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SKELETAL REGULATIONS (D GADDY, SECTION EDITOR)

The NPY System and its Neural and Neuroendocrine Regulation of Bone

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Abstract The past decade has seen a significant expansion of our understanding of the interaction between the neural system and bone. While innervation of bone was long appreciated, the discovery of central relays from the hypothalamus to the cells of bone has seen the identification of a number of efferent neural pathways to bone. The neuropeptide Y (NPY) system has proven to represent a major central pathway, regulating the activity of osteoblasts and osteoclasts, through signaling of central and peripheral ligands, through specific receptors within the hypothalamus and the osteoblast. Moreover, this pathway is now recognized as acting to coordinate both skeletal and energy homeostasis. This review examines the mechanism and actions of the NPY pathway to regulate bone mass and bone cell activity.

Keywords Neuropeptide Y · NPY · Peptide YY · Bone remodeling · Hypothalamus · Osteoblast · Sympathetic nervous system · Mouse model

Introduction

Bone remodeling is a tightly coupled process of bone resorption followed by bone formation [1]. The balance between bone formation and resorption is essential for bone homeostasis, with disruption of this balance toward excessive bone resorption leading to bone fragility and susceptibility to fracture, as evident so commonly in osteoporosis [2]. As such, further insights into mechanisms of bone homeostasis will aid in identifying potential therapeutic targets for bone diseases. Bone remodeling has long been viewed as predominantly an endocrine- and paracrine-regulated process. Recently, the discovery of bone regulation by neural signals represents an emergent area of study that is identifying novel regulatory axes between the nervous system and bone cells. Current understanding is that bone can be modulated by central and peripheral actions of the nervous system. Neural signaling in the hypothalamus mediates the central regulation of bone. Locally, bone tissue is well innervated by both sensory and autonomic nerve fibers [3] where neurotransmitters can directly mediate their effects on bone tissue. One such neural system of particular importance to bone metabolism is the neuropeptide Y (NPY) system. This review discusses central and peripheral actions of NPY in the regulation of skeletal tissue.

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The Neuropeptide Y System

Originally identified in pig brain, NPY is a 36-amino acid peptide widely expressed in the central and peripheral nervous systems of mammals [4]. NPY plays an important role in the regulation of numerous physiological processes, including skeletal and energy homeostasis, and immune and endocrine function. In the central nervous system (CNS), NPY is widely expressed in the hypothalamus, cerebral cortex, brain stem, striatum, and limbic structures [5]. However, the hypothalamic arcuate nucleus contains the highest expression of NPY [6]. In the peripheral nervous system, NPY is found in the sympathetic nervous system, co-stored and co-released with noradrenaline during nerve stimulation [7]. Interestingly, NPY expression is increasingly being identified in peripheral tissues including both osteoblasts

and adipocytes [8–10]. Other NPY-related peptides include peptide YY (PYY) and pancreatic polypeptide (PP), which are also 36-amino acid peptides and all share a common hairpin-like loop structure, the PP-fold [11]. This PP-fold is essential in regulating the binding to their Y receptors [11, 12].

Y receptors are a family of G-protein-coupled receptors, of which five subtypes have been identified to date: Y1, Y2, Y4, Y5, and y6 [6]. NPY and PYY have similar Y-receptor binding profiles with greatest affinity for the Y2 receptors, followed by Y1, Y5, and the least affinity for Y4 receptors [13, 14]. In contrast, PP has a high affinity to the Y4 receptor with much lower affinity to all other Y receptors [13]. Each receptor displays varying distributions across central and peripheral tissues to reflect their specific functions in NPY signaling. Y1 receptors have the widest distribution in the brain with particular expression in the paraventricular nucleus (PVN) of the hypothalamus [15]. The arcuate nucleus, along with its high NPY expression, expresses Y2 receptors. On the other hand, Y4 is found in both the brain and peripheral tissues [13]. The Y5 receptor is predominantly expressed in the CNS and is consistently co-localized with the Y1 receptor [15–17]. Intriguingly, despite the fact that it is functional in mice and rabbits, the y6 receptor is nonfunctional in humans due to a single base-pair deletion [18, 19]. In addition to neural tissue expression, adipocytes express both Y1 and Y2 receptors [9] and osteoblasts express Y1 receptors [20••, 21], indicating the potential for local and even autocrine effects as NPY is expressed in the same cells [8–10].

