Biased Agonism at the Parathyroid Hormone Receptor: A Demonstration of Functional Selectivity in Bone Metabolism

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Abstract: 'Biased agonism' refers to the ability of a ligand to selectively recruit different intracellular signaling proteins to elicit distinct phenotypic effects in cells. While conventional G protein-coupled receptor (GPCR) agonism and antagonism can be regarded as modulating the *quantity* of efficacy, functionally selective or 'biased' ligands *qualitatively* change the trafficking of information flowing across the plasma membrane. The concept of ligand directed signaling fundamentally raises the potential of pharmacologic agents with novel therapeutic profiles possessing improved therapeutic efficacy or reduced side effects. Currently, there is little experimental evidence that biased ligands offer advantages over conventional agonists/antagonists *in vivo*. Recent work examining biased agonism at the type I parathyroid hormone receptor (PTH1R) demonstrates that selective activation of G protein-independent arrestin-mediated signaling pathways elicits a physiologic response in bone distinct from that induced by the conventional PTH1R agonist PTH(1-34). While intermittent (daily) administration of PTH(1-34) (teriparitide) is effective in increasing bone formation, PTH(1-34) administration is also associated with increases in bone resorption and a propensity to promote hypercalcemia/hypercalcuria. In contrast, D-Trp12,Tyr34-bPTH(7-34) (PTH-βarr), an arrestin pathway-selective agonist for the PTH1R, induces anabolic bone formation independent of classic G protein-coupled signaling mechanisms. Unlike PTH(1-34), PTH-βarr appears to 'uncouple' the anabolic effects of PTH1R activation from its catabolic and calcitropic effects. Such findings offer evidence that arrestin pathway-selective GPCR agonists can elicit potentially beneficial effects *in vivo* that cannot be achieved using conventional agonist or antagonist ligands.

Keywords: β-arrestin, Biased agonism. Bone metabolism, G protein-coupled receptor, Parathyroid hormone, Parathyroid hormone receptor type 1.

INTRODUCTION

Heptahelical G protein-coupled receptors constitute the largest and most diverse superfamily of cell surface receptors, accounting for as many as 1000 discrete receptor proteins [1]. Members of this superfamily of seven transmembrane receptors (7TMRs) are believed to have evolved from a common ancestor. While retaining their heptahelical structure and their functional coupling to heterotrimeric G proteins, this class of receptors has evolved into subfamilies characterized by distinct structural elements and diverse modes of ligand binding, receptor activation, and receptor regulation [2, 3]. This evolutionary diversity translates into the capacity for GPCRs to detect an enormous array of extracellular stimuli. GPCR signaling is fundamental to numerous biologic functions including vision, taste, and odorant sensory detection, neurotransmission, endocrine control of physiological homeostasis and reproduction, and regulation of hemodynamics and intermediary metabolism. As a result, modulating GPCR activity has been a principal focus of pharmaceutical development. It is estimated that almost half of all drugs in clinical use target this ubiquitous family of receptors [4, 5].

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Early conventional models of GPCR signaling envisioned the receptor existing in equilibrium between two states: an inactive state (R) and an active state (R*) which is stabilized by agonist binding [6-8]. Here the binding of an agonist triggers conformational changes that coupled the receptor to its cognate G proteins, thereby regulating the activity of enzymatic effectors, such as adenylate cyclases, phospholipase Cβ isoforms, and ion channels. The subsequent generation of small molecule "second messengers" activates a cascade of events that alter the physiological function of the cell. In this early allosteric model of GPCR signaling, the intrinsic efficacy of a ligand is a reflection of its ability to alter the R-R* equilibrium. *Agonists* stabilize the R* conformation, pulling the equilibrium toward the 'on' state; true 'neutral' *antagonists* bind indiscriminately to both R and R*, producing no physiological response but blocking the response to agonists. The eventual discovery of constitutively activating GPCR mutations allowed the detection of receptor activity in the absence of ligand and enabled the identification of ligands that suppress basal receptor activity [9-11], or *inverse agonists*. Thus, inverse agonists appear as antagonists when basal receptor activity is low, but have the added property of reducing constitutive receptor activity by binding preferentially to R and pulling the equilibrium toward the 'off' state (Fig. **1**).

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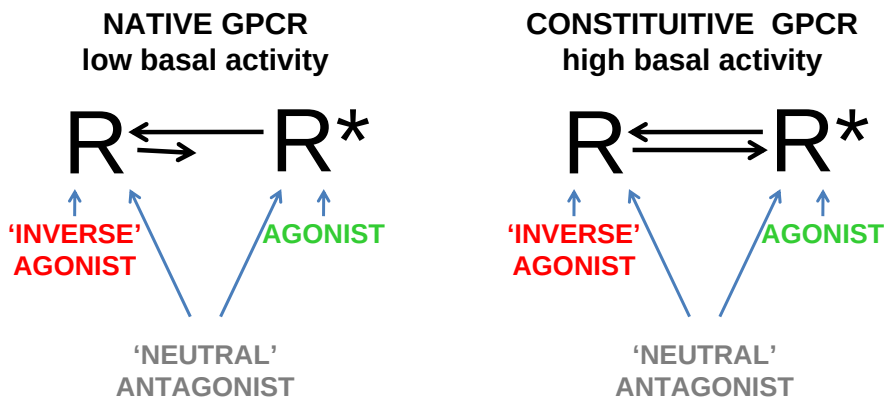


