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REVIEW

'Biasing' the parathyroid hormone receptor: A novel anabolic approach to increasing bone mass?

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'Functional selectivity' refers to the ability of a ligand to activate and/or inhibit only a subset of the signals capable of emanating from its cognate G-protein-coupled receptor (GPCR). Whereas conventional GPCR agonism and antagonism can be viewed as modulating the *quantity* of efficacy, functionally selective or 'biased' ligands *qualitatively* change the nature of information flow across the plasma membrane, raising the prospect of drugs with improved therapeutic efficacy or reduced side effects. Nonetheless, there is little experimental evidence that biased ligands offer advantages over conventional agonists/antagonists *in vivo*. Recent work with the type I parathyroid hormone receptor (PTH_1R) suggests that biased ligands that selectively activate G-protein-independent arrestin-mediated signalling pathways may hold promise in the treatment of osteoporosis. Parathyroid hormone (PTH) is a principle regulator of bone and calcium metabolism. In bone, PTH exerts complex effects; promoting new bone formation through direct actions on osteoblasts while simultaneously stimulating bone loss through indirect activation of osteoclastic bone resorption. Although the conventional PTH_1R agonist teriparatide, PTH(1–34), is effective in the treatment of osteoporosis, its utility is limited by its bone-resorptive effects and propensity to promote hypercalcaemia/hypercalcuria. In contrast, D-Trp^{12},Tyr^{34}-bPTH(7–34) (PTH-βarr), an arrestin pathway-selective agonist for the PTH_1R, induces anabolic bone formation independent of classic G-protein-coupled signalling mechanisms. Unlike PTH(1–34), PTH-βarr appears to 'uncouple' the anabolic effects of PTH_1R 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.

Abbreviations

DPD, deoxypyrodiniline; IP3, inositol-1,4,5-trisphosphate; OR, oestrogen receptor; OPG, osteoprotegrin; PTH, parathyroid hormone; PTH-βarr, D-Trp^{12},Tyr^{34}-bPTH(7-34); PTH1R, type I parathyroid hormone receptor; RANKL, receptor activator of NF-κB ligand; SORM, selective oestrogen receptor modulator

Early allosteric models of GPCR activation envisioned the receptor as existing in equilibrium between an 'off' state (R) that was silent and an 'on' (R*) state that elicited a measurable response (Karlin, 1967; Thron, 1973; Kenakin, 1996). Since spontaneous adoption of the R* state would be an exceedingly rare event, molecules that preferentially bound and stabilized the otherwise rare R* state were described as agonists, while those that had no discernable effect on receptor activity but competitively inhibited agonist binding were classified as antagonists. The subsequent discovery of constitutively activating GPCR mutations that generated measurable levels of receptor activity in the absence of ligand not

only lent credence to the allosteric model, it also enabled the detection of ligands that suppress basal receptor activity (Costa and Herz, 1989; Samama *et al.*, 1993; Weiss *et al.*, 1996). In such models, 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 towards the 'on' state; true 'neutral' *antagonists* bind indiscriminately to both R and R*, producing no physiological response but blocking the response to agonists; while *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 towards the 'off' state.

Despite their power, such models are based on simplifications that limit their utility. In a 'two-state' model, receptor conformation is the sole determinant of information flow; that is, ligand binding can alter the fraction of receptors in the R* state but cannot qualitatively change the nature of that state. If true, it follows that the classification of a ligand as agonist, antagonist or inverse agonist would be independent of the assay used to measure receptor activity; that is, the relative order of potency for a series of ligands should not vary when two or more assays are employed. There is, however, no *a priori* reason to assume that a receptor conformation that enables coupling to one downstream effector (e.g. G-protein class) will necessarily couple the receptor to all of its possible downstream effectors. And if receptors can indeed adopt more than one R* conformation, there is no *a priori* reason to assume that chemically distinct ligands will stabilize an identical population of active states or mimic the spontaneously formed active state.