The Y receptor family signal transduction pathways involve G_i or G_o protein signaling via suppression of cyclic adenosine monophosphate (cAMP) generation [13]. In addition to affecting cAMP levels, it was also observed that the Y1 receptors mediate intracellular calcium signaling by mobilizing intracellular calcium stores and activating or blocking calcium channels, in a cell type–specific manner [22–24]. Potassium channels can also be activated or inhibited by signaling through Y receptors [25]. Furthermore, Y1 receptor signaling has been shown to promote cell proliferation by activating the mitogen-activated protein kinase pathway, extracellularly regulated kinase [26–28]. This effect has been shown to be dependent on phosphatidylinositol-3-kinase [29] and also to involve intermediary protein kinase C [30, 31].

NPY and Bone

Initial thoughts of a role of NPY in bone regulation arose from the discovery of NPY-immunoreactive fibers in bone tissue–associated blood vessels adjacent to bone lining and bone marrow cells [32–36]. This indicated the possibility of neuroendocrine actions on bone cells. In addition to its direct effects, NPY has a central role in regulating bone homeostasis as part of its well-defined role as a central mediator of energy homoeostasis involving leptin signaling on NPY-ergic neurons in the hypothalamus [37, 38]. Mice deficient in leptin (*ob/ob*) or its receptor (*db/db*) had higher cancellous bone volume associated with increased bone turnover [39]. The notion of a central axis of leptin signaling on bone mass was supported by rescue experiments using intracerebroventricular injection of leptin or leptin-producing adenovirus in *ob/ob* mice [39, 40]. Moreover, the abolishment of central leptin signaling by targeted destruction of *Ob-Rb*–positive hypothalamic neurons increased cancellous bone mass [41]. Taken together, the identification of skeletal effects in response to altered central leptin signaling indicated the possibility of central actions of NPY. Since then, with the use of mouse models (Table 1), significant progress has been made in establishing the NPY system as a neural and neuroendocrine regulator of bone homeostasis.

Central Actions of NPY in Skeletal Homeostasis

Hypothalamic NPY

Since the elucidation of hypothalamic control in bone homeostasis by NPY-associated leptin [39], there is significant evidence that NPY has an independent role to that of leptin in regard to bone regulation [42, 43]. Intracerebroventricular infusion of NPY into the cerebral spinal fluid led to a significant reduction in cancellous bone volume in wild-type mice [39]. Subsequent studies using hypothalamus-specific NPY overexpression in the neural tissue of the arcuate nucleus in wild-type mice support the anti-osteogenic effects of hypothalamic NPY [43, 44]. Furthermore, germline NPY knockout mice displayed greater trabecular and cortical bone volume, associated with enhanced osteoblast activity [45••]. These changes are supported by increased expression of bone osteogenic factors, alkaline phosphatase, RUNX2, and Osterix [45••], indicating a critical role of NPY in the control of bone formation. In contrast, another study found no skeletal changes in NPY null mice [46]. A number of factors may have been involved in the lack of bone phenotype such as a possible redundancy from other NPY ligands, PYY and PP. On the other hand, the enhanced activity of the hypothalamic-pituitary-adrenal axis in NPY null mice may play a role, as hypercortisolemia is capable of reducing anabolism in these mice to wild-type levels (Unpublished data). Importantly, in addition to the established central role of NPY in fat metabolism [45••], these studies demonstrate the ability of central NPY to regulate both bone and fat tissue homeostasis, with evidence that these processes are coordinated [8, 45••].

