i.e.*, allowing for weekly rather than daily injections leading to prolonged cycles of intermittent exposure to the hormone, was shown to exert potent anabolic effects on both trabecular and cortical bone in intact and ovariectomized rodents – actually much more potent effects than intermittent PTH(1-34) – despite having hypercalcemic effects that precluded its clinical development (4). Hence PTH stimulates bone formation rather independently of the mode of exposure or pharmacokinetic profile of the derived compounds. However, continuous exposure to the hormone stimulates bone resorption to levels that may eventually exceed its bone-forming effects at the tissue level,

resulting in net bone mineral loss. The magnitude of PTH bone-resorbing effects therefore depends on the intensity and duration of intracellular cAMP signaling in osteoblasts, which regulates the expression of numerous cytokines involved in osteoblast-osteoclast coupling: most prominently, receptor activator of nuclear factor kappa B ligand (RANKL), which promotes osteoclastogenesis, and its antagonist, osteoprotegerin (OPG), which inhibits osteoclast development, activity and survival by preventing RANKL from binding RANK on precursor and mature osteoclasts (5). The RANKL/OPG ratio increases with the duration of exposure to and concentration of PTH, eventually leading to chronic hypercalcemia and bone loss, as observed in primary hyperparathyroidism. Bone loss in this case is predominant in the cortical bone compartment, due to accelerated intracortical (Haversian) and endocortical bone remodeling, resulting in cortical thinning and porosity.

Regulation of PTH Intracellular Signaling

At the cellular level, the effects of PTH are further regulated following PTH/PTHrP receptor activation, first through desensitizing/resensitizing mechanisms involving phosphorylation of serine and threonine residues in the intracytoplasmic domains of the receptor, particularly the C-terminal tail and 3rd intracellular loop. In turn, the phosphorylated receptor interacts with cytoplasmic arrestins, primarily β-arrestin2, which prompts termination of receptor-mediated intracellular cAMP and Ca/IP3 signaling through sterical inhibition of G protein binding and phosphodiesterase activation; ligand-receptor complex internalization through clathrin-coated vesicles; and its degradation or recycling (Fig. 1) (6;7).

In the absence of β-arrestin binding, intracellular signaling in response to agonists is prolonged and sustained. Proper PTH/PTHrP receptor agonists, such as PTH(1-84), PTH(1-34), PTHrP(1-141) and PTHrP(1-36), eventually induce conformational changes in the receptor, particularly in intracellular loop 3, which enable β-arrestins to bind and uncouple the receptor from G proteins (8). PTH- and PTHrP-derived molecules, such as Bpa1-PTHrP(1-36), which induce activated but not β-arrestin-bound receptor conformations, in turn trigger continuous cAMP signaling from receptors remaining at the cell surface, and which become refractory to re-exposure to agonist, *i.e.*, chronic desensitization (8). Recent findings indicate that native PTH and PTHrP may actually trigger different receptor conformations, leading to different ligand-receptor outcomes and signaling profiles. Hence Ferrandon *et al*. recently used FRET analyses to describe at least two distinct PTH receptor conformations, R^o and RG (9). The former is induced and/or stabilized by PTH and is associated with continuous cAMP signaling from the internalized receptors – an unprecedented and surprising finding. In turn, this prolonged wave of cAMP signaling could explain the catabolic actions on bone of PTH-like analogs favoring the R^o conformation (10). In contrast, PTHrP favors the RG conformation, which is also associated with receptor internalization but a more immediate and/or definite inhibition of cAMP signaling. This phenomenon might therefore explain why PTHrP was observed to retain anabolic activity in animals while displaying less bone-resorbing/hypercalcemic effects compared to PTH (11). Since both PTH and PTHrP recruit β-arrestin to the receptor and promote its endocytosis through clathrin-coated vesicles (6;7), it remains possible that the dissociation constant of β-arrestins differs between PTH- and PTHrP-activated receptor conformations. We speculate that a more stable association of β-arrestins with PTHrP-bound receptor could definitely terminate cAMP signaling at the cell membrane whereas a more rapid release of β-arrestins from the PTH-bound complex (7) could allow a second wave of intracellular cAMP signaling from endocytic vesicles. As discussed above, this prolonged cAMP signal would in turn induce the gene expression changes responsible for PTH catabolic effects on bone. This interpretation, *i.e.*, the more stable interaction of β-arrestins with PTHrP- than with PTH-bound receptors would also be