As increasingly sophisticated biochemical and biophysical approaches to measuring ligand–receptor interactions were developed, it became clear that the relative activity of agonists does not always adhere to the predictions of simple receptor theory; that is, structurally distinct ligands can stabilize different conformational populations and elicit unique ligand-specific efficacy signatures (Maudsley *et al.*, 2005; Kenakin and Miller, 2010). The first formal model to account for these digressions postulated that it is the ligand–receptor complex, not the receptor alone, that specifies the active state (Kenakin, 1995). In this case, the formation of agonist-selective active states can 'bias' the coupling of the receptor to different signalling 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 dramatic. Functional selectivity can range from relatively modest deviations in rank order of potency to frank reversal of efficacy, wherein the characterization of a ligand as agonist, antagonist or inverse agonist becomes assay-dependent.

The promise of functional selectivity

Conventional GPCR pharmacology deals with changing the *quantity* of efficacy (e.g. increasing receptor activity in the setting of low endogenous agonist concentration or decreasing it in the presence of elevated levels of the native ligand). In contrast, efforts to exploit functional selectivity for pharmaceutical design revolve around changing the *quality* of efficacy in a manner that either produces increased therapeutic benefit or diminishes unwanted side effects. An apt analogy from outside the GPCR arena might be selective oestrogen receptor modulators (SORMs), oestrogen receptor (OR) ligands that exhibit oestrogen-like activity in some tissues while inhibiting oestrogen action in others (McDonnell *et al.*, 2002). As with GPCRs, the active conformation of OR subtypes is influenced by ligand structure, allowing different ligands to recruit a unique complement of receptor-associated co-modulators, proteins that enhance or repress OR transcriptional activity (i.e. co-activators and co-repressors). Because the pattern of co-modulator expression varies between different oestrogen-responsive tissues, it has been possible to develop tissue-selective OR ligands for the treatment of breast cancer (e.g. tamoxifen) and postmenopausal osteoporosis (e.g. raloxifen).

If one considers GPCRs as capable of signalling via multiple downstream effectors (e.g. G-protein pools or non-G-protein effectors), the analogy to SORMs, which 'bias' OR signalling by recruiting different co-modulators, becomes clear. Carrying it a step further, it is also apparent that the ability of the ligand to 'bias' signalling extends only as far as its ability to influence receptor coupling to its proximate effectors. With GPCRs, this implies certain signalling functions can be dissociated (e.g. those arising from activation of different G-protein pools), whereas others may remain linked (e.g. effects arising downstream of the same G-protein pool). In the case of SORMs, the practical effect has been that it is possible to dissociate the effects of oestrogen on liver and bone from those on breast and endometrial tissue, but not thus far to dissociate its potentially beneficial effects on hepatic lipoprotein metabolism from its pro-thrombotic effects on the synthesis of clotting factors.

Unlike SORMs, biased GPCR drugs have yet to find their way into the clinic. While 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 (Azzi *et al.*, 2003; Wisler *et al.*, 2007), no currently used pharmaceuticals are known to possess unique clinical efficacy based on their ability to bias GPCR signalling.

The pluridimensionality of GPCR signalling

While all conventional GPCRs share the ability to function as ligand-activated guanine nucleotide exchange factors for heterotrimeric G-proteins, it is increasingly clear that GPCR signal transduction is far more complex and context-dependent than classically envisioned. Many GPCRs have been shown to couple to two, three or more unrelated G-protein classes at physiological levels of expression, enabling a single receptor to engage multiple effectors simultaneously or activate them differentially in a tissue-selective manner (Laugwitz *et al.*, 1996; Maudsley *et al.*, 2005). Moreover, a host of other protein–protein interactions are known

to affect the specificity, selectivity and time course of signalling by the minimal GPCR–G-protein–Effector module. These include the formation of GPCR dimers (Angers *et al.*, 2002), the interaction of GPCRs with receptor activity-modifying proteins (Foord and Marshall, 1999) and the binding of PDZ domain-containing and non-PDZ domain scaffold proteins to the intracellular loops and C-termini of receptors (Brady and Limbird, 2002; Hall and Lefkowitz, 2002; Bockaert *et al.*, 2003). To the extent that ligand binding can alter these myriad interactions, functional selectivity has the potential to modify GPCR signalling.