Table 1 Summary of bone phenotypes from gene knockout mouse models on the NPY system

Gene deletion	Bone phenotype	Site of action	References
Y1R	↑ Bone volume	Osteoblasts	[8, 20••, 21, 66]
Y2R	↑ Bone volume ↓ Osteoblast Y1R	Hypothalamus	[21, 51, 52, 53•, 68]
Y4R	None	None	[70]
Y5R	Not reported	Not reported	Not reported
y6R	Not reported	Not reported	Not reported
Y1R, Y2R	↑ Bone volume	Central and peripheral tissues	[8]
Y2R, Y4R	↑↑ Bone volume	Hypothalamus, adipose tissues (leptin)	[70]
NPY	↑ Bone volume	Central and peripheral tissues	[45••]
PYY	↓ Bone volume	Not reported	[71]
PP	None	None	[71]

NPY neuropeptide Y; *PP* pancreatic polypeptide; *PYY* peptide YY

Y2 Receptors of the Hypothalamus

Y2 receptors are widely expressed in areas of the brain such as the hypothalamus, hippocampus, and brain stem [15, 47], with Y2 receptors representing approximately two thirds of the total binding capacity of NPY in the CNS [48]. Interestingly, Y2 receptors may act as an autoreceptor, as 80% of the NPY-expressing neurons in the arcuate nucleus co-express the Y2 receptor [49] and act to regulate and reduce the release of NPY by NPY-ergic neurons [50].

The germline deletion of Y2 receptors in mice exhibited a twofold increase in trabecular bone volume due to increased osteoblast activity [51]. There were no changes in circulating hormones known to regulate bone physiology, notably leptin, thyroid hormones, insulin-like growth factor 1, corticosterone, or testosterone, which suggests that hypothalamic Y2 receptors modulate bone formation by neural mechanisms, rather than endocrine mechanisms [51]. Hypothalamus-specific Y2 receptor deletion in adult mice produced an identical bone anabolic phenotype to germline Y2 receptor deletion [51, 52]. Furthermore, chemical lesioning studies of the arcuate nucleus, using monosodium glutamate, elevated bone formation [46]. This demonstrates that Y2 receptor signaling in the hypothalamus plays a critical role in the regulation of bone mass.

Surprisingly, although expressed in a vast majority of NPY-ergic neurons [49], Y2 receptors mediate bone regulation primarily through non-NPY neurons [53•]. The targeted deletion of hypothalamic Y2 receptors in NPY-ergic neurons produced only moderate increases in trabecular bone volume [53•]. Preliminary data from our laboratory suggest that sympathetic neurons emanating from the PVN, the site for arcuate NPY signaling, may be responsible for the efferent pathway (Unpublished data).

Y1 Receptors of the Hypothalamus

In the CNS, Y1 receptors are highly concentrated in the PVN region of the hypothalamus, a region supplied with NPY by NPY-ergic neurons of the arcuate nucleus [15, 54]. Thus, changes in NPY expression in the arcuate nucleus can alter NPY-mediated Y1 receptor signaling in the PVN. Similar to the Y2 receptor knockout mice, germline Y1 receptor deficiency produced greater cortical and cancellous bone volume with a generalized increase in osteoblast activity [8]. Consistent with the lack of significant NPY/Y2 receptor-mediated effects in bone from the arcuate nucleus [53•], the specific loss of hypothalamic Y1 receptors in the PVN did not alter bone homeostasis [8]. Interestingly, the double deletion of Y1 and Y2 receptors in mice showed no additive effects on the bone phenotype [8]. This suggests that Y1 and Y2 receptors may share a common pathway in bone homeostasis. Nevertheless, it appears that hypothalamic Y1 receptors do not play a central role in bone regulation and the observed bone phenotype in germline Y1 receptor knockout mice was due to non-hypothalamic signaling.