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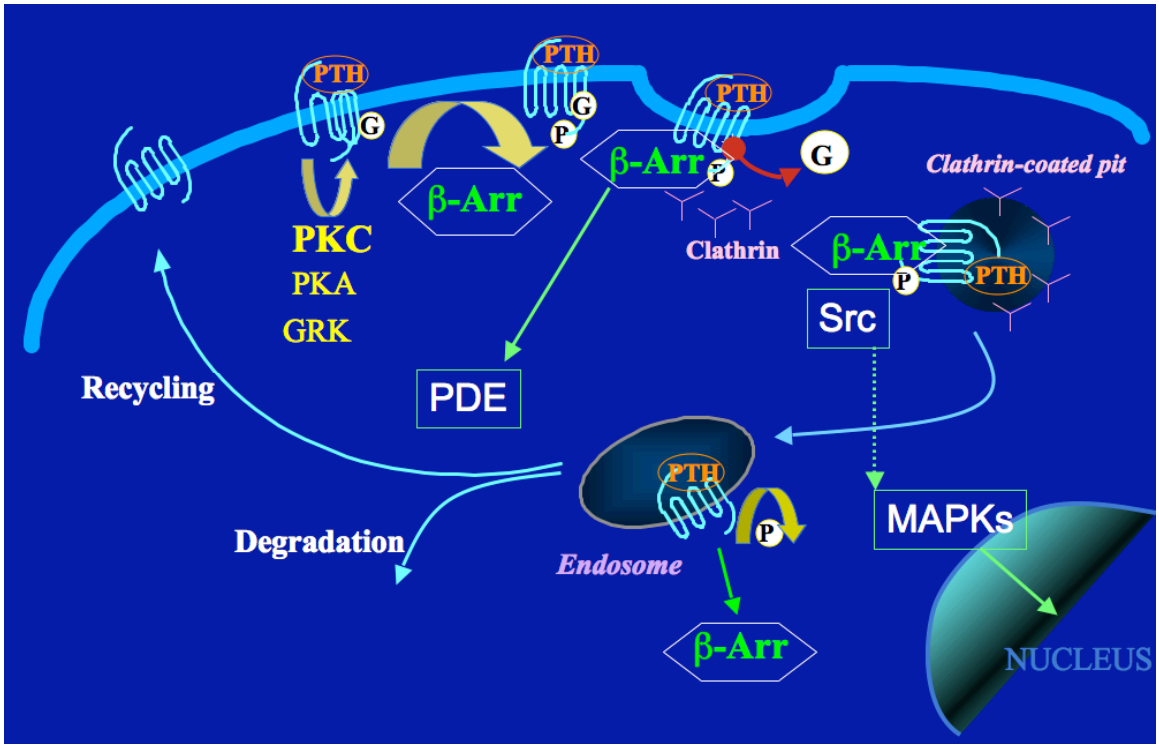

Fig. 1. Regulation of PTH/PTHrP receptor trafficking and signaling by β-arrestin.

consistent with the observation that PTHrP dissociates more readily from the receptor (9), since we observed that ligands that do not trigger β-arrestin binding to the PTH/PTHrP receptor also had the longest dissociation constants (8).

