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Review

The control of bone remodeling by neuropeptide Y receptors

S.J. Allison [a], P.A. Baldock [a], H. Herzog [b,*]

[a] Bone and Mineral Research Program, Garvan Institute of Medical Research, St. Vincent's Hospital, 384 Victoria Street, Darlinghurst, Sydney, NSW 2010, Australia

[b] Neurobiology Program, Garvan Institute of Medical Research, St. Vincent's Hospital, 384 Victoria St., Darlinghurst, Sydney, NSW 2010, Australia

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ABSTRACT

An important role for the neuropeptide Y receptor system in the regulation of bone formation was recently revealed with a significant elevation in trabecular bone formation and bone volume following germline or hypothalamus-specific deletion of neuropeptide Y2 receptors in mice. Subsequent studies have now demonstrated that this central pathway is distinct from that of the other centrally regulated bone formation pathway mediated by leptin. This review discusses these recent findings and outlines how these new pathways could translate into potential novel targets for the treatment of bone disease.

Contents

1. Introduction

The role of the neuropeptide Y (NPY) system in the regulation of food intake and energy homeostasis is well established, however, evidence for an important role for this system also in the regulation of bone formation has recently emerged. Germline deletion of neuropeptide Y2 receptors produces a two-fold increase in trabecular bone volume associated with a

* *Corresponding author*. Tel.: +61 2 9295 8296; fax: +61 2 9295 8281.
E-mail address: h.herzog@garvan.org.au (H. Herzog).

Abbreviations: NPY, neuropeptide; PTH, parathyroid hormone; icv, intracerebroventricular; BMD, bone mineral density; SNS, sympathetic nervous system; DBH, β-hydroxylase; IGF-1, insulin-like growth factor-1

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greater rate of bone formation by osteoblasts [5]. Interestingly, this bone formation phenotype can be reproduced by conditional deletion of Y2 receptors solely from the hypothalamus of adult mice, demonstrating the response in peripheral bone tissues to be regulated by centrally located Y2 receptors. A second bone acting pathway involving mediation via a hypothalamic mechanism involves leptin [12]. The known interaction between NPY and leptin in their regulation of energy homeostasis suggests possible interaction also in their regulation of bone formation. However, this proposal contrasts with evidence from several recent studies suggesting their regulation of bone formation occurs via distinct pathways [4]. The findings from these studies are discussed later in this review; however, to comprehend the significance of these findings it is necessary first to understand the process of bone remodeling and the consequences when it is inappropriately regulated.

2. Regulation of bone remodeling

In the normal adult, bone is in a state of constant turnover through the actions of two major cell types; osteoclasts, which resorb bone at multiple discrete sites, and osteoblasts, which form new bone following bone resorption. This turnover process is called bone remodeling, and is essential to sustain requirements for ion homeostasis, to replace damaged bone, and to respond to stimuli such as mechanical loading. The process of bone remodeling is normally very tightly regulated, such that the total amount of bone resorbed equals the amount of bone formed, maintaining a constant bone mass. Multiple hormonal and endocrine factors are known to regulate bone remodeling and the dysfunction or cessation of these factors often leads to bone disease. A prime example of this is the hormone estrogen, which plays an important protective role in bone. Cessation of estrogen production by the ovaries following menopause in females results in a marked increase in the number and activity of bone resorbing osteoclasts, resulting in excess bone resorption compared with bone formation [20]. This subsequently leads to the deterioration of bone structures and to the development of the bone disease osteoporosis. Osteoporosis is the most common disease of bone and is characterized by a low bone mineral density (BMD), deterioration of bone microarchitecture, and increased risk of low trauma fractures [29]. Current treatments for osteoporosis inhibit osteoclast resorption and can effectively prevent further bone loss. However, the use of such antiresorptive treatments are often limited as osteoporosis is most frequently diagnosed once significant bone loss has already occurred, and thus the prevention of further bone loss is really of little benefit to the patient as fracture risk remains high. An anabolic treatment which could stimulate the formation of new bone by osteoblastic cells would allow treatment to be initiated after the diagnosis of osteoporosis, with the potential to replace or repair already lost bone. Recent studies demonstrating the control of osteoblast function by neuronal factors *in vivo* has revealed potential new anabolic pathways to target for the treatment of deteriorative bone disease such as osteoporosis.

