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NPY regulation of bone remodelling

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ABSTRACT

Neuropeptide Y (NPY), a classic neuronal regulator of energy homeostasis, is now also known to be involved in the control of bone homeostasis. Of the five known Y receptors through which the NPY family of ligands signals, the Y1 and Y2 receptors have so far been implicated in the control of osteoblast activity and thus bone formation. Analysis of brain specific NPY overexpressing and Y receptor knockout models has revealed a powerful anabolic pathway likely involving hypothalamic Y2 receptors and osteoblastic Y1 receptors. Furthering our understanding of the mechanisms underlying the involvement of the NPY system in the control of bone could lead to the development of therapies to improve bone mass in patients with diseases such as osteoporosis.

Contents

1. Introduction

Bone is a complex and dynamic tissue with a mineralised extracellular matrix and the ability to adapt to its functional demands and repair itself. The functions of bone extend beyond mechanical support and include providing protection for vital organs and the bone marrow, providing a site for muscle attachment for locomotion and serving as a reservoir for ions, especially calcium and phosphate (Baron, 1999; Marks and Odgren, 2002; Rodan, 2003). These ions are released through remodelling, a process which is constantly occurring, firstly as part of bone growth, and then continuously throughout life via bone turnover. In a normal adult skeleton, bone remodelling is a tightly coupled process of bone resorption followed by bone formation (Baron, 1999). The balance between bone formation and resorption is essential for bone homeostasis. Disruption of this balance can diminish bone mass and micro-architectural integrity of bone tissue resulting in an increase in bone fragility and susceptibility to fracture, as evident so commonly in osteoporosis (Sambrook and Cooper, 2006). Osteoporotic fractures can cause significant pain, deformity, long-term debility and mortality making osteoporosis a serious health issue and a major economic burden on health-care systems worldwide (Johnell and Kanis, 2006). Although anti-resorptive therapies can

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be successful in reducing further bone loss and lowering the fracture risk by 30–50% (Meunier et al., 2004) there is a pressing need for the development of new, effective anabolic agents capable of rebuilding bone that has already been lost.

It is now known that humoral factors, as well as communication amongst cells of the bone environment, and between bone cells and the brain, are all important in regulating bone resorption and formation. Modern research techniques are enabling us to obtain new insights into the control of bone remodelling and the search for effective therapies to control bone mass in humans. Neuropeptide Y (NPY), a 36-amino acid neurotransmitter, is one of the most abundant neuropeptides and was first isolated from pig brain by Tatemoto et al. (1982). The classic role of NPY is as a major central regulator of food consumption and energy homeostasis. However, recently an important role for the NPY system in the regulation of bone metabolism has also been identified providing a potential target for the development of therapeutic treatments for osteoporosis.

2. Bone remodelling

Bone remodelling is remarkably precise, with bone resorption and formation tightly balanced in time, space and quantity (Zaidi, 2007). It is a coordinated process that occurs at approximately two million microscopic sites throughout the adult skeleton (Rodan, 2003). However, bone is only remodelled in discrete packets or bone remodelling units and does not occur at random (Mundy, 1999). Instead, mechanical effects on bone including changes in body weight may play an important role in coordinating bone remodelling (Harada and Rodan, 2003).

The major effector cells of bone turnover or remodelling are the bone-resorbing osteoclasts and the bone-forming osteoblasts. Osteoclasts are terminally differentiated, multinucleated cells derived from hematopoietic stem cells through the monocyte/macrophage lineage. Once differentiated, mononuclear osteoclasts on the bone surface fuse together to form multinucleated cells capable of migrating from one resorption site to another (Duong and Rodan, 2001). In contrast, bone-forming osteoblasts are derived from mesenchymal stem cells (MSCs), which also give rise to chondrocytes, adipocytes, fibroblasts and myocytes (Harada and Rodan, 2003). Each phenotypic pathway requires an exact program of gene expression yet some plasticity remains in that committed cells can sometimes trans-differentiate into cells of the other lineages (Zaidi, 2007). The production of the transcription factor, runt-related transcription factor 2 (Runx2; also known as cbfa-1) is an essential determinant of a fully differentiated osteoblast and is also necessary for the synthesis and secretion of bone matrix (Komori et al., 1997; Otto et al., 1997). Other transcription factors essential to osteoblastic differentiation include osterix which acts downstream of Runx2, and the homeobox-containing transcription factors msh homeobox homologue 2 (Msx2) and distal-less homeobox 5 (Dlx5) which are expressed in the early stages of osteoblast differentiation (Harada and Rodan, 2003).

