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Osteoblastic Actions of the Neuropeptide Y System to Regulate Bone and Energy Homeostasis

Harry Horsnell[1,2] · **Paul A. Baldock**[1,3,4]

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Abstract Neural pathways are now a well-appreciated factor in the regulatory milieu controlling the maintenance of bone mass. A number of neural pathways from the brain to bone have been identified. These pathways often involve elements of the energy homeostatic apparatus, indicating links between the regulation of bone metabolism and energy balance. Neuropeptide Y is one such factor that co-regulates these two processes. Initial studies outlined the skeletal actions of NPY from within the brain and the interactions with energy homeostatic processes. However, in recent years, an appreciation for the actions of NPY within bone cells has expanded. Cells of the osteoblastic lineage express both NPY ligand and a cognate receptor NPY, Y1R. Murine studies have demonstrated that both ligand and receptor actively control bone mass and osteoblast activity and interact with mechanical signals to integrate with the local loading environment. Local NPY signalling regulates osteoprogenitor production and differentiation, to cover the entire osteoblastic lineage. In addition, several recent studies have demonstrated extra-skeletal actions of osteoblastic NPY signalling, to regulate energy expenditure and with it adiposity, and in a separate study, to control release of a factor-controlling beta cell mass and insulin production/release and with it glucose tolerance. Thus, osteoblastic neuropeptide production and signalling illustrates the rapidly widening sphere of influence of skeletal tissue, and suggests a far more complex and interconnected physiology then is currently appreciated.

Keywords Osteoblast · Neuropeptide Y · Mouse model · Hypothalamus · Energy balance

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✉ Paul A. Baldock
p.baldock@garvan.org.au; http://www.garvan.org.au

[1] Osteoporosis and Bone Biology Division, Garvan Institute of Medical Research, St Vincent's Hospital, 390 Victoria St, Darlinghurst, Sydney, NSW 2010, Australia

[2] Department of Biology and Biochemistry, Bath University, Claverton Down Rd, Bath, North East Somerset BA2 7AY, UK

[3] School of Medicine, The University of Notre Dame Australia, 160 Oxford St, Darlinghurst, Sydney, NSW 2010, Australia

[4] Faculty of Medicine, University of New South Wales, Sydney, NSW 2052, Australia

Introduction

The Neuropeptide Y System

The traditional view of bone remodelling as being primarily regulated by endocrine, paracrine and mechanical processes has expanded in recent years to encompass the importance of central and peripheral neuronal signals to the maintenance of bone mass. Interestingly, these studies have often focussed upon pathways involved in energy homeostasis, such as leptin [1]. Thus, in addition to highlighting the role of neural signals in bone metabolism, they have also demonstrated the regulatory interconnection between bone and adipose tissue. One such system that controls both bone and energy metabolism through pathways involving central and peripheral neural signals is the Neuropeptide Y (NPY) system. NPY is widely expressed in the central (CNS) and peripheral (PNS) nervous systems and is one of the most powerful modulators of whole body energy balance. Within the hypothalamus, NPY acts to coordinate signals from a wide variety of sources, including endocrine, such as

leptin, but also neural, environmental and behavioural inputs; responding by inducing a positive energy balance through increased appetite and reduced energy utilisation. In the CNS, NPY is found in various regions such as the cerebral cortex, brain stem and striatum [2] with the highest expression found in the arcuate nucleus of the hypothalamus [3]. Peripherally NPY is found in the sympathetic nervous system, being co-stored and release with noradrenaline [4], as well as increasingly identified in tissues, such as bone [5]. Due to its wide expression and role in energy balance, it is unsurprising that it regulates a number of important physiological processes including appetite, thermoregulation and glucose homeostasis [3]. Interestingly, NPY has also been demonstrated to be involved in a wider network of activities, including among them bone metabolism [6], where NPY acts to coordinate the action of bone remodelling with the requirements of whole body energy balance [7]. Furthermore, NPY and the NPY, Y1 receptor (Y1R) have been identified in bone tissues such as in osteoblasts, osteocytes, as well as adipocytes and adipose tissue macrophages [5, 8–10], indicating the potential for local actions in addition to those dictated by central signalling axes.