Among the most studied of these 'novel' GPCR signalling mechanisms is the phenomenon of 'G-protein-independent' signalling by GPCR-arrestin 'signalsomes'. The arrestins are a family of four GPCR binding proteins that regulate receptor desensitization and endocytosis. Arrestins bind tightly and specifically to agonist-occupied receptors that have been phosphorylated by GPCR kinases (Lefkowitz, 1993). Once arrestin-bound, GPCRs are sterically precluded from further G-protein activation. The two non-visual arrestin isoforms also regulate the agonist-promoted internalization of most GPCRs (Ferguson, 2001). β-Arrestins 1 and 2 contain C-terminal motifs that engage clathrin and the β2 adaptin subunit of the AP-2 complex leading to the clustering and internalization of desensitized receptors via clathrin-coated pits. It was the discovery that arrestins serve as adapters not only in the context of GPCR sequestration but also in linking activated receptors to other enzymatic effectors (Luttrell *et al.*, 1999), which forced a change in our view of GPCR signal transduction. It is now clear that a number of catalytically active proteins bind arrestins and are recruited to agonist-occupied GPCRs, among them Src family 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. It is via these interactions that arrestin-binding confers unique signalling properties upon agonist-occupied GPCRs, opening up a broad realm of previously unappreciated GPCR signal transduction (Luttrell and Gesty-Palmer, 2010).

Since arrestin binding uncouples the receptor and G-protein, the G-protein-dependent and arrestin-dependent signalling states of the receptor are mutually exclusive; that is arrestin binding 'switches' the GPCR between two temporally discrete signalling modes (Figure 1). Although, under physiological conditions, arrestin signalling commences in the setting of concurrent G-protein activation, it is clear that at least some arrestin-mediated signals do not require prior G-protein activation. For example, complementary data obtained using G-protein-uncoupled receptor mutants and arrestin pathway-selective biased ligands have shown that arrestin-dependent activation of ERK1/2 by the angiotensin AT_{1A}, β_2 adrenergic and parathyroid hormone (PTH_1) receptors is G-protein-independent (Azzi *et al.*, 2003; Wei *et al.*, 2003; Gesty-Palmer *et al.*, 2006). While the true scope of physiologically relevant arrestin signalling remains largely unknown, a growing literature supports the concept that arrestin-bound effectors perform numerous functions, among them enhancing second messenger degradation, regulating

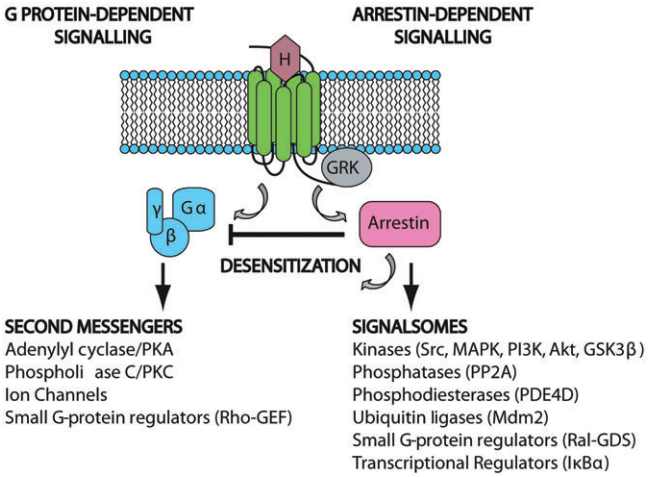

Figure 1

Arrestin binding switches GPCRs between two signalling states. Agonist binding to a GPCR stimulates the intrinsic guanine nucleotide exchange factor activity of the receptor, activating heterotrimeric G-proteins and initiating second messenger-dependent 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 signalling 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.

cytoskeletal dynamics controlling GCPR endocytosis, post-endocytic receptor trafficking, vesicle exocytosis and cell migration, and impacting more distant processes such as protein translation and gene transcription (Luttrell and Gesty-Palmer, 2010).

The complex actions of PTH

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 regulated is tightly linked to serum calcium level. 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 D2 to its active form $1,25(OH)_2$-vitamin D3, which in turn enhances intestinal calcium absorbtion. The physiological 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 (Dobnig and Turner, 1995; Schmidt *et al.*, 1995). At the same time, PTH stimulates bone resorption by increasing the recruit-