Fig. (1). Efficacy in a two-state system. Most native GPCRs exhibit low basal activity, that is the equilibrium between the 'off' state of the receptor (R) and the 'on' state (R*) heavily favors R. Ligands with agonist activity preferentially stabilize R* pulling the equilibrium toward the 'on' state. Antagonists lack intrinsic efficacy. A true 'neutral antagonist' would bind equivalently to R and R*, thus would have no effect on basal activity, but will competitively reduce activity measured in the presence of an agonist ligand. In systems with low basal activity, ligands that preferentially bind R cannot be distinguished from ligands that bind equivalently to both states. However, in systems with high basal activity, e.g. constitutively active GPCRs, a detectable quantity of R* exists in the absence of agonist. In this setting it is possible to demonstrate that some ligands, termed 'inverse agonists', are selective for R, enabling them to lower the basal activity of the system.

With the development of increasingly sophisticated biochemical and biophysical approaches for measuring ligand-receptor interactions emerged evidence that receptors can in fact assume many conformations [12]. Each of these conformations can potentially interact with a ligand in a highly selective manner. In turn, these discrete receptor conformations are capable of selectively interacting with specific intracellular signaling complexes and eliciting unique ligand-specific efficacy signatures [5, 13]. Recognition that the relative activity of agonists does not always adhere to the predictions of the simple "two-state" model, has rendered the conventional allosteric model of GPCR signaling increasingly limited in its utility to describe 7TMR signaling events.

The first formal model to account for these departures from classical "inactive/active state" receptor theory postulated that the ligand-receptor complex, not the receptor alone, specifies the active state [14] (Fig. **2**). Here, the formation of agonist-selective active states can 'bias' the coupling of the receptor to different signaling pathways. Many terms have been used to describe this phenomenon including 'stimulus-trafficking', 'functional dissociation', 'biased agonism', 'biased inhibition', 'differential engagement', 'discrete activation of transduction', and 'functional selectivity'. Whatever term is applied, the implications for signal transduction are striking. Functional selectivity can range from relatively modest deviations in potency to frank reversal of efficacy, such that the characterization of a ligand as agonist, antagonist or inverse agonist becomes assay-dependent. Conventional GPCR pharmacology addresses changing the *quantity* of efficacy; that is increasing or decreasing receptor activity. In contrast, functional selectivity revolves around changing the *quality* of efficacy by amplifying desired signaling effects while diminishing other unwanted effects. Thus development of ligands with novel GPCR stimulus trafficking characteristics is of significant pharmacologic interest in relation to drug design.

MULTIPLE DIMENSIONS OF GPCR SIGNALING

While all conventional GPCRs share the ability to function as ligand-activated guanine nucleotide exchange factors for heterotrimeric G proteins, it is increasingly apparent that GPCR signal transduction is far more complex than classically envisioned. Many GPCRs have been shown to couple to two, three or more unrelated G protein classes, enabling a single receptor to engage multiple effectors simultaneously or activate them differentially in a tissue-selective manner [13, 15]. Moreover, a host of protein-protein interactions are known to affect the specificity, selectivity and temporal signaling of GPCRs and their effector components. These include the formation of GPCR dimers [16], the interaction of GPCRs with Receptor Activity-Modifying Proteins (RAMPs) [17, 18], and the binding of PDZ domain-containing and non-PDZ domain scaffold proteins to the intracellular loops and C-termini of receptors [19-21]. To the extent that ligand binding can alter this diverse array of protein interactions, functional selectivity has the potential to modify GPCR signaling.

The arrestins, a family of four GPCR binding proteins that regulate receptor desensitization and endocytosis, are among the most studied protein modulators of GPCR signaling. Arrestins bind tightly and specifically to agonist-occupied GPCRs that have been phosphorylated by GPCR kinases [22] and sterically inhibit the receptor from further G protein activation. The two non-visual arrestin isoforms, β-arrestins 1 and 2, also regulate the agonist-induced internalization of desensitized GPCRs via clathrin-coated pits [23].

The discovery that arrestins serve as adapters not only in the context of GPCR sequestration, but also in linking activated receptors to additional effectors of downstream signaling [24], advanced our understanding of GPCR signal transduction. It is now recognized that a number of catalytically-active proteins bind arrestins and are recruited to agonist-occupied GPCRs. Among these are Src family