Bone loss occurs when the processes of bone resorption and bone formation are not matched. A major cause of remodelling imbalance is oestrogen deficiency after menopause, which results in increased osteoclastogenesis and a subsequent loss of cancellous bone mass. Other causes include hyperparathyroidism, reduced mechanical loading and genetic predispositions (Sambrook and Cooper, 2006). Bone resorption occurs much faster than bone formation: it will take osteoblasts at least 3 months to rebuild an area of bone that has been broken down in 2–3 weeks (Harada and Rodan, 2003). Consequently, when bone resorption increases, bone formation lags even further behind. Thus, the need for effective anabolic agents capable of enhancing bone formation and rebuilding bone that has been lost.

3. Central control of bone

In addition to the brain's influence on bone through neuroendocrine pathways, recent evidence suggests that bone metabolism is also under the direct control of centrally-mediated neuronal pathways. Both sensory and autonomic nerve fibres innervate bone tissue (Gordeladze and Reseland, 2003). Furthermore, the presence of a number of neurotransmitters and neuropeptides in bone including adrenaline, noradrenaline, substance P (SP), calcitonin-gene related peptide (CGRP), vasoactive intestinal peptide (VIP) and NPY supports a direct signalling system between the brain and bone (Bjurholm, 1991; Tabarowski et al., 1996). Accumulating evidence suggests that these neuropeptides are directly involved in the regulation of bone remodelling. Indeed, receptors for many of these neuropeptides including CGRP, SP, and VIP have not only been found on bone cells but, additionally, the activity of bone cells can be altered *in vitro* by stimulation of these receptors (Lundberg and Lerner, 2002; Mori et al., 1999; Shih and Bernard, 1997).

Substantial progress has been made in the elucidation of neural control of bone metabolism following the finding that leptin can inhibit bone formation via signalling in the hypothalamus (Ducy et al., 2000). Since this time, considerable effort has been made to further characterise this relationship. Central leptin signalling within the hypothalamus has now been shown to have a direct neural connection to osteoblasts via sympathetic neurons, which alter osteoblast activity by modulation of β2 adrenergic receptors expressed on these cells (Takeda et al., 2002). Another interesting aspect of the bone phenotype in leptin-deficient *ob/ob* mice was the suggestion that leptin could be affecting bone resorption via central effects on the neuropeptide cocaine amphetamine regulated transcript (CART). CART mRNA expression is significantly decreased in *ob/ob* mice but can be restored by intraperitoneal treatment with leptin (Kristensen et al., 1998). Despite having a normal bone formation rate, CART knockout mice are osteoporotic due to an increase in bone resorption as evidenced by an increase in osteoclast number and surface as well as an increase in urinary elimination of deoxypyridinoline, a marker of osteoclast function (Elefteriou et al., 2005). They also showed an up regulation in receptor activator for nuclear factor (NF)-κB ligand (RANK-L) expression, which may be the means by which CART affects bone resorption. Conversely, elevated hypothalamic CART expression in melanocortin 4 receptor (Mc4r) deficient mice has been shown to be necessary for their low bone resorption (Ahn et al., 2006). However, *in vitro* osteoclast differentiation experiments suggest that the effect of CART is not bone cell autonomous (Elefteriou et al., 2005). Therefore, further work is