The role of arrestins in the regulation of PTH activity is not limited to moderating G protein-mediated signaling. They also serve as a scaffold for the internalized receptor to initiate G protein-independent, Src-mediated MAPK signaling and transcriptional activity (Fig. 1) (12;13). Recently a main gene network centered on MAPK p38 that requires β-arrestin2 for up- or down-regulation by intermittent PTH has been identified in osteoblastic cells (14). This network comprises genes such as C/EBPδ (*Cebpd*), which mediates estradiol's inhibitory effects on IGF-1 expression in response to other hormones, *i.e.*, PTH. In addition, there is evidence that MAPK p38 mediates PTH effects on the stimulation of alkaline phosphatase and matrix mineralization by osteoblasts (15). Although previous experiments using N-terminally truncated PTH molecules, such as PTH(3-34) (a Gq signaling selective agonist) and PTH(7-34) (an antagonist at the PTH/PTHrP receptor), have demonstrated that loss of cAMP-stimulating activity reduced their *in vivo* anabolic properties, these molecules also lose their ability to recruit β-arrestins and therefore to elicit MAPK-signaling. These observations have raised the intriguing hypothesis that "biased agonists", *i.e.*, PTH- and PTHrP-derived molecules that induce β-arrestin-bound receptor conformations without triggering the active/G protein-coupled receptor state, could elicit bone forming responses (16). In particular these biased agonists could regulate some anabolic genes without necessarily up-regulating expression of osteoclast-activating genes like RANKL (since the latter is mediated by cAMP and repressed by β-arrestins, see above), hence resulting in a limited activation of bone resorption.

Regulation of PTH Activity by β-Arrestins In Vivo

The potential importance of arrestins in the bone anabolic response to intermittent PTH

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is further illustrated by the gene expression profile of primary osteoblasts from mice lacking β-arrestin2. When these osteoblasts are exposed to intermittent PTH *in vitro,* expression of nearly 50% of the genes that were normally up- or down-regulated by PTH is lost in the absence of β-arrestin2 (14). It is therefore not surprising that the *in vivo* response to both intermittent and continuous PTH is altered in β-arrestin2 KO mice (Table 1). These alterations are complex, reflecting changes in the ratio of RANKL/OPG depending on the presence/absence of β-arrestin2 (17), but also of other genes implicated in the coupling of osteoblast-osteoclast activities, including the ephrin/ephrin receptor system expressed at their surface. Hence β-arrestin2 KO mice are more sensitive to the bone remodeling effects of PTH on cortical and trabecular bone surfaces, due to a higher RANKL/OPG ratio and therefore a greater osteoclastogenic response to PTH (17;18). On another side, prolonged cAMP signaling in osteoblastic cells lining quiescent (*i.e.*, modeling) bone surfaces, such as the periosteum, could explain the greater bone formation on this surface in response to intermittent PTH (19).

Table 1. Summary of PTH effects on trabecular and cortical bone in β-arrestin2-deficient mice

Mouse model	♀ mice (19)		♂ mice (18)		♂ mice (17)		♀ ovariecto-mized mice (17)		♂ mice (20)		♂ mice (20)	
Treatment or condition	iPTH		iPTH		low Ca^{2+} diet (secondary HPT)		ovariectomy		iPTH		iPTH-βArr	
β-arrestin2 status (global)	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-	+/+	-/-
Trabecular bone	↑	↑	↑	=	(↓)	↓	(↓)	↓	↑	↑	↑	↓
Cortical bone	(↑)	↑↑	↑	(↑)	no Δ	↓	(↓)	(↓)	↑	no Δ	no Δ	↓