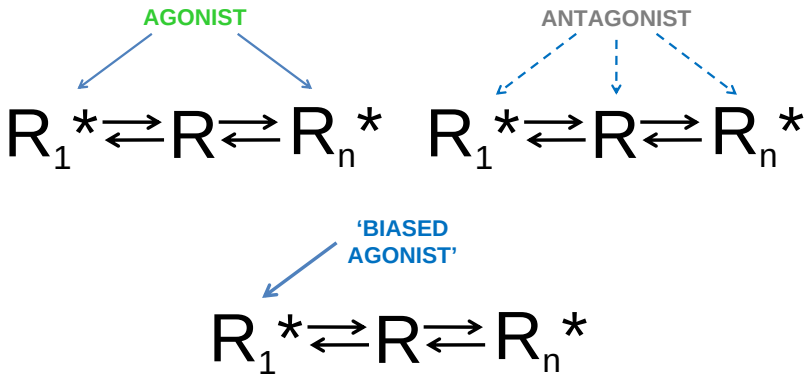


Fig. (2). Biased agonist model. This model of GPCR signaling takes into account the fact that receptors can employ multiple "active" state conformations, R_1^*, R_2^*....R_n^*. Thus GPCRs signal through different pathways with different efficacies. Ligands with agonist activity preferentially stabilize R* 'active' states. Antagonists lack intrinsic efficacy and bind equivalently to R and R*, thereby, competitively reducing activity measured in the presence of an agonist ligand. A biased agonist binds preferentially to one of the active states and stabilizes it, thus initiating one set of signal transduction events downstream of the receptor without activating others.

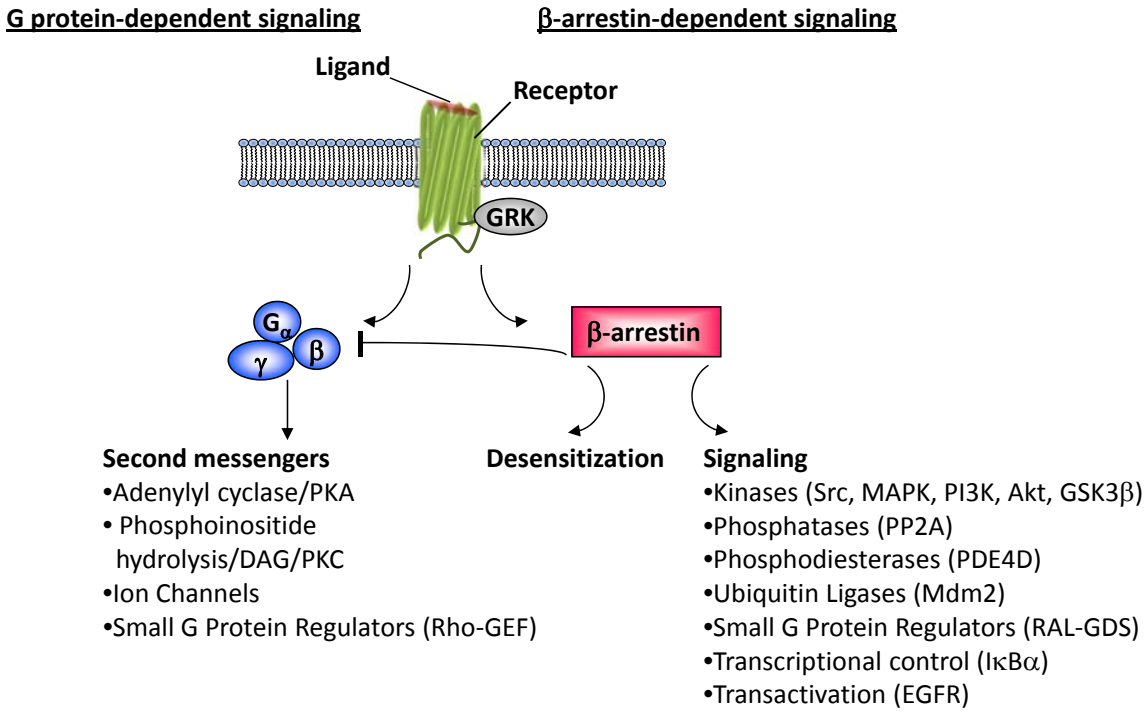


Fig. (3). Two GPCR signaling states modulated by arrestin binding. Agonist binding to a GPCR classically activates heterotrimeric G protein-dependent second messenger pathways. The activated receptor is recognized by GPCR kinases (GRK), which phosphorylate the receptor and facilitate the binding of arrestins which promote desensitization of G protein mediated signaling and internalization of the receptor. Arrestins also function as ligand-regulated scaffolds, recruiting a number of catalytic proteins into the receptor-arrestin complex to initiate a second wave of 'signalsome-dependent' events.

tyrosine kinases, components of the ERK1/2 and c-Jun N-terminal kinase 3 mitogen-activated protein kinase (MAPK) cascades, the E3 ubiquitin ligase, Mdm2, the cAMP phosphodiesterases (PDE), PDE4D3/5, diacylglycerol kinase, the inhibitor of nuclear factor (NF)-κB, IκBα, the Ral-GDP Dissociation Stimulator, Ral-GDS, and the Ser/Thr protein phosphatase (PP)2A [24-31]. It is through these interactions that arrestin-binding confers unique signaling properties upon agonist-occupied GPCRs, opening up a broad realm of previously unappreciated GPCR signal transduction [27] (Fig. **3**).