Table 1

Efficacy profiles of conventional and biased PTH_1R ligands

Ligand	G-protein-coupling	β-Arrestin-coupling	References
PTH(1–34)	Gs and Gq/11	β-Arrestin 1/2	Juppner *et al.* 1991; Abou-Samra *et al.*, 1992; Bringhurst *et al.*, 1993; Iida-Klein *et al.*, 1997; Gesty-Palmer *et al.*, 2006
PTH(1–31)	Gs selective	ND	Jouishomme *et al.* 1994; Whitfield and Morley, 1995
PTH(3–34)	Gq selective	ND	Jouishomme *et al.* 1992; Azarani *et al.*, 1996; Takasu *et al.*, 1999
PTH(28–42)	Gq selective	ND	Azarani *et al.* 1996; Takasu *et al.*, 1999
PTH(28–48)	Gq selective	ND	Azarani *et al.* 1996; Takasu *et al.*, 1999
Trp^1PTHrp(1–36)	Gs selective	Antagonist	Gesty-Palmer *et al.* 2006
Bpa^1PTHrp(1–36)	Gs selective	Antagonist	Bisello *et al.* 2002; 2004
D-Trp^{12},Tyr^{34}-bPTH(7–34)	Inverse Gs agonist	β-Arrestin 1/2	Gardella *et al.* 1996; Gesty-Palmer *et al.*, 2006; 2009

ND, not determined.

ment, 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 NF-κB 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 hypercalcaemia (Tam *et al.*, 1982; Hock and Gera, 1992; Dobnig and Turner, 1995; Ishizuya *et al.*, 1997; Qin *et al.*, 2004).

PTH acts principally through the PTH_1R, a class II GPCR that is highly expressed in kidney and bone. Most of its known effects are mediated by classic G-protein signalling mechanisms, including G_s-mediated activation of adenylyl cyclase, resulting in cAMP production and PKA activation, and $G_{q/11}$-mediated activation of phospholipase-Cβ, leading to inositol-1,4,5-trisphosphate (IP_3) production, calcium mobilization and PKC activation (Juppner *et al.*, 1991; Abou-Samra *et al.*, 1992; Bringhurst *et al.*, 1993; Iida-Klein *et al.*, 1997; Koh *et al.*, 1999). In renal tubular epithelium, PDZ domain-mediated binding of Na^+/H^+ exchanger regulatory factor 2 to the PTH_1R C-terminus permits the receptor to engage $G_{i/o}$ proteins, leading to inhibition of adenylyl cyclase while simultaneously enhancing receptor coupling to $G_{q/11}$ (Mahon *et al.*, 2002). PTH activates the ERK1/2 MAPK cascade through both PKA and PKC in a cell-specific and G-protein-dependent manner (Verheijen and Defize, 1997; Cole 1999; Lederer *et al.* 2000). PTH-stimulated MAPK activation is known to have proliferative and differentiative effects in bone (Garcia-Ocana *et al.*, 1998; Swarthout *et al.*, 2001).

Recent work has shown that in primary osteoblasts the PTH_1R also signals by coupling to arrestins (Gesty-Palmer *et al.*, 2009). PTH_1R 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 (Ferrari *et al.*, 1999; Vilardaga *et al.*, 2002; Gesty-Palmer *et al.*, 2006). Thus, PTH(1–34) stimulates ERK1/2 by two temporally distinct mechanisms; a conventional G-protein-dependent pathway that involves PKA and/or PKC, and a G-protein-independent pathway mediated by arrestins (Gesty-Palmer *et al.*, 2006).

Biased agonism at the PTH receptor

The PTH_1R has long served as a model for the study of functional selectivity in GPCR signalling, as its pleiotropic downstream signalling events are sensitive to changes in ligand structure. The C-terminal truncated PTH (1–34) fragment possesses all of the known biochemical and physiological properties of the native hormone, acting as a conventional/full agonist with respect to activation of G_s and $G_{q/11}$ signalling and arrestin-dependent receptor desensitization and internalization. Other PTH fragments exhibit marked variations in coupling PTH_1R to downstream effectors, including in some cases frank reversal of efficacy. For example, shorter N-terminal fragments of the PTH peptide [e.g. PTH(1–31)] preferentially cause G_s coupling (Jouishomme *et al.*, 1994; Whitfield and Morley, 1995), while N-terminal truncations [e.g. PTH(3–34)] promote $G_{q/11}$ coupling while failing to activate G_s (Jouishomme *et al.*, 1992; Azarani *et al.*, 1996; Takasu *et al.*, 1999). 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 (Gesty-Palmer *et al.*, 2006). Additionally, Bpa^1-PTHrp-(1–36) has been shown to induce sustained G_s coupling without promoting arrestin-dependent receptor desensitization (Bisello *et al.*, 2002; 2004). More extreme (D-Trp^{12}, Tyr^{34})-PTH(7–34) (PTH-βarr) acts as an inverse agonist for G_s coupling (Gardella *et al.*, 1996; Gesty-Palmer *et al.*, 2006; 2009) yet is capable of signalling via a G-protein-independent β-arrestin-mediated signalling pathway. Table 1 summarizes the efficacy profiles attainable using several reported PTH_1R biased agonists.