Peripheral/Neuroendocrine Actions of NPY in Skeletal Homeostasis

NPY Ligand

Thus far, we have shown the impact of hypothalamic NPY signaling on bone homeostasis. It is interesting to note that increased osteoblastic activity seen in the NPY knockout mice was not completely restored to wild-type levels by the replenishment of NPY into the hypothalamus of NPY knockout mice [45••]. This suggests that hypothalamic NPY is not solely responsible for the bone anabolic phenotype observed in NPY knockout mice, and that non-hypothalamic NPY may play a more direct, neuroendocrine role in bone formation.

Several in vitro studies have suggested a direct action of NPY within bone tissues. NPY treatment in osteoblastic cell lines inhibited the cAMP response to parathyroid hormone (PTH), noradrenaline, and forskolin [55, 56, 57•]. In addition, NPY was shown to inhibit isoprenaline-induced osteoblast

differentiation from bone marrow stromal cell (BMSC) cultures [58]. Furthermore, NPY treatment of calvarial osteoblasts from wild-type mice led to a marked decrease in cell numbers, whereas NPY treatment in osteoblast cultures from Y1 receptor knockout mice had no effect upon cell number [8]. Similarly, NPY treatment in calvarial osteoblasts reduced the expression of late-stage genes (osteocalcin and DMP-1) and reduced mineral deposition, consistent with an increase in Y1 receptor expression with maturation in these cells [57•]. These studies support the presence of functional Y receptors on bone cells and a direct suppressive regulatory role for NPY in the periphery.

There are several sources of NPY in the periphery. NPY is co-released with noradrenaline from sympathetic nerves [59] and by the adrenal medulla [60]. It is also expressed in vascular smooth muscle cells and pancreatic cells [61]. Importantly, NPY is expressed in osteoblasts, osteocytes [20••, 57•], and osteoclasts (Unpublished data). Given the presence of Y1 receptors in osteoblasts, peripheral NPY may act in a paracrine/autocrine fashion in bone cells.

Apart from osteoblastic bone formation, there is some evidence that the NPY system may also be able to regulate osteoclastic bone resorption. Despite the lack of change in osteoclast number, germline $Y1^{-/-}$ mice exhibit an increase in osteoclast surface [8]. NPY has also been shown to indirectly inhibit receptor activator of nuclear factor-κB ligand (RANKL)–induced osteoclastogenesis by selectively inhibiting isoprenaline- and PTH-induced RANKL expression in mouse BMSCs via the Y1 receptor [58]. Isoprenaline acts via β-adrenergic receptors on osteoblastic cells stimulating them to produce RANKL, which in turn acts to induce osteoclast differentiation [62]. The stimulation of RANKL production by both isoprenaline and PTH is mediated via the cAMP/protein kinase A pathway [58]. Consequently, it was suggested that NPY inhibited osteoclastogenesis by interfering with β-adrenergic agonist–induced cAMP production by stromal cells. However, in a more recent in vitro study, it was shown that NPY treatment directly inhibited RANKL expression by osteoblastic cells [63]. In addition, NPY treatment on BMSCs resulted in a transient increase in osteoprotegerin expression, the decoy receptor that inhibits RANKL signal transduction [63]. Therefore, the effects of NPY on the regulation of bone resorption appear to be at least partially mediated by alterations in osteoblast behavior. The direct effect of NPY on osteoclasts remains to be identified.

Peripheral Y1 Receptors

Y1 receptors have a broad distribution in peripheral tissues including the colon, pancreas, adipose tissues, kidney, adrenal gland, heart, and placenta [10, 61, 64, 65]. In relation to bone, Y1 receptors are expressed in the osteoblastic lineage, increasing with differentiation [20••, 57•].