2.1. *The NPY system in bone—early evidence*

Several early studies provided evidence that NPY-immunoreactive fibers were present in bone, most commonly associated with blood vessel walls, leading to the proposal of a primarily vasoregulatory role in bone, rather than the regulation of bone cell activity [1,16,19,31]. The report that NPY was produced by megakaryocytes within bone marrow supported this proposed role [15]. However, NPY-immunoreactive fibers were also identified associated with bone lining and marrow cells, while studies using RIA demonstrated the presence of NPY in rat periosteum, bone tissue and bone marrow [2,16]. Subsequent studies demonstrated that NPY treatment in osteoblastic cell lines inhibited the cAMP response to PTH and norepinephrine [7,8], suggesting the presence of Y receptors on bone cells and revealing a possible role for NPY in the regulation of osteoblast activity. However, reports of Y receptors in bone are contradictory, with some studies reporting the presence of an Y receptor on human osteoblastic and osteosarcoma-derived cell lines and bone marrow cells [23,35], while others did not detect Y receptor transcripts in primary osteoblastic cultures [5], thus a role for NPY in the direct regulation of bone cell activity via receptor interactions is yet to be firmly established.

2.2. *Regulation of bone formation by Y receptors*

A definitive role for the neuropeptide Y receptor system in the regulation of bone was first revealed in 2002, following germline deletion of the Y2 receptors. Analysis of bone sections from the distal femur of germline Y2 knockout mice ($Y2^{-/-}$) revealed a two-fold greater trabecular bone volume in both male and female $Y2^{-/-}$ compared with wild type mice, associated with significant increases in both trabecular number and thickness (Fig. 1a) [5]. Tetracycline-based dyes incorporated into newly mineralized bone were used to obtain a dynamic measurement of osteoblast activity following fluorescence-based microscopy. This revealed that the increase in bone volume observed in $Y2^{-/-}$ mice resulted from a greater rate of bone formation due to elevated osteoblast activity, demonstrating this pathway to be anabolic (Fig. 1b). Parameters of bone resorption were unchanged except for a modest elevation in osteoclast number. Importantly, the bone formation and bone volume response seen in germline $Y2^{-/-}$ mice was achieved to a similar extent within just 5 weeks following conditional deletion of hypothalamic Y2 receptors in adult mice, revealing the potency by which this pathway modulates osteoblast activity, and demonstrating a role for central Y2 receptors in this process. The increase in bone volume observed in both the germline and conditional $Y2^{-/-}$ mice occurred in the absence of measurable changes in the concentration of insulin-like growth factor-1 (IGF-1), free T4, calcium, leptin and testosterone, with corticosterone levels increased only in conditional $Y2^{-/-}$ mice compared with control. These findings suggested that the anabolic effects of Y2 receptor deletion on bone were not mediated indirectly by these known effectors of bone turnover, and provided strength to the hypothesis that the Y2-mediated anabolic pathway acts via a previously un-described neuronal mechanism. Most importantly, the ability of conditional