Anabolic Effects of an Arrestin-Biased PTH Agonist

To investigate the effects of selective activation of the β-arrestin pathway, a recent study by Gesty-Palmer *et al*. (20) investigated a biased agonist for the type I PTH/PTHrP receptor, (D-Trp^{12},Tyr^{34})-PTH(7-34), termed PTH-βarr due to its ability to activate β-arrestin but not classical G-protein signaling pathways (13). In male wild-type mice, daily intraperitoneal injection of PTH(1-34) or PTH-βarr for 8 weeks significantly increased lumbar spine BMD relative to vehicle-treated mice, whereas only PTH(1-34) increased femoral BMD. Consistent with these observations, μCT analysis confirmed a significant increase in trabecular bone volume in the vertebrae and tibiae of wild-type mice treated with either PTH(1-34) or PTH-βarr. In contrast, only PTH(1-34), but not PTH-βarr, induced anabolic effects in the cortical compartment, with increased cortical thickness and periosteal circumference compared to vehicle. In mice null for β-arrestin2 (*βarr2* (-/-)), PTH(1-34) treatment induced slower (compared to wild-type), but significant increases in lumbar spine and femoral BMD relative to vehicle-treated mice, with corresponding increases in trabecular bone volume by μCT. In contrast, the anabolic effect of PTH-βarr seen in wild-type mice was absent in *βarr2*(-/-) mice. In fact, *βarr2* (-/-) mice treated with PTH-βarr exhibited a *decline* in lumbar spine BMD and μCT-assessed vertebral trabecular bone volume relative to controls. In *βarr2*(-/-) mice, treatment with PTH-βarr also led to decreased mid-femoral cortical thickness relative to vehicle-treated controls. These PTH negative effects in the absence of both cAMP signaling and arrestins could be explained by reverse-agonist effects on the PTH/PTHrP receptor, which has more constitutive activity in the absence of β-

arrestin2 (7) . These findings further indicate that the bone anabolic effects of PTHR1 simulation have distinct G-protein-mediated and β-arrestin-mediated components.

Histomorphometric analyses of the vertebral body confirmed these observations, such that wild-type mice treated with PTH(1-34) or PTH-βarr exhibited increased osteoblast parameters and bone formation. In *βarr2*(-/-) mice, only PTH(1-34) treatment increased osteoblastic activity and bone formation. In comparison, osteoclast number was increased by PTH(1-34) but not by PTH-βarr in wild-type mice, suggesting that selective activation of β-arrestin signaling was insufficient to induce an increase in the RANKL/OPG expression ratio (see above). In comparison, in *βarr2*(-/-) mice treatment with PTH(1-34) led to a slight (but non-significant) increase, whereas PTH-βarr led to a marked *decrease* in osteoclast surface, again suggesting reverse agonist activity. The observation that both PTH(1-34) and PTH-βarr increased osteoblast activity in wild-type mice, whereas only PTH(1-34) increased osteoblast activity in *βarr2*(-/-) mice implies that *both* G-protein-mediated and β-arrestin-mediated pathways promote osteoblast activity. In contrast, stimulation of osteoclastic activity seems to be regulated mainly by G-protein-mediated pathways.

Summary and Perspective

These new observations highlight the important role of arrestins in regulating PTH activity. The biased PTH agonist assayed by Gesty-Palmer *et al*. (20) provides a proof-of-concept that β-arrestin-mediated, G protein-independent signaling may translate partial and/or selective PTH anabolic effects on bone. Why selective activation of the β-arrestin/ERKs pathway induced by PTH-βarr exerts anabolic effects on the cancellous but not the cortical bone compartment, and which genes are involved in these effects, remain to be elucidated.

Whereas the advantages of (D-Trp^{12},Tyr^{34})-PTH(7-34) over daily PTH(1-34) remain unclear, future developments of PTH- and PTHrP-derived analogs and "biased agonists" triggering/stabilizing distinct receptor conformations, such as RG (see above (9), and R_{ds} (arrestin-bound (8)), associated with selective intracellular signaling pathways and regulation of anabolic gene profiles could provide advantages over currently approved PTH(1-84) and teriparatide molecules for osteoporosis treatment. Hence better understanding of the molecular mechanisms regulating PTH activity at the cellular level should prompt the development of "designer drugs" for metabolic bone diseases.

Conflict of Interest: Dr. Ferrari reports that he receives research support from Amgen and is an advisory committee member and lectures occasionally at conference symposia for Merck Sharp & Dohme, the Alliance for Better Bone Health (sanofi aventis/P&G), Amgen, Eli Lilly (Switzerland), Servier (Switzerland), and Novartis (Switzerland). Dr. Bouxsein: none reported.

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