A highly novel feature of at least some arrestin-dependent signals is that they can be initiated independent of heterotrimeric G protein activation [32-34]. Downstream signals emanating from GPCRs in the absence of G protein coupling has been demonstrated for several different 7TMRs using G protein-uncoupled receptor mutants and arrestin pathway-selective biased ligands. Examples such as the

angiotensin AT_{1A}, β_2 adrenergic and PTH1 receptors show that arrestin-dependent activation of ERK1/2 is temporally distinct from that which is activated via G protein dependent mechanisms [32-35].

The true scope of physiologically-relevant arrestin signaling remains largely unknown. However a growing literature supports the concept that arrestin-bound effectors perform numerous functions, among them: enhancing second messenger degradation; regulating cytoskeletal dynamics controlling GPCR endocytosis, post-endocytic receptor trafficking, vesicle exocytosis, and cell migration; and impacting more distant processes such as protein translation and gene transcription [27]. In addition, some clinically useful GPCR ligands, e.g. the β adrenergic receptor 'antagonists' propranolol and carvedilol, have been shown in retrospect to exhibit a degree of functional selectivity [32, 35]. While, no currently used pharmaceuticals are known to possess unique clinical efficacy based on their ability to bias GPCR signaling, the opportunities for drug discovery are obvious.

PHARMACOLOGIC IMPLICATIONS OF FUNCTIONAL SELECTIVITY APPLIED TO METABOLIC BONE DISORDERS

Osteoporosis, a significant health threat among our aging population, is characterized by a decrease in bone mineral density (BMD) and a deterioration in the microarchitecture of bone resulting in an increased susceptibility to fractures [36]. The etiology of osteoporosis is complex, representing the net imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone-resorption. Currently employed anti-resorptive therapies such as bisphosphonates, selective estrogen modulators (SERMs), calcium and vitamin D, are not sufficient to regenerate lost trabecular bone architecture. Thus efforts are needed to identify anabolic agents that target osteoblast-mediated bone formation. Ideally these therapies would uncouple bone formation from osteoclast-mediated bone resorption and improve mineral content and bone quality. Several approaches, including the use of fluoride [37-39], human growth hormone [40, 41], prostaglandins [42, 43] and PTH analogs [44], have been explored as prospective agents to induce bone formation. Of these, PTH analogs are the most efficacious anabolic therapies developed to date. Presently, the conventional PTH1R agonist, PTH(1-34) is the only FDA-approved anabolic approach to stimulate bone formation. However its clinical utility is hampered by its effects on bone resorption and propensity to produce hypercalcemia and hypercalcuria with prolonged administration. The development of PTH receptor agonists which promote osteoblastic bone formation without stimulating bone resorption may have clinical utility for the treatment of metabolic bone disease such as osteoporosis [45-47]. A PTH analog that activates β-arrestin-mediated signaling in the absence of G protein signaling dependent signaling shares this desired pharmacologic profile [47]. The recent identification of "β-arrestin-biased" agonists may form the basis for pharmacologic agents with enhanced therapeutic specificity and efficacy [5, 45-47].

THE COMPLEX ACTIONS OF PARATHYROID HORMONE

PTH is an 84-amino acid peptide hormone that serves as the primary systemic regulator of calcium homeostasis. Expressed principally in the parathyroid gland, PTH secretion is tightly linked to serum calcium levels. The calcium-sensing receptor in parathyroid cells negatively regulates PTH secretion, such that a fall in calcium leads to increased PTH secretion. The principle targets of PTH in the periphery are kidney and bone, where its actions promote a rise in serum calcium. In the kidney, PTH regulates renal tubular calcium resorption through a well-characterized cAMP-dependent mechanism. It also regulates renal expression of the 1α-hydroxyase necessary to convert 25(OH)-vitamin D3 to its active form $1,25(OH)_2$-vitamin D3, which in turn enhances intestinal calcium absorbtion. The physiologic actions of PTH on bone are complex. At the cellular level, PTH directly stimulates osteoblasts to build bone by increasing osteoblast number and activity, promoting the deposition of new bone matrix and accelerating the rate of mineralization [48, 49]. At the same time, PTH stimulates bone resorption by increasing the recruitment, differentiation and activity of osteoclasts. The effects of PTH on bone resorptive osteoclasts are indirect. Lacking PTH receptors, osteoclasts respond to factors, such as receptor activator of NFκB ligand (RANKL) and osteoprotegrin (OPG), secreted by osteoblasts in response to PTH. Because the anabolic and catabolic effects of PTH are coupled, the net effect of PTH on bone is dependent upon the kinetics of receptor activation, with intermittent exposure leading to a net increase in bone formation, while continuous exposure produces net bone loss and possible hypercalcemia [44, 48, 50-52].

PTH acts principally through the PTH1R, a class II GPCR that is highly expressed in kidney and bone. Most of its known effects are mediated by classic G protein signaling mechanisms, including G_s-mediated activation of adenylyl cyclase, resulting in cAMP production and PKA activation, and $G_{q/11}$-mediated activation of phospholipase-C β, leading to inositol-1,4,5-trisphosphate (IP_3) production, calcium mobilization and PKC activation [53-57]. In renal tubular epithelium, PDZ domain-mediated binding of Na^+/H^+ exchanger regulatory factor 2 to the PTH1R C-terminus permits the receptor to engage $G_{i/o}$ proteins, leading to inhibition of adenylyl cyclase while simultaneously enhancing receptor coupling to $G_{q/11}$ [58]. PTH also activates the ERK1/2 MAPK cascade through both PKA and PKC in a cell-specific and G protein-dependent manner [59-61]. PTH stimulated MAPK activation is known to have proliferative and differentiative effects in bone [62, 63].