NPY, Y1R and Y2R Central Regulation of Bone Mass

The effects of NPY are mediated by a family of G-protein-coupled receptors. Five major receptors have been identified; Y1R, Y2R, Y4R, Y5R and y6R (present only in certain species) [3] with varying distributions and well as differing affinities for NPY, Y2 > Y1 > Y5,Y4 = y6, as well as the other two ligands in this family peptide YY and pancreatic polypeptide [11]. Both Y1 and Y2 receptors have been demonstrated to regulate bone mass, but at differing locations. Both are abundant in CNS. The arcuate nucleus of the hypothalamus shows the greatest expression of NPY, and these NPY-ergic neurons have express high levels of Y2R. These arcuate NPY neurons project to other regions, such as the paraventricular nucleus which has abundant Y1R expression [12]. In recent years, Y receptors have been increasingly identified in peripheral tissues. Adipocytes express both Y1R and Y2R [13] and in osteoblasts Y1R is expressed throughout the osteoblast lineage and in osteocytes [14, 15], as is NPY ligand itself [5], suggesting that the NPY system may have local effects in these tissues. In this review, we will briefly discuss previous knowledge of the central actions of NPY, but primarily, present evidence of NPY action in the osteoblast, to modulate skeletal and non-skeletal processes.

NPY is a key regulator in bone metabolism as well as a coordinator of whole body energy balance. Extremely powerful central pathways exist for both of these aspects, as evidenced by the doubling of body weight and 7-fold reduction in cortical bone formation just 3 weeks after specific elevation of NPY expression in the arcuate nucleus of adult mice [16]. Both Y1R and Y2R are expressed in the hypothalamus [17], however, only Y2R are critical to the central NPY pathway [16, 18, 19]. Whether Y2R were globally deleted or conditionally deleted from hypothalamic neurons, there was a marked increase in the cortical and cancellous bone volume associated with increased mineral apposition rates [19]. Importantly, this bone anabolic response was in the absence of changes in serum markers such as leptin and IGF-1 and the absence of Y2 receptor mRNA in bone tissue, indicating a neural pathway from the brain [19].

As Y1R has also been identified in the hypothalamus, it was also implicated in central bone regulation. When Y1R was globally deleted there was an increase in cortical and cancellous bone volume in association with increased osteoblast activity, as well as metabolic phenotypes of adiposity and hyperinsulinemia [20–22]. However, when conditionally knocked out from the hypothalamus there was no bone phenotype, furthermore Y1R and Y2R double knockouts show no additive phenotype [8]. This indicated that NPY likely acts through a pathway of Y2R and Y1R receptors but at different locations in the system. This was reinforced when mouse osteoblasts lining cancellous and cortical bone surfaces showed expression of Y1R yet Y2R were absent [15].

A global knockout of NPY supported the Y2R and Y1R deletion studies, as it revealed a similar bone phenotype, with increased in cortical and cancellous bone volume associated with greater osteoblast activity [7]. Runx2 and Osterix were upregulated in the absence of NPY reinforcing the histological findings that NPY mediates an anti-anabolic bone response through modulation of osteoblast activity (Fig. 1a) [7]. In order to isolate the location of the NPY regulating bone, NPY expression was re-established in the arcuate nucleus of NPY null mice using a viral vector. NPY expression in the arcuate of NPY null mice reduced the cortical and cancellous bone mass, however the original phenotype was not completely restored [7], indicating multiple sources of NPY regulating bone mass.

Peripheral NPY-Skeletal Actions

As described above, hypothalamic NPY has important regulatory effects on osteoblasts activity. In addition, however, these investigations suggested an important role for peripheral NPY in bone homeostasis. In the periphery, NPY can come from several sources. It is released into the circulation by sympathetic nerves [23] and the adrenal medulla [24] and is produced within peripheral tissues such as pancreatic cells [25]. Most importantly, NPY is present in osteoblasts and osteocytes [5, 14, 26]. Given the presence of Y1R on osteoblasts, there is a possibility for peripheral NPY to have local regulation of bone cells. Further to this, the absence of Y2R in bone suggests a direct anabolic effect on osteoblastic cells by the NPY system through locally expressed Y1R. Previous studies have indicated that NPY can act directly on bone cells. NPY has been shown to inhibit osteoblast-like cell formation