The germline deletion of Y1 receptors produced a high bone mass in mice that was independent of the actions of hypothalamic Y1 receptors [8]. Interestingly, ex vivo culture of Y1 receptor knockout BMSCs showed increased proliferation and differentiation [20••]. Furthermore, the direct osteoblast actions from Y1 receptors was demonstrated as osteoblast-specific Y1 receptor knockout mice recapitulated the bone phenotype of germline Y1 receptor–deficient mice both in vitro and in vivo [66]. These findings confirm the direct and functional role of Y1 receptor in inhibiting mineral deposition by mature osteoblasts, as well as reinforcing the negative effects of NPY on bone cells.

Peripheral Y2 Receptors

Y2 receptors are expressed in white and brown adipose tissue, liver, muscle, spleen, and intestine [67–69]. Unlike Y1 receptors, Y2 receptors are not expressed in osteoblasts [21].

A recent study from conditional peripheral Y2 receptor knockdown demonstrates that peripheral Y2 receptors are not involved in the regulation of bone mass. These conditional knockout mice did not show significant changes in any bone indices [68]. This finding supports the critical role of hypothalamic Y2 receptors on neuronal control of bone formation by NPY-ergic neurons and particularly non-NPY-ergic neurons [49, 53•]. However, Y1 receptors may be the link to peripheral regulation of bone in central Y2 receptor signaling. More specifically, osteoblastic Y1 receptors may be directly involved in the development of a bone anabolic phenotype of germline Y2 knockout mice. Cultured BMSCs isolated from Y2 receptor knockout mice displayed an increase in the number of osteoprogenitor cells and increased mineralization [21]. This increased activity was associated with a significant decrease in BMSC Y1 receptor expression, indicating a positive role of Y2 receptors on osteoblastic Y1 receptor expression [21]. No additive skeletal changes were identified in Y1 and Y2 double knockout mice, indicating the possibility of Y1 and Y2 receptors sharing a common pathway from the hypothalamus to the periphery, to regulate bone formation [8].

Other NPY Family Ligands and Receptors in Bone Homeostasis

PP in Bone Homeostasis

To date, PP does not appear to have a major influence on bone mass in vivo. Neither transgenic overexpression nor gene knockout models in mice demonstrated any bone phenotypes [70, 71].

PYY in Bone Homeostasis

The gastrointestinal peptide, PYY, was previously shown to influence bone metabolism as well as to regulate nutrient intake, similar to NPY [72–74]. PYY was linked to anorexia nervosa–induced osteopenia, as high levels of PYY were correlated with reduced bone mineral density in anorexic women [75, 76]. These studies suggest that high levels of PYY may contribute to bone loss, consistent with NPY and Y receptor knockout mice studies. A recent cross-sectional study in healthy premenopausal women demonstrated that PYY levels have a significant, negative association with total body and hip bone mass [77], explaining nearly 9% of the variance in hip bone mineral density in these women. However, PYY deletion in mice resulted in an osteopenic phenotype with reductions in vertebral cancellous bone mass and bone strength [71]. Further investigation is required on osteoblastic and osteoclastic activity and its role