Influence of β-arrestin2 on bone formation and turnover

β-arrestin2 has been shown to affect bone remodelling and the skeletal response to endogenous PTH (Bouxsein *et al.*,

2005; Ferrari *et al.*, 2005; Gesty-Palmer *et al.*, 2009; Pierroz *et al.*, 2009). β-arrestin2$^{-/-}$ mice lack both β-arrestin2-dependent desensitization of PTH-stimulated G-protein activation and β-arrrestin2-mediated signalling. *In vivo*, β-arrestin2$^{-/-}$ mice have normal serum calcium levels and no gross alterations in skeletal morphology or size compared with 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 (Pi *et al.*, 2005), possibly a compensatory mechanism to maintain physiological calcium homeostasis in the setting of impaired PTH_1R desensitization. Also, β-arrestin2$^{-/-}$ mice exhibit higher basal rates of bone turnover. 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 RANKL mRNA expression, osteoclast surface, marrow osteoclast precursors and bone turnover markers such as urine deoxypyrodiniline (DPD) (Gesty-Palmer *et al.*, 2009; Pierroz *et al.*, 2009). Although their trabecular bone mineral densities and bone volume are comparable, the knockout mice show micro-architectural differences, such as increased trabecular thickness and decreased trabecular number, likely representing the net effect of increased bone formation that is offset by accelerated bone resorption. These results clearly demonstrate that β-arrestin2 in not required for skeletal patterning and development and suggest that a major function in bone is to dampen heterotrimeric G-protein signalling, consistent with its ubiquitous role in GPCR desensitization.

However, when one overrides compensatory physiological mechanisms by exposing β-arrestin2$^{-/-}$ mice to pharmacological levels of PTH(1–34), differences emerge that suggest that arrestins in bone may play roles beyond desensitization (Bouxsein *et al.*, 2005; Ferrari *et al.* 2005; Gesty-Palmer *et al.* 2009; Pierroz *et al.* 2009). 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 increases in markers of osteoclast recruitment and bone resorption: increased RANKL mRNA expression, osteoclast number and urine DPD, reflecting the PTH-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 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.* (2005) 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. The lack of effect in the male β-arrestin2$^{-/-}$ mice was attributed to the loss of β-arrestin-mediated desensitization of G-protein-coupled signalling, increased and sustained cyclic AMP, and exaggerated osteoclastogenesis resulting from an increased RANKL/OPG ratio in the knockout animals (Bouxsein *et al.*, 2005; Ferrari *et al.* 2005).

Skeletal effects of an arrestin pathway-selective agonist of the PTH receptor

The early embryonic lethality of β-arrestin1/2 double knockout animals (Kohout *et al.*, 2001), 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-arrestin$^{-/-}$ mice could arise from impaired PTH_1R 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 arrestin pathway-selective biased agonists for the PTH_1R provides a means to examine the contribution of arrestin-dependent signalling to bone remodelling *in vivo*, independent of its role in the desensitization of PTH_1R-mediated G-protein activation. Administering a arrestin pathway-selective PTH analogue to wild-type mice allows one to examine the contribution of β-arrestin-mediated signalling to skeletal metabolism in the absence of pharmacological activation of G-protein pathways, whereas the same experiment performed in β-arrestin2$^{-/-}$ would reveal skeletal effects that might arise from the transient *inhibition* of G-protein signalling resulting from competitive inhibition of endogenous PTH signalling. Conversely, administering PTH(1–34) to the β-arrestin2$^{-/-}$ animals allows separation of the effects of G-protein signalling from β-arrestin signalling because PTH(1–34) activates both pathways in wild-type animals, but only G-protein signalling in the knockout.