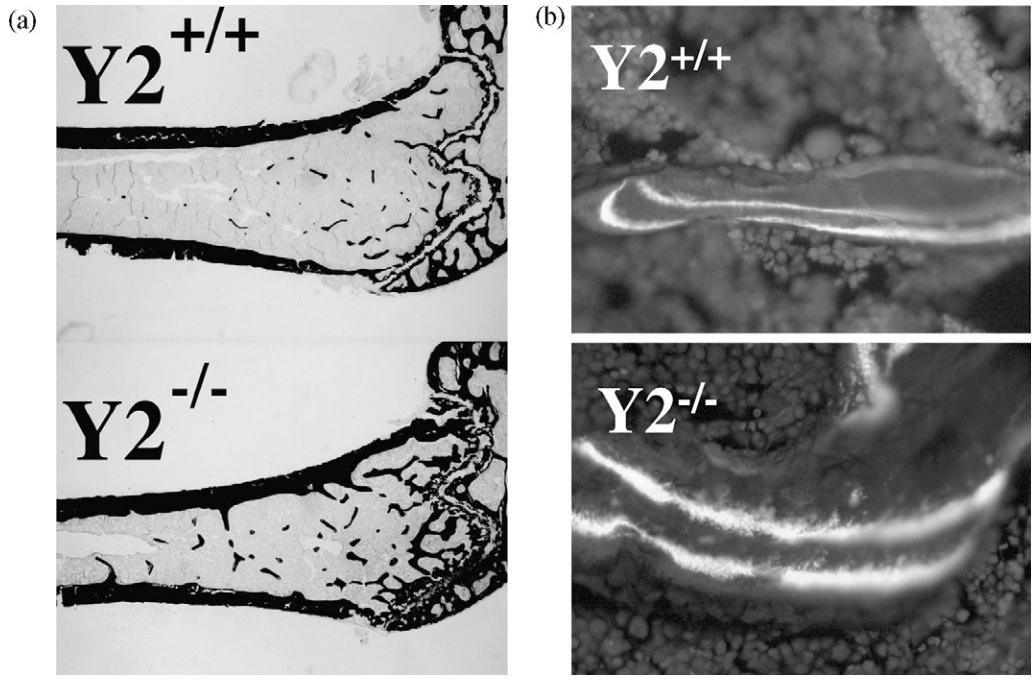

Fig. 1 – Deletion of Y2 receptors increased osteoblast activity and trabecular bone volume. Histological sections from distal femurs of wild type ($Y2^{+/+}$) and germline Y2 knockout ($Y2^{-/-}$) mice. Greater amount of darkly stained trabecular bone in $Y2^{-/-}$ mice compared with wild type (a). Greater distance between tetracycline-based dyes demonstrates greater osteoblast activity in $Y2^{-/-}$ mice (b).

deletion of hypothalamic Y2 receptors to activate the bone anabolic response to double trabecular bone volume in skeletally mature adult mice within such a short period of time has significant clinical implications for the treatment of bone disease such as osteoporosis.

2.3. *The central regulation of osteoblast activity*

These findings revealed a previously unknown role for the neuropeptide Y receptor system in the regulation of bone cell activity, and further revealed that this regulation was controlled by central Y2 receptors present within the hypothalamus. At about the same time as the Y2 receptor-mediated anabolic pathway was revealed; another rodent model in which osteoblast activity was modulated by a hypothalamic circuit was also reported, involving the adipocytic hormone leptin. Mice deficient in leptin (ob/ob), or its receptor (db/db), were reported to have a doubling in trabecular bone volume, and, similar to the phenotype of the $Y2^{-/-}$ model, this was again attributable to an elevation in osteoblast activity, which activated bone formation sufficiently to overcome elevated bone resorption [12]. Importantly, intracerebroventricular (icv) administration of leptin caused a dose-dependent reduction in trabecular bone volume in both ob/ob and wild type mice, demonstrating leptin's actions on bone cell activity to be mediated by a central circuit [12]. These findings were supported by a further study in which one mouse of a parabiosed pair of ob/ob mice received icv infusion of leptin. A decrease in bone mass occurred only in the leptin recipient mouse, and not the contralateral mouse, further demonstrating the regulation of osteoblast activity on trabecular surfaces to be mediated by actions of leptin within the hypothalamus [33].

Despite the marked differences in body weight of the $Y2^{-/-}$ and ob/ob models, the similarities in the bone formation response observed in the absence of either Y2 receptor or leptin signaling suggested these two anabolic pathways might regulate osteoblast activity by a common pathway. Indeed, NPY and leptin are known to interact to regulate food intake and energy homeostasis. The hormone leptin, is released from adipocytes, with serum leptin levels positively correlated with adipose mass [11]. It acts at specific receptors within the hypothalamus to regulate energy homeostasis. NPY acts downstream of leptin, and is negatively regulated by serum leptin, such that when adipose levels are depleted, reduced leptin signaling results in increased expression of NPY which contributes to the homeostatic response to stimulate appetite and conserve energy [14]. Interestingly, Y2 receptors and leptin receptors are co-localized on NPY-ergic neurons in the arcuate nucleus, indicating a role for the Y2 receptor in the regulation of energy homeostasis by leptin [6,10]. Indeed, Y2 receptors have been demonstrated to modify the adipose response due to leptin deficiency, with deletion of Y2 receptors reducing the obese phenotype of the ob/ob model [28].