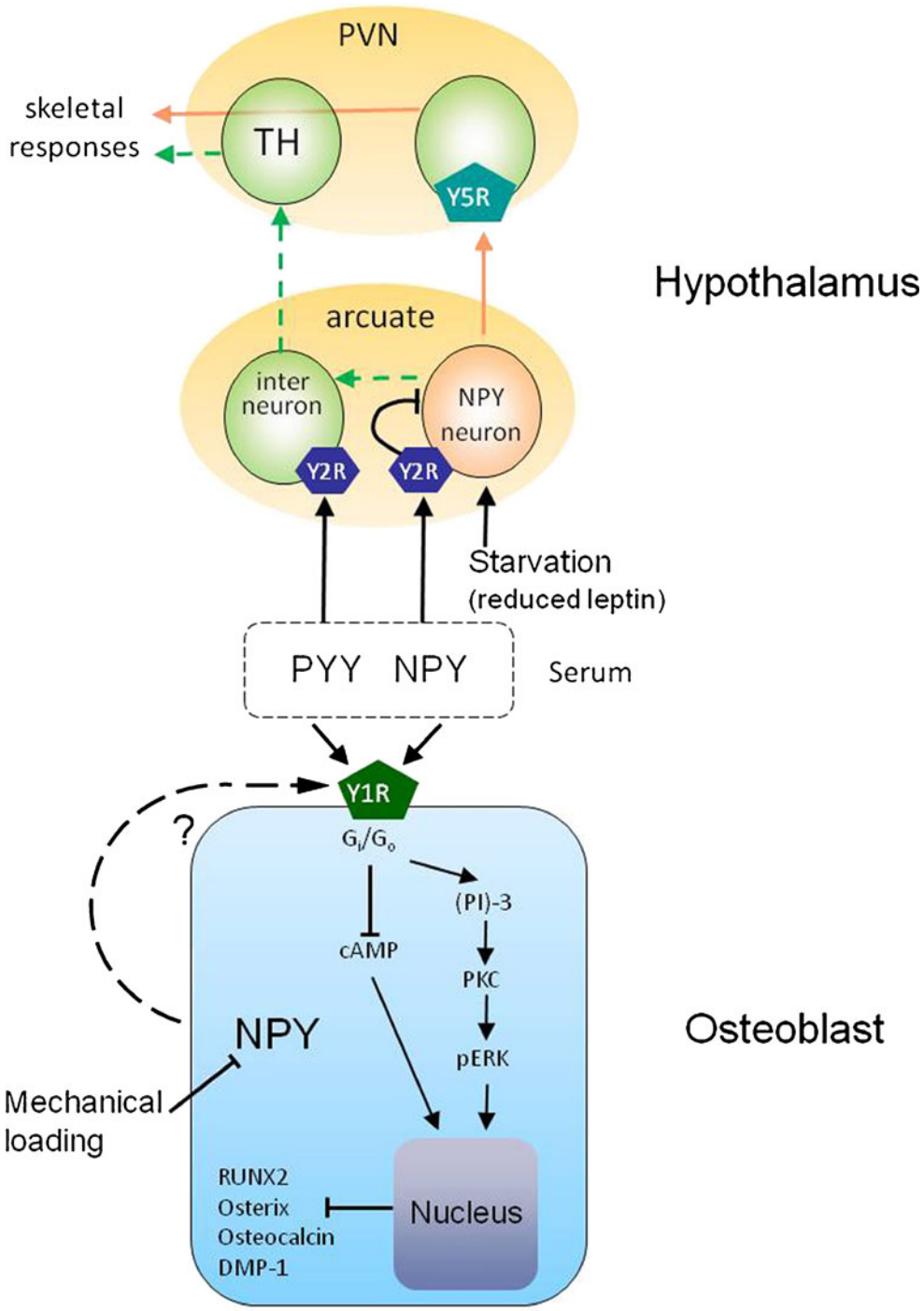

Fig. 1 The NPY system components as key regulators of bone homeostasis in a neural and neuroendocrine manner. In the hypothalamus, NPY can mediate skeletal responses possibly through the Y5 receptors in the PVN. In addition, central bone regulation can be mediated through the actions of Y2 receptors on non-NPY-ergic neurons to activate TH. In the osteoblast, NPY, co-secreted with noradrenaline from sympathetic nerves or produced by the adrenals, or PYY from the gastrointestinal tract can activate Y1 receptors expressed upon osteoblast and osteoprogenitor populations to inhibit differentiation. See Conclusions in text for additional details. cAMP—cyclic adenosine monophosphate; NPY—neuropeptide Y; PI-3—phosphatidylinositol-3-kinase; PKC—protein kinase C; PVN—paraventricular nucleus; PYY—peptide YY; TH—sympathetic neurons

in Y receptor signaling to identify the exact role of PYY in bone homeostasis.