The results of such an experiment (Gesty-Palmer *et al.*, 2009), performed in congenic male β-arrestin2$^{-/-}$ mice and wild-type C57BL/6 controls, is summarized in Figure 2. Despite the antagonism of G-protein signalling, wild- type animals treated with PTH-βarr exhibited increased bone formation, associated with increased osteoblast number, osteocalcin mRNA expression and serum osteocalcin level, increased trabecular number and thickness 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 signalling 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 analogue does not elicit the full PTH_1R signalling response in bone (Figure 3). 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-

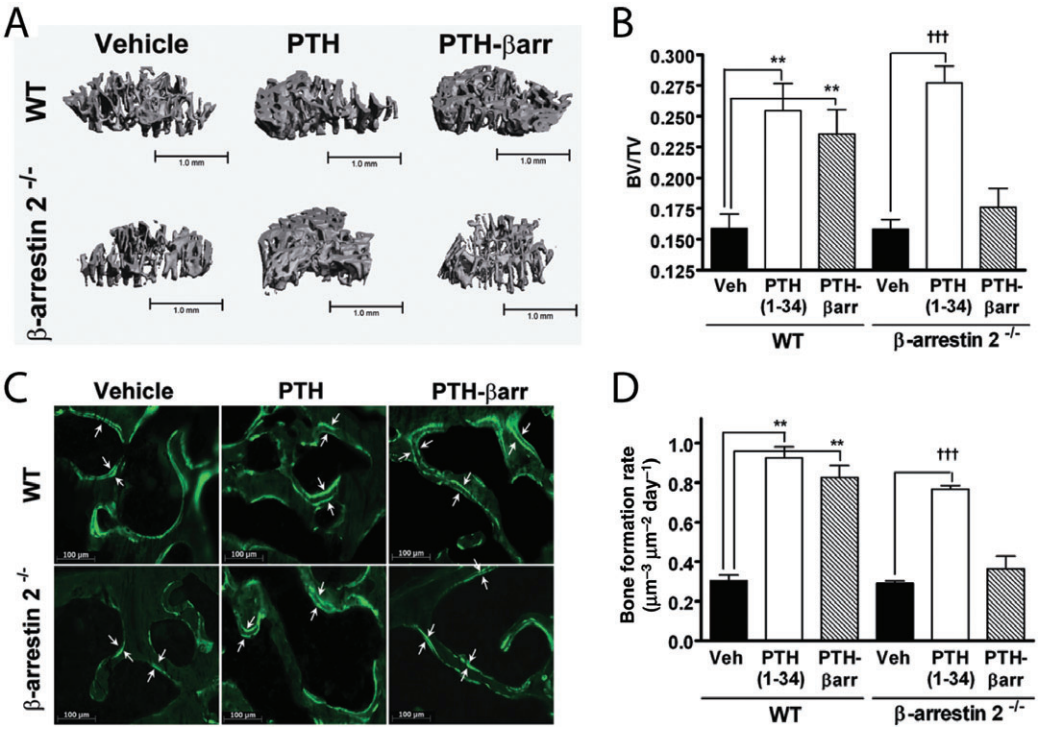

Figure 2

Arrestin pathway-selective biased agonism at the PTH_1R 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^{-1} kg^{-1} day^{-1}) 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-labelled, non-decalcified, 10 μm sections of lumbar vertebrae from male WT and β-arrestin2$^{-/-}$ mice treated for 8 weeks with either vehicle, PTH or PTH-βarr. Scale bar = 100 μm. Bone formation rates are determined by measuring the distance between calcein-labelled layers. (D) Quantitation of bone formation rates from calcein-labelled 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.

type animals, PTH(1–34) stimulated osteoblast/osteoclast coupling and bone resorption, as evidenced by increases RANKL mRNA, osteoclast number and markers of bone resorption including urinary calcium and DPD. β-arrestin2$^{-/-}$ animals treated with PTH(1–34) exhibited an exaggerated increase in osteoclast number and urine DPD, supporting the conclusion that PTH_1R-mediated bone resorption is principally mediated via G-protein-dependent signalling pathways that are not activated by the arrestin-selective PTH analogue.