Recent work has shown that in primary osteoblasts the PTH1R also signals by coupling to arrestins [47]. PTH1R activation by conventional agonists like PTH(1-34) promotes translocation of both β-arrestin1 and β-arrestin2 to the plasma membrane, association of the receptor with β-arrestins, internalization of receptor-β-arrestin complexes and arrestin-dependent activation of ERK1/2 [33, 64, 65]. Thus, PTH(1–34) stimulates ERK1/2 by multiple distinct mechanisms; conventional G protein-dependent pathways that involves PKA and/or PKC, and newly recognized G

protein-independent pathway mediated by arrestins [33, 59, 61]. Moreover, it has been shown that these discrete G protein-dependent and G protein-independent/β-arrestin mediated signaling pathways are temporally distinct. G protein-dependent ERK1/2 activation is rapid and transient over the course of minutes. Whereas the time course of G protein-independent ERK1/2 activation, mediated by β-arrestins, is delayed and sustained over the course of hours [33].

BIASED AGONISM AT THE PTH RECEPTOR

The PTH1R has long served as a model for the study of functional selectivity in GPCR signaling. Through the generation of numerous PTH peptide analogs, it has been demonstrated that the pleiotropic downstream signaling events induced by the PTH1R are very sensitive to alterations in the hormone agonist's amino acid sequence and structure. Select examples of pathway-selective PTH1R biased agonists are highlighted in Table **1**. To illustrate, the C-terminal truncated PTH (1-34) fragment possesses all of the known biochemical and physiologic properties of the native hormone, acting as a conventional/full agonist with respect to activation of G_s and $G_{q/11}$ signaling and arrestin-dependent receptor desensitization and internalization. In addition, PTH(1-34) also stimulates β-arrestin dependent downstream signaling pathways. Other PTH fragments exhibit marked variations in coupling PTH1R to downstream effectors, including in some cases reversal of efficacy. For example, shorter N-terminal fragments of the PTH peptide, e.g. PTH(1-31) and ^{125}I-[Aib^{1,3},M]PTH(1–15) preferentially promote G_s coupling [66-68], while N-terminal truncations, e.g. PTH(3-34) promote $G_{q/11}$ coupling while failing to activate G_s [69-71]. Agonists have also been identified that distinguish the contributions of arrestin proteins to PTH1R signaling. Trp^1-PTHrp-(1-36) has been shown to activate ERK1/2 solely through a G_s/PKA dependent pathway that is unaffected by β-arrestin expression [33]. Additionally, Bpa^1-PTHrp-(1-36), has also been shown to induce sustained G_s coupling without promoting arrestin-dependent receptor desensitization [72, 73]. More novel, (D-Trp^{12}, Tyr^{34})-PTH(7-34) (PTH-βarr) is a PTH analog previously identified as an inverse agonist that uncouples G protein activation [33, 47, 74], that remains capable of signaling via a G protein-independent/β-arrestin mediated pathway [33, 47]. Thus PTH-βarr is a β-arrestin-pathway selective biased agonist. This newly recognized ability of β-arrestins to serve as signal transducers in the absence of G protein coupling represents an innovative paradigm of receptor signaling which can be targeted to induce a subset of physiologic responses [33, 47]. Identification of a β-arrestin-pathway biased agonist such as PTH-βarr allows for the investigation of G protein-independent/β-arrestin mediated signaling pathways and their relevance to bone and calcium metabolism.

INFLUENCE OF β-ARRESTIN2 ON BONE FORMATION AND TURNOVER

β-arrestin2 has been shown to affect bone remodeling and the skeletal response to endogenous PTH [47, 75-77]. β-arrestin2$^{-/-}$ mice lack both β-arrestin2-dependent desensitization of PTH-stimulated G protein activation and β-arrrestin2-mediated signaling. *In vivo*, β-arrestin2$^{-/-}$ mice have normal serum calcium levels and no gross alterations in skeletal morphology or size compared to congenic wild type mice. However the loss of β-arrestin2 does alter underlying bone metabolism. Circulating levels of endogenous PTH are suppressed in β-arrestin2$^{-/-}$ mice [78], possibly a compensatory mechanism to maintain physiologic calcium homeostasis in the setting of impaired PTH1R desensitization. Also, β-arrestin2$^{-/-}$ mice exhibit higher basal rates of bone turnover compared to wild type mice. Osteoid surface and osteocalcin mRNA levels are increased, consistent with an overall increase in the rate of bone formation, while at the same time bone resorption is accelerated, as evidenced by increased osteoclast surface and bone turnover markers such as urine deoxypyrodiniline (DPD) excretion [47, 77]. Although the trabecular bone