Y4 in Bone Homeostasis

PP has a high affinity to Y4 receptors [13]. Consistent with PP studies in bone, bone mass was unaltered by germline deletion of Y4 receptors in mice [70]. Interestingly, the double knockout of Y2 and Y4 receptors showed a synergistic increase in trabecular bone volume in male mice compared to Y2 receptor knockout mice [70], suggesting a relationship between Y2 and Y4 receptor pathways in the regulation of bone homeostasis. Notably, female Y2 and Y4 double knockout mice did not show this bone phenotype [70]. The sex difference in bone phenotypes was possibly due to reduced leptin levels in males but not in females [70], thereby indirectly indicating an additive action on bone of Y2 receptor deletion and leptin deficiency.

Y5 and y6 Receptors in Bone Homeostasis

To date, little is known about the involvement of Y5 and y6 receptor signaling in the control of bone metabolism. The gene for the Y5 receptor shares a common promotor region with the Y1 receptor gene in humans [78]. Additionally, Y5 receptors normally co-localize with Y1 receptors in the CNS [15–17], and thus are ideally placed to transduce the NPY-ergic signals from the arcuate nucleus. Although not consistently shown to be expressed in BMSCs or osteoblasts [20••], it was recently reported that the Y5 receptor was significantly downregulated in BMSCs from aging rats. In addition, NPY-induced cell proliferation was reduced in aging BMSCs, which was improved with Y5 receptor overexpression [79]. This finding may prove to be important in bone homeostasis in regard to self-renewal of bone marrow progenitor cells.

In humans, the y6 receptor is truncated and nonfunctional. It is fully functional in mice which, interestingly, shares 51% sequence homology to the human Y1 receptor [13]. Further studies are needed to determine a potential a role for the y6 receptor in the control of bone mass in these animals. This will allow for better interpretation of results from mouse models when applying to the human setting.

Clinical Importance of NPY Regulation in Bone

The fact that a body fat phenotype is often associated with the bone phenotype in NPY system knockout mice demonstrates that body weight and bone mass regulation are closely linked [80]. This concept is supported by studies indicating that bone mass is more closely related to fat mass (r=0.5) than lean mass (r=0.2) [81]. This is of clinical relevance as studies have identified body mass as a strong predictor of fracture in both men and women, particularly for hip fracture [82–84]. Furthermore, there is a marked increase in fracture risk at lower body weights. A recent meta-analysis showed a twofold increase in hip fracture risk with a decline in BMI from 25 to 20 kg/m^2 [84]. Interestingly, weight loss is a strong risk factor for bone loss, which itself is an independent predictor of fracture [82, 85]. Therefore, treatments to increase both body weight and bone mass are likely to translate into a reduction in fracture, and thus, morbidity and mortality. These findings highlight that antagonizing Y receptors may not only have a benefit as an antiobesity treatment, it can also increase bone mass in osteoporosis and consequently reduce the risk of fractures.

Conclusions

Taken together, these studies have identified NPY and Y1 and Y2 receptors as key regulators of bone homeostasis in a neural and neuroendocrine manner (Fig. 1). In the hypothalamus, two efferent pathways exist, one in response to altered NPY expression (acting likely through Y5 receptors), a second resulting from Y2 receptor action on non-NPY-ergic neurons to activate sympathetic neurons. However, these efferent, sympathetic signals are modulated by local processes. In the osteoblast, NPY, co-secreted with noradrenaline from sympathetic nerves or produced by the adrenals, or PYY from the gastrointestinal tract can activate Y1 receptors expressed upon osteoblast and osteoprogenitor populations. In this manner, hypothalamic NPY is ideally placed to modulate bone mass in a generalized manner throughout the skeleton. In addition, NPY is locally expressed in bone cells in which it can act in an autocrine and paracrine fashion to Y1 receptors. The existence of these efferent neural pathways has identified a novel action for the hypothalamic nuclei in the control of peripheral tissue homeostasis. This will provide new avenues in identifying novel therapeutic targets in bone biology.

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