The antiosteogenic actions of leptin have more recently been demonstrated to act via the sympathetic nervous system [33]. Leptin deficient ob/ob mice have decreased sympathetic tone, leading to the finding that dopamine β-hydroxylase (DBH)-deficient mice which are unable to produce the catecholamine ligands for adrenergic receptors, epinephrine and norepinephrine, also had a high trabecular bone mass phenotype [33]. Moreover, icv infusion of leptin to DBH mice

failed to reduce their bone mass, indicating that a functional autonomic nervous system is necessary for the antiosteogenic actions of leptin. Further studies demonstrated that administration of the β-agonist isoproterenol reduced bone mass in both wild type and ob/ob mice while administration of the non-selective β-adrenergic receptor antagonist propranolol increased bone mass in wild type mice and prevented ovariectomy-induced bone loss [33], providing the first substantial evidence that modulation of sympathetic nervous system (SNS) activity is able to affect bone remodeling. Supporting evidence arose with the characterization of β2-adrenergic receptor knockout (Adrb2$^{-/-}$) mice, which also had a high bone mass phenotype and was unaffected by icv delivery of leptin [13]. Thus, these studies have convincingly demonstrated that the central antiosteogenic actions of leptin on osteoblastic cells were mediated by downstream sympathetic signaling to control bone formation and bone mass. Consistent with these studies, β-adrenergic receptors have been identified on osteoblasts and in osteoblast-like cell lines from human, rat and mouse [17,21,33,35], suggesting that modulation of sympathetic activity may directly modulate osteoblast function. This suggestion is supported by a number of *in vitro* studies using β-agonists and antagonists to assess the influence of sympathetic activity on downstream signaling pathways and bone cell activity [17,21,34].

Recent studies have now also revealed a role for leptin in the regulation of bone resorption. Adrb2$^{-/-}$ mice were found to have decreased bone resorption in addition to their increased bone formation, which was again not normalized by icv leptin administration [13]. Transplantation experiments in which bone marrow from wild type mice was transplanted into Adrb2$^{-/-}$ mice normalized the bone resorption of Adrb2$^{-/-}$ mice while transplantation of bone marrow from Adrb2$^{-/-}$ mice into wild type mice reduced bone resorption [13], suggesting that leptin also regulates bone resorption through the sympathetic nervous system, most likely indirectly through cells of the osteoblastic lineage.

However, despite these *in vitro* studies and *in vivo* analyses of rodent models, the practicalities of modulating sympathetic activity to treat bone loss in humans is still a matter for debate. Since the above-mentioned studies in rodents, the effects of β-adrenergic antagonists on bone turnover, BMD, and fracture risk have been assessed in human based studies with mixed findings. Some studies have reported β-blocker use to be associated with reduced fracture risk and increased BMD in women and men [24,30], while another study found β-blocker use to be associated with increased fracture risk and reduced markers of bone formation [26]. Therefore, there is mixed evidence regarding the potential benefits of β-blocker use in humans for the treatment of osteoporosis, and placebo-controlled randomized clinical trials are necessary to resolve these contradictory findings.

2.4. *Interaction between Y2 receptors and leptin in the regulation of bone*

As mentioned above, the NPY and leptin systems are known to interact to regulate energy homeostasis, with leptin and Y2-receptors co-expressed on NPY-ergic neurons within the arcuate nucleus. The demonstration that Y2 receptor signaling is able to modify the regulation of energy homeostasis by leptin gives rise to the question of whether a similar interaction could also occur in bone.

NPY is present in both sympathetic and parasympathetic nerve fibers where it has a well-established role in vasoconstriction [22,25]. In addition, the predominantly pre-synaptic Y2-receptor is known to suppress neurotransmitter and norepinephrine release [9], and is therefore itself able to regulate sympathetic activity.