Table 1. Profiles of PTH1R Conventional and Biased Ligands

Ligand	G Protein-coupling	β-arrestin-coupling	References
PTH(1-34)	G_s and $G_{q/11}$	β-arrestin 1 and β-arrestin 2	[33, 53-56]
^{125}I-[Aib^{1,3},M]PTH(1–15)	G_s only	not determined	[66]
PTH(1-31)	G_s only	not determined	[67, 68]
PTH-(3-34)	G_q only	not determined	[69-71]
PTH-(28-42)	G_q only	not determined	[70, 71]
PTH-(28-48)	G_q only	not determined	[70, 71]
Trp^1-PTHrp(1-36)	G_s only	Antagonist	[33]
Bpa^1-PTHrp(1-36)	G_s only	Antagonist	[72, 73]
D-Trp^{12},Tyr^{34}-bPTH(7-34)	Inverse G_s agonist (uncouples G_s)	β-arrestin 1 and β-arrestin 2	[33, 47, 74]

Signaling profiles of the conventional agonist, PTH(1-34) and select biased PTH1R agonists. The binding of a biased ligand results in the activation of a select subset of available signaling pathways that are known to be activated by the receptor.

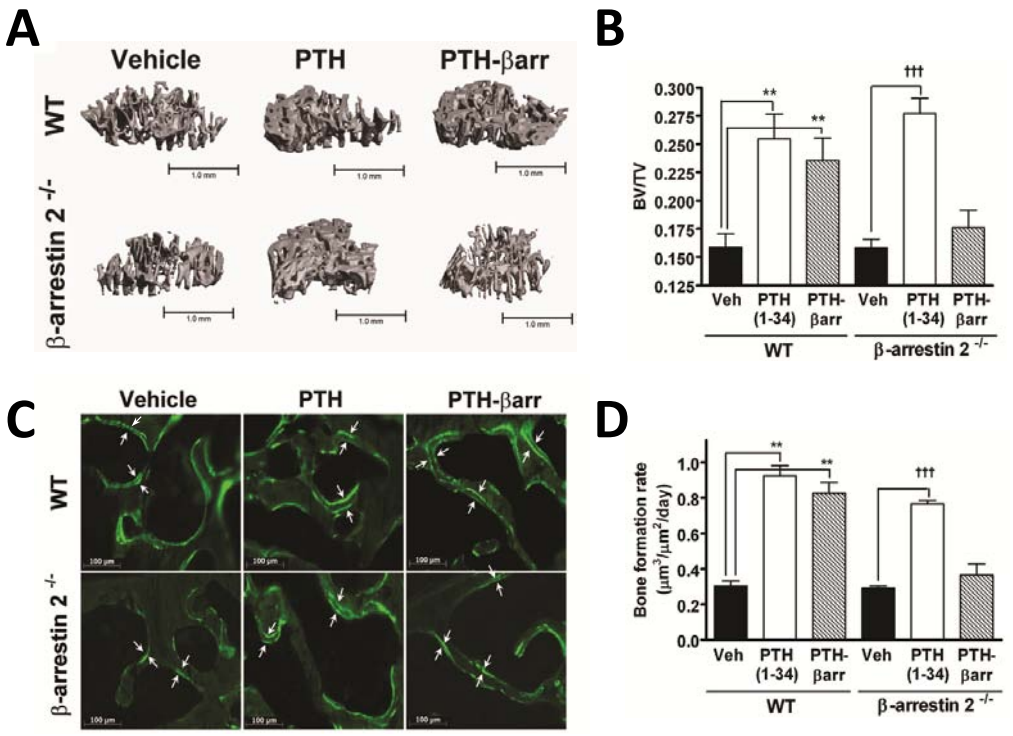
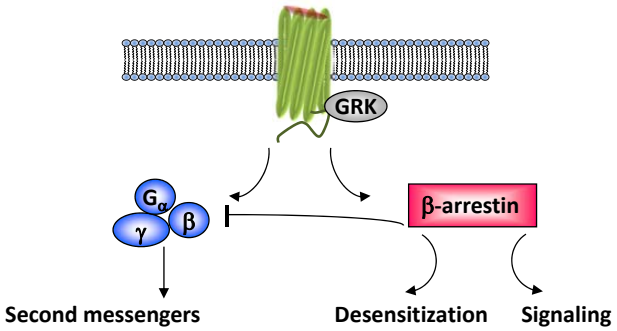


Fig. (4). Arrestin pathway-selective biased agonism at the PTH1R promotes bone formation independent of G protein activation. (**A**) Representative quantitative micro CT (qCT) of proximal tibia from male wild type (WT) and β-arrestin2$^{-/-}$ mice treated for 8 weeks with daily injections of vehicle, or (40 μg/kg/day) of PTH(1-34) or PTH-βarr. Scale bar =1.0 mm. (**B**) qCT of proximal tibia was used to determine the effects of intermittent PTH(1-34) or PTH-βarr on trabecular bone (Tb) volume fraction (BV/TV). Data represent the mean ± SEM of measurements taken from at least seven male mice. (**C**) Representative calcein double-labeled, non-decalcified, 10 μm sections of lumbar vertebrae from male WT and β-arrestin2$^{-/-}$ mice treated for 8weeks with either vehicle, PTH, or PTH-βarr. Scale bar = 100 μm. Bone formation rates are determined by calcein-double labeling (arrows). (**D**) Quantitation of bone formation rates from calcein-labeled trabecular bone. Data represent the mean ± SEM of measurements from four mice. *P < 0.05; **P < 0.01; ***P < 0.001 compared with vehicle-treated mice. †P < 0.05; ††P < 0.01; †††P < 0.001 compared with vehicle-treated β-arrestin2$^{-/-}$ mice. Significance determined with one-way ANOVA with Bonferroni correction. Figure adapted with permission from Science-Translational Medicine. 2009; **1:**1ra1.