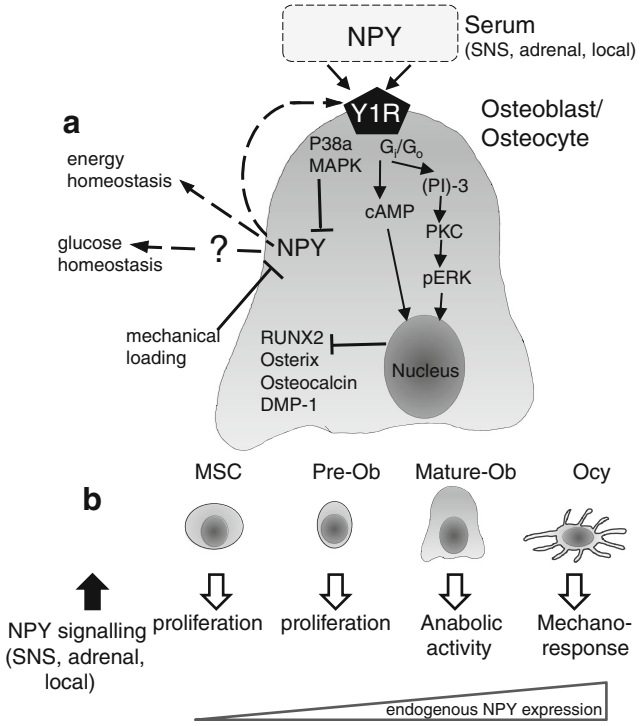

Fig. 1 Effect of NPY signalling on osteoblast function and differentiation. **a** NPY from exogenous sources, mainly SNS and adrenal glands, as well as from local production, acts upon Y1 receptors to inhibit cAMP and ERK pathways to downregulate activity and osteoblastic gene expression. NPY from osteoblasts/osteocytes is able to regulate energy and glucose homeostasis, through as yet undefined pathways. **b** NPY affects multiple aspects of the osteoblast lineage, reducing proliferation of mesenchymal stem cells (MSC) and osteoprogenitor (Pre-Ob), as well as inhibiting bone formation in osteoblasts and mechano-responsiveness of osteocytes

in bone marrow stromal cell (BMSC) cultures treated with isoprenaline [27]. Yet when Y1R were deleted from the osteoblasts, cultures treated with NPY had no change in cell number [8]. Furthermore, NPY-treated calvarial osteoblasts have a reduced expression of late stage genes, such as osteocalcin and DMP-1, with diminished activity represented by reduced mineral deposition, which is consistent with an upregulation in Y1R expression through differentiation [5].

Y1 Receptor in the Periphery

Germline deletion of the Y1R showed that osteoblast activity is increased on cortical and cancellous bone surfaces leading to greater bone mass [8]. BMSC cultured from global Y1R null mice displayed improved mineralization in vitro and greater bone mass and osteoblast activity in vivo [14], which was attributed at least in part, to an upregulation of proliferation and differentiation of mesenchymal progenitor cells (Fig. 1b) [14]. Interestingly, the bone anabolic phenotype present in the Y2R null mice may involve osteoblastic Y1R. In BMSCs cultured from global Y2R null mice there was also an increase in mineralisation and osteoprogenitor cell numbers, in the absence of neural stimulation [15]. The increase in activity was attributed to the decrease in Y1R expression in the BMSCs cultured from Y2R null mice [15], suggesting that control of cell lineage fate and proliferation may be an important linkage point between central and peripheral NPY circuits to bone.

Importantly, lineage-specific deletion of Y1R in early [28••] or late osteoblasts/osteocytes [29] in vivo produced increased cancellous and cortical bone mass. The bone cell changes associated with loss of osteoblastic Y1R were consistent with those following loss of central Y2R, with greater bone volume resulting from increased mineral apposition, not greater mineralised surface or osteoblast number [19, 29]. The osteoblast specificity of the effect was confirmed in early osteoblast Y1R null mice, when bone marrow ablation and reconstitution with wild type marrow completely corrected the high bone mass phenotype [28••]. Osteocytes were also identified as expressing higher levels of NPY, as well as expressing the Y1R [5]. Importantly, expression of NPY was reduced following exposure of calvarial osteoblast cultures to fluid shear stress in vitro, indicating mechanical loading produces reduction in the suppression of osteoblast activity through reducing NPY expression. Consistent with this notion, NPY exposure to these same cultures reduced markers of osteoblast differentiation, such as BSP, osteocalcin and DMP-1 [5]. This study indicates that NPY action is integrated with local mechanical activity, and may reflect the requirement for a local NPY circuit in addition to the powerful central axis. The potential for pharmacological inhibition of Y1R has been modelled, with the orally administrable, long-acting Y1R antagonist, BIBO3304 able to increase bone mass and promote bone formation [30•], however, caution may be required during fracture repair [31].