Together with the similar anabolic bone phenotype produced by deletion of either leptin or Y2 receptor signaling, and the known role of the adrenergic system in mediating the effects of leptin on bone cell activity, these findings suggest that the leptin and Y2-mediated anabolic pathways interact via a common pathway. Furthermore, as central leptin signaling inhibits NPY production in leptin-responsive NPY neurons [32], and Y2 receptors also inhibit the production of NPY [18], lack of either leptin or Y2 receptor signaling would result in increased hypothalamic NPY. Indeed, expression of hypothalamic NPY is elevated in both the leptin or Y2 receptor deficient anabolic models [27,28,36], suggesting this to be a possible common mechanism by which these two pathways regulate osteoblast activity. However, continuous icv infusion of NPY into wild type mice actually decreased bone volume [12], raising the possibility that the mechanisms by which leptin regulates energy homeostasis and bone mass might be separate. This study did not, however, address the possibility that the observed reduction in osteoblast activity could also be a consequence of elevated leptin signaling, secondary to their greater adiposity induced by the elevation in NPY.

In order to further investigate whether the Y2 receptor and leptin anabolic pathways are mediated by a common or distinct pathway, osteoblast activity was investigated in Y2$^{-/-}$ob$^{-/-}$ double knockout mice to determine the extent to which deletion of Y2 receptor signaling would affect the leptin-deficient anabolic response. Interestingly, analysis of distal femur from Y2$^{-/-}$ob$^{-/-}$ mice revealed a phenotype similar to that of an ob/ob mouse, with elevated trabecular bone volume compared with wild type, associated with a greater rate of bone formation [4]. Trabecular bone volume was reduced compared with germline Y2$^{-/-}$ mice, however, this was attributable to greater bone resorption, likely conveyed by leptin-deficiency. The lack of an additive effect following Y2 receptor deletion in leptin-deficient mice indicates the presence of either a shared osteoblast regulatory feedback loop, or alternatively suggests that a minimal permissive level of leptin might be required for activation of the Y2-mediated anabolic pathway [4].

Interestingly, a synergistic anabolic effect was reported in male Y2$^{-/-}$Y4$^{-/-}$ double knockout mice, with a three-fold increase in trabecular bone volume, significantly greater than the two-fold increase seen in Y2$^{-/-}$ mice alone [27]. This synergistic increase in bone volume was only evident in male mice, in which plasma leptin was significantly reduced, suggesting that the synergistic increase in bone volume observed in male Y2$^{-/-}$Y4$^{-/-}$ mice might be due to an additive effect of the Y2 receptor and leptin mediated antiosteogenic pathways in the absence of functional Y4 receptors. These findings suggest that the Y2 and leptin antiosteogenic pathways do not share a common feedback loop, and therefore