mineral densities and bone volume fraction of β-arrestin2$^{-/-}$ and wild type mice are comparable, the β-arrestin2$^{-/-}$ show micro-architectural differences, such as increased trabecular thickness and decreased trabecular number. These differences likely represent the net effect of increased bone formation that is offset by accelerated bone resorption. Collectively these findings clearly demonstrate that β-arrestin2 is not required for skeletal patterning and development, and suggest that a major function in bone is to dampen heterotrimeric G protein signaling, consistent with its ubiquitous role in GPCR desensitization.

However, when one overrides compensatory physiologic mechanisms by exposing β-arrestin2$^{-/-}$ mice to pharmacologic levels of PTH(1-34), differences emerge that suggest that arrestins in bone may play roles beyond desensitization [47, 75-77]. In wild type animals, intermittent administration of PTH(1-34) produces the expected increases in indices of bone formation: increased osteoblast number and osteoid surface, increased osteocalcin mRNA and serum osteocalcin level. PTH(1-34) also produces the expected increase in osteoclast activity marked by increased in RANKL mRNA expression, increased osteoclast surface and increased urine DPD excretion. Together these opposing responses reflect the PTH1R-dependent coupling of osteoblastic bone formation to osteoclastic bone resorption.

The net effect is increased bone formation, as evidenced by the increase in trabecular bone volume, trabecular number and trabecular thickness within cancellous bone. Additional increases in periosteal circumference and cortical thickness in the femur indicate a net increase in cortical bone formation in response to PTH(1-34). In contrast, the response to intermittent PTH(1-34) in β-arrestin2$^{-/-}$ mice is more complex, and is marked by attenuated bone formation at trabecular and endocortical bone surfaces and increased markers of bone resorption. Such findings suggest that the loss of β-arrestin2 might impair new bone formation in addition to accelerating bone loss. Ferrari, *et al*. reported that intermittent administration of PTH(1-34) increased bone mass in female β-arrestin2$^{-/-}$ mice but failed to exert an anabolic effect in male animals [76]. The lack of effect in the male β-arrestin2$^{-/-}$ mice was attributed to the loss of β-arrestin-mediated desensitization of G protein coupled signaling, increased and sustained cyclic AMP, and exaggerated osteoclastogenesis resulting from an increased RANKL/OPG ratio in the knockout animals [75, 76].



PTH(1-34)-Agonist Signaling
Activates G protein and
β-arrestin mediated in signaling

Increase:
•Trabecular bone volume fraction
•Trabecular *thickness*
•Bone formation rate
•Bone forming osteoblast number
•Bone resorbing osteoclast activity
•Serum osteocalcin
•Urine DPD
•Urine calcium

PTH-βarr-β-arrestin-biased signaling
Activates β-arrestin mediated in signaling
Acts as an inverse agonist to G protein signaling

Increase:
•Trabecular bone volume fraction
•Trabecular *number*
•Bone formation rate
•Bone forming osteoblast number

• Serum osteocalcin

Fig. (5). Effects of conventional and arrestin pathway-selective biased PTH1R agonists on bone metabolism. Stimulation of the PTH1R results in the activation of two distinct signaling pathways: one G protein-mediated and the other β-arrestin-mediated. Concomitantly, βarrestins also desensitize the G-protein activated response. The binding of the conventional PTH1R agonist, PTH(1-34) results in the activation of G protein- and β-arrestin-dependent signaling, whereas PTH-βarr activates only the β-arrestin dependent pathway. PTH-βarr stimulates anabolic bone formation through a β-arrestin dependent mechanism independent of G protein activation. Summarized are the effects of PTH(1-34) and PTH-βarr on markers of osteoblast mediated bone formation and osteoclast mediated bone resorption.

SKELETAL EFFECTS OF AN ARRESTIN PATHWAY-SELECTIVE AGONIST OF THE PTH RECEPTOR

The early embryonic lethality of β-arrestin1/2 double knockout animals [79], along with the inherent duality of arrestin function, presents challenges to deciphering their roles in the regulation of bone metabolism. The blunted anabolic response to PTH(1-34) in β-arrestin2$^{-/-}$ mice could arise from impaired PTH1R desensitization, leading to excessive G protein-dependent stimulation of osteoclasts. Alternatively, the phenotype might reflect the loss of β-arrestin2-mediated signals required for an optimal response to PTH(1-34), or some combination of effects. The relative contributions of these two processes cannot be distinguished using β-arrestin2$^{-/-}$ animals, since both functions of β-arrestin are absent and cannot be independently reconstituted.