Murine models altering NPY or Y receptor signalling have been typified by alteration of osteoblast activity; however, changes in bone resorption have been reported. Global loss of Y1R resulted in increased osteoclast number and osteoclast surface [8], unlike the deletion osteoblastic Y1R, which did not alter the osteoclast lineage [29]. Indeed, the germline Y1R null mice displayed greater surface extent of bone resorption, but no coupled response in mineralising surface, suggesting a cell autonomous effect [8]. The change in osteoclast surface in the germline deletion has been suggested to result from an indirect affect attributed to changes in NPY altering osteoclastogenesis by affecting the production of cAMP, RANK-ligand and OPG in mouse bone marrow cells [27, 32], however RANKL:OPG was not altered in global or osteoblast-specific Y1R null models, nor was resorption altered following over expression of NPY in osteoblasts [33•], suggesting osteoblast signalling is not the cause of the osteoclast phenotype in global Y1R null mice [29]. More work is required to ascertain the impact of altered NPY signalling upon bone resorption, and the source of the NPY involved, however, chronic stress increased osteoclast number in wild type mice,

but not in NPY null mice, suggesting a possible role for sympathetic-derived NPY [34].

The Source of Bone-Regulatory NPY

The deletion of Y1R from osteoblasts confirms that NPY signalling occurs locally to control bone mass, however, it does not indicate the source of the NPY, whether osteoblastic and/or from sympathetic neurons. Two studies have investigated this using specific over expression models. Over expression of NPY in late osteoblasts and osteocytes, using the 2.3 col1α1 Cre driver, similar to the one used to delete Y1R above, produced a 2–3-fold increase in NPY expression in bone [29], without a change in circulating levels [33•]. Consistent with the stimulation evident with loss of Y1R [29], increasing NPY reduced cortical and cancellous bone volume in female mice where expression change of NPY was greatest, with similar patterns in male mice [33•]. This is the first evidence of local autocrine/paracrine action of NPY in vivo. In vitro osteoblastic cultures, where NPY expression levels were markedly increased, demonstrated reductions in mineralisation and osteoblastic gene expression of 5-fold or more [33•]. The contribution of NPY from sympathetic neurons, where it is co-released with noradrenaline, was examined in NPY null mice with a specific reintroduction of NPY in dopamine hydroxylase-expressing neurons. Dopamine hydroxylase catalyses the production of noradrenaline, marking sympathetic nerves [35]. Reintroduction of NPY into sympathetic neurons had no effect upon the elevated cancellous bone volume of the underlying NPY null phenotype. However, when mice were exposed to chronic stress, NPY null mice lost substantial amounts of bone, but those with sympathetic NPY were protected from loss [34]. This finding is consistent with NPY's anxiolytic actions, with higher NPY levels known to protect against PTSD and anxiety and depression [36, 37]. These findings indicate that osteoblastic NPY is involved in the regulation of bone mass, acting to suppress bone formation activity of osteoblasts. However, this system is complicated by the context in which NPY production by neurons may be increased, such as chronic stress or depression, when circulating levels may play an increasing role.

Together this data outlines a system whereby NPY can be centrally modulated, primarily in the arcuate nucleus, to induce marked changes in the action of osteoblasts. In the periphery, signals are modulated by local processes involving Y1 receptors expressed on osteoblastic, osteocytic and osteoprogenitor cell populations. NPY produced locally in bone cells such as osteocytes provides a mechanism by which bone can respond to load [5] and efferent NPY neural signalling to fine tune bone homeostasis.

Osteoblastic NPY and Control of Energy Homeostasis

NPY not only plays an important role in bone homeostasis but is also a very potent modulator of adiposity through control of energy expenditure and appetite. While studies of the central regulatory pathways to bone have identified coordination of bone and energy homeostasis by NPY-ergic neurons, recent studies have revealed that the NPY system in osteoblasts also contributes to the regulation of whole body energy and glucose homeostasis. This represents a quantum shift in our view of homeostasis in general; moving from a hypothalamus-centric view to increasing incorporation of additional inter-organ pathways. In addition, it expands our view of the role of the skeleton; from a tissue primarily responding to external influences, to an endocrine organ in its own right, able to regulate critical aspects of whole body physiology, such as obesity and glucose tolerance.