Developing novel agents for the treatment of metabolic bone disease

Osteoporosis is a significant health threat among our aging population, leading to susceptibility to fractures, increased morbidity, mortality and rising medical costs (Orsini *et al.*, 2005). The aetiology of osteoporosis is complex, representing the net imbalance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption, leading to low bone mass, loss of bone microarchitecture and bone fragility. Currently employed anti-resorptive therapies 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, improve mineral content and bone quality. Several approaches, including the use of fluoride (Caverzasio *et al.*, 1997; Lau and David, 1998; Vestergaard *et al.*, 2008), human growth hormone (Sugimoto *et al.*, 2002; Giustina *et al.*, 2008), prostaglandins (Quarles *et al.*, 1993; Soper *et al.*, 2001) and PTH analogues (Qin *et al.*, 2004) have been explored. Of these, PTH analogues are the most efficacious anabolic therapies developed to date. Presently, the conventional PTH_1R agonist PTH(1–34) is the only U.S. Food and Drug Administration-approved anabolic approach to stimulate bone formation, yet its clinical utility is hampered by its effects on bone resorption and propensity to produce hypercalcaemia and hypercalcuria with prolonged administration.

Pathway-selective PTH analogues have proven to be valuable tools for determining the contribution of different PTH_1R signalling pathways to bone metabolism both *in vitro* and *in vivo*. Our recent work using an arrestin pathway-

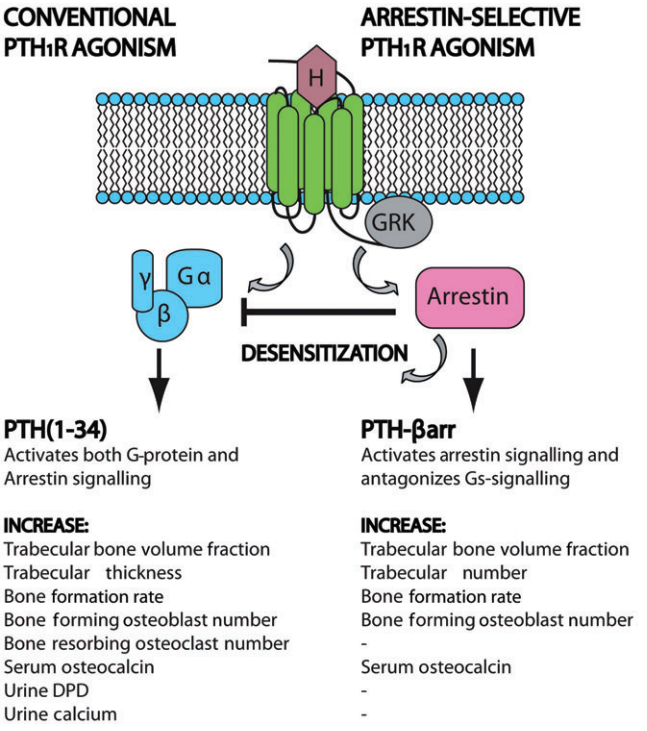

Figure 3

Effects of conventional and arrestin pathway-selective biased PTH_1R agonists on bone metabolism. Stimulation of the PTH_1R results in the activation of two distinct signalling pathways: one G-protein-mediated and the other β-arrestin-mediated. Concomitantly, β-arrestins also desensitize the G-protein-activated response. The binding of the conventional PTH_1R agonist, PTH(1–34) results in the activation of G-protein- and β-arrestin-dependent signalling, 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.

selective PTH_1R agonist *in vivo* suggests that activation of arrestin signalling 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 PTH_1R efficacy to achieve a desired response profile.

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 biological actions as well as improved specificity and efficacy. Ligands that direct signalling towards 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) (Bohn *et al.*, 1999). By contrast, relatively little is known about the physiological roles of arrestin-mediated signalling, and as a result, we have much to learn before we can even identify those settings where arrestin selectivity might confer therapeutic advantage (Luttrell and Gesty-Palmer, 2010). Still, the demonstration that an arrestin pathway-selective biased agonist of the PTH_1R can accelerate bone formation *in vivo* offers the best evidence to date that biased activators of G-protein-independent signalling can achieve biological responses that cannot be attained with conventional non-selective agonists or antagonists and offers hope that the study of arrestin signalling will lead to the development of novel therapeutics.

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Conflict of interest

The authors have no conflicts of interest.

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