The identification of arrestin pathway-selective biased agonists for the PTH1R provides a means to examine the contribution of arrestin-dependent signaling to bone remodeling *in vivo*, independent of its role in the desensitization of PTH1R-mediated G protein activation. Administering an arrestin pathway-selective PTH analog to wild type mice allows one to examine the contribution of β-arrestin-mediated signaling to skeletal metabolism in the absence of pharmacologic activation of G protein pathways. The analogous experiment performed in β-arrestin2$^{-/-}$ mice would reveal skeletal effects that might arise from the transient *inhibition* of G protein signaling resulting from competitive inhibition of endogenous PTH signaling. Conversely, administering PTH(1-34) to the β-arrestin2$^{-/-}$ animals allows separation of the effects of G protein signaling from β-arrestin signaling because PTH(1-34) activates both pathways in wild type animals, but only G protein signaling in the knockout.

The results of such an experiment [47], performed in congenic male β-arrestin2$^{-/-}$ mice and wild type C57BL/6 controls, is summarized in (Fig. **4**). Despite the antagonism of G protein signaling, wild type animals treated with PTH-βarr exhibited increased bone formation, associated with increased osteoblast number, osteocalcin mRNA expression and serum osteocalcin levels, as well as increased trabecular number and greater bone volume fraction. In β-arrestin2$^{-/-}$ mice administered PTH-βarr, the skeletal effects on bone formation were either opposite or unchanged, indicating that they did not result from inhibition of G protein signaling mediated by endogenous PTH. Despite the similarity to the anabolic response to PTH(1-34) in wild type animals, it is clear that the arrestin-selective PTH analog does not elicit the full PTH1R signaling response in bone (Fig. **5**). Unlike PTH(1-34), the anabolic effect of PTH-βarr appeared to be confined to the trabecular bone compartment. Moreover, selective activation of the β-arrestin2 pathway by PTH-βarr did not significantly increase any indices of osteoclastic bone

resorption. In wild type animals, PTH(1-34) stimulated osteoblast/osteoclast coupling and bone resorption, as evidenced by increases in RANKL mRNA expression, osteoclast number, and markers of bone resorption including urinary calcium excretion and urinary DPD. β-arrestin2$^{-/-}$ animals treated with PTH(1-34) exhibited an exaggerated increase in osteoclast number and urine DPD, supporting the conclusion that PTH1R-mediated bone resorption is principally mediated via G protein dependent signaling pathways that are not activated by the arrestin-selective PTH analog.

β-ARRESTIN PATHWAY-SELECTIVITY AS A STRATEGY FOR DRUG DESIGN

The phenomenon of biased agonism presents the opportunity to develop drugs that target GPCRs with unique biologic actions as well as improved specificity and efficacy. Ligands that direct signaling toward individual G protein pathways may prove useful in a variety of settings. G protein-selective ligands that signal without producing arrestin-dependent desensitization have seemingly obvious applications, e.g. targeting opioid receptors for the management of chronic pain [80]. In contrast, relatively little is known about the physiologic roles of arrestin-mediated signaling, and as a result we have much to learn before we can even identify those settings where arrestin selectivity might confer therapeutic advantage [27].

Pathway-selective PTH analogs have proven to be valuable tools for determining the contribution of different PTH1R signaling pathways to bone metabolism both *in vitro* and *in vivo*. Recent work using an arrestin pathway-selective PTH1R agonist *in vivo* suggests that activation of arrestin signaling is sufficient to promote bone formation but is unable to stimulate bone resorption, meaning that it uncouples the bone forming effects of PTH on osteoblasts from its previously non-dissociable effects on osteoclastic bone resorption. Although considerable additional work will be required to understand the mechanistic basis of PTH-βarr actions in bone and determine whether it has efficacy in various preclinical models of metabolic bone disease, these results suggest a novel therapeutic strategy that capitalizes on functionally selective ligands to tailor PTH1R efficacy in order to achieve a desired response profile. This demonstration that an arrestin pathway-selective biased agonist of the PTH1R can accelerate bone formation *in vivo* offers the best evidence to date that biased activators of G protein-independent signaling can achieve biological responses that cannot be attained with conventional non-selective agonists or antagonists. This *in vivo* evidence for functional selectivity at the PTH1R in bone provides impetus that the study of arrestin signaling will lead to the development of novel therapeutics targeting GPCRs.

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STATEMENT OF CONFLICT OF INTEREST

The authors have no conflicts of interest.

ABBREVIATIONS

GPCR	=	G protein-coupled receptor
7TMRs	=	Seven transmembrane receptors
PTH1R	=	Type I parathyroid hormone receptor
PTH	=	Parathyroid hormone
PTH-βarr	=	D-Trp12,Tyr34-bPTH(7-34)
RAMPs	=	Receptor Activity-Modifying Proteins
MAPK	=	Mitogen-activated protein kinase
BMD	=	Bone mineral density
RANKL	=	Receptor activator of NFκB ligand
OPG	=	osteoprotegrin
DPD	=	deoxypyrodiniline

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