It has been known for some time that NPY can act directly on white adipose tissues in the periphery through the Y1R and Y2R found on adipocytes. This can function in a local manner, with NPY secreted by visceral adipose tissue and local macrophages to promote proliferation in adipocyte cell populations [9]. Peripheral Y1R on adipocytes has also been shown to be a critical regulator of fat mass and lipid oxidation [38]. Y1R peripheral knockdowns show resistance to diet-induced obesity, whereas global Y1R null mice show no such resistance and become obese with increasing age [38]. This is similar to the antagonism seen in Y2R between central and peripheral receptors; hypothalamic Y1R deletion reduces obesity and adipocytic Y1R acts to stimulate fat accretion.

While roles for adipocyte NPY signalling have been defined, surprisingly, recent studies have indicated actions of osteoblastic NPY to control adiposity and other aspects of homeostasis. A recent study examining mice with an early osteoblast lineage (Osterix Cre) deletion of p38 MAPK noted marked reductions in adipose tissue weights associated with increased energy expenditure [39••]. Serum insulin, osteocalcin and FGF23 were unaltered; however, circulating NPY was reduced by almost half. Importantly, osteoblasts were the only cells to display a reduction in NPY expression, and i.p. administration of NPY partially corrected the adipose tissue deficits in terms of tissue weight and adipocyte cell size [39••].

Another recent paper has identified osteoblastic Y1R signalling as responsible for regulating whole body glucose homeostasis and beta cell mass [28••]. An early lineage (3.6 col1α1 Cre) deletion of Y1R resulted in the increase in bone mass similar to late lineage deletion [29], however, with the addition of a glycaemic phenotype. Early lineage Y1R deficient mice had significantly reduced pancreatic islet number, beta cell mass and circulating insulin levels, associated with mildly reduced glucose tolerance. High fat feeding raised insulin levels in mutant and wild type mice, but the reduced level was maintained in Y1R deficient mice, leading to improved glucose tolerance in obese mice. The local nature of the glycaemic effect was confirmed following loss of the phonotype in marrow ablated mice reconstituted with wild type marrow. Moreover, the direct nature of the effect was

confirmed following loss of the stimulatory effect of osteoblast-conditioned media on the beta cell line MIN6, when conditioned media was applied from Y1R deficient cultures was used. One glycaemic pathway from bone has been identified, involving osteocalcin signalling [40]. However, gene expression for regulators the osteocalcin pathway were not altered and osteocalcin was not present in the conditioned media from cultured cells of either genotype, nor was this effect evident in late osteoblast/osteocyte Y1R null mice, indicating a novel regulator of glucose homeostasis. Thus, osteoblasts appear to be newly identified endocrine regulators of energy homeostatic processes involving energy expenditure and partitioning through glucose metabolism. This insight may have relevance to off target effects of bone active therapies, but also may provide novel therapeutic possibilities.

Conclusion

Neuropeptide Y has emerged as a complex and wide reaching regulatory factor not only of bone homeostasis but also from bone to other systems. Once considered predominantly a centrally active molecule regulating appetite and energy homeostasis. Studies in bone have revealed a number of unexpected actions for NPY. Firstly, its local expression in the osteoblastic lineage where it suppresses bone formation and does so in response to mechanical stimuli. The expression of Y1R across the lineage, enabling it to regulate progenitor commitment as well as bone formation activity, not only from local sources, but also from the circulation in conditions such as stress.

Secondly, the extra-skeletal effects are the most surprising and informative. Early indications suggest that osteoblastic NPY has a number of roles outside the skeleton. Signalling within early osteoblasts through the Y1R, NPY alters secretion of a factor that markedly alters beta cell mass and insulin secretion, thereby modulating glucose tolerance, most particularly in a setting of obesity, and may be of utility in type 2 diabetes. In addition, by secretion from osteoblasts, NPY regulates whole body energy utilisation and thereby adipose depot volumes. Thus, a reduction in osteoblastic NPY production would simultaneously increase bone mass, reduce adiposity and benefit glucose tolerance. Unlocking the potential of bone-centric factors involved in the NPY pathway may therefore provide therapeutic benefit in those with metabolic disturbances.

Compliance with Ethical Standards

Conflict of Interest The authors of this paper declare they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article contains no studies with human or animal subjects performed by the author.

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