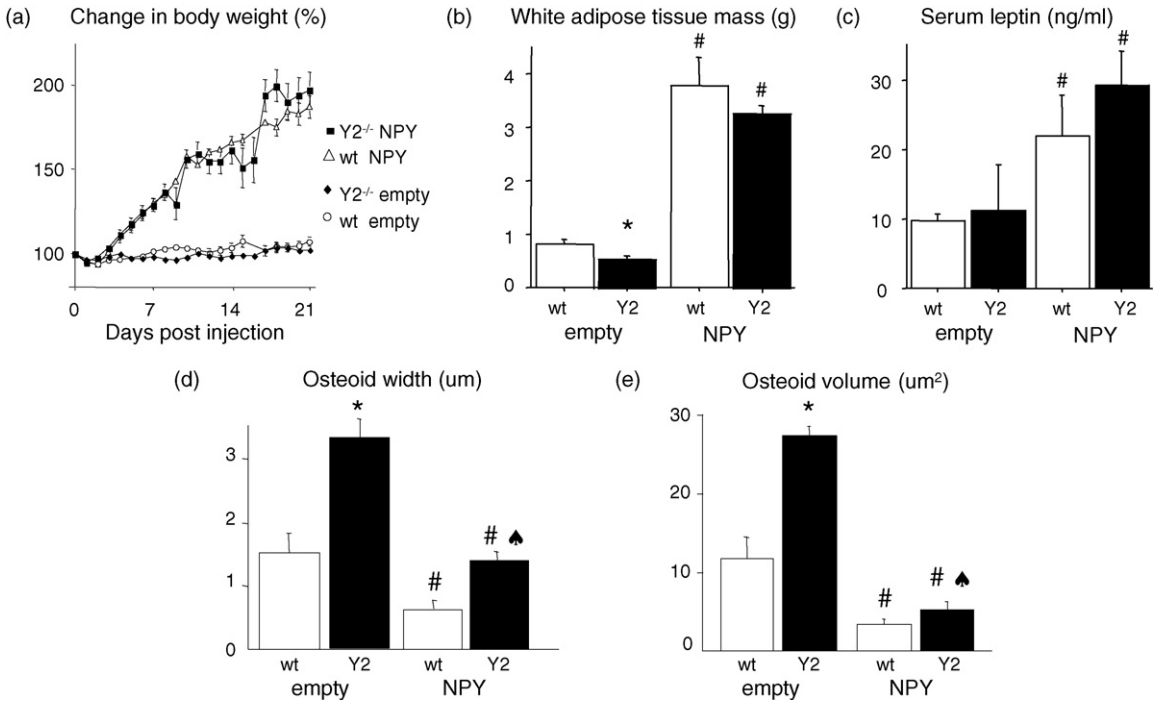

Fig. 2 – Y2-anabolic activity is distinct from leptin antiosteogenic action. Injection of NPY-expressing adeno-associated virus into the hypothalamus of wild type and $Y2^{-/-}$ mice increased body weight (a), white adipose tissue mass (b), and serum leptin (c). Elevated NPY and leptin reduced osteoblast activity, evident as decreased osteoid width and volume in both wild type and $Y2^{-/-}$ mice, but osteoid parameters remained significantly greater in $Y2^{-/-}$ mice compared to wild type (d and e). $^{*}p < 0.05$ vs. AAV-empty wild type; $^{\#}p < 0.05$ vs. AAV-empty; $^{\spadesuit}p < 0.05$ wild type AAV-NPY vs. $Y2^{-/-}$ AAV-NPY.

support the hypothesis that a permissive level of leptin may be required for the Y2-mediated anabolic response to be active.

As a consequence of these findings, the effect of Y2 receptor deficiency on bone cell activity was investigated under conditions of elevated leptin and NPY by over-expressing NPY in the hypothalamus of wild type and $Y2^{-/-}$ mice using recombinant adeno-associated viral vector. This produced a marked increase in body weight, adipose mass, and thus leptin levels (Fig. 2a–c) [4]. As expected from earlier studies [12], overexpression of NPY and leptin produced a corresponding decrease in parameters of bone formation in mice of both genotypes. Importantly, however, $Y2^{-/-}$ mice maintained their two-fold greater osteoblast activity compared with wild type mice (Fig. 2d and e) [4], demonstrating that although not entirely resistant to the antiosteogenic actions of leptin, the anabolic activity of the $Y2^{-/-}$ model was maintained, suggesting distinct actions of the Y2 and leptin pathways in their regulation of osteoblast activity, with elevated levels of leptin decreasing bone formation, while Y2 deletion consistently activated osteoblast activity.

Recent studies of cortical bone have also provided evidence of distinct actions of the leptin and Y2-mediated pathways, with ablation of Y2 receptor signaling activating osteoblast activity and increasing cortical bone mass, while in contrast leptin deficiency reduced cortical bone mass and density [3]. These studies reveal opposing activities of the leptin and Y2 pathways in cortical bone, and support a model in which the actions of these two pathways are also distinct in cancellous bone.

3. Conclusions

These studies have revealed that the regulation of bone formation by central Y2 receptors is likely to be distinct from that of the other known central regulator of bone formation, leptin, and therefore opens new avenues for potential osteoporosis treatment. It is yet to be established however, whether the adrenergic system also plays a role in the regulation of bone formation by Y2 receptors. The downstream signaling pathways involved in the Y2-mediated anabolic effect are also yet to be revealed. Together, however, these studies have shown that complex pathways within the central hypothalamus exist which are able to control bone cell activity, opening up exciting new possibilities for osteoporosis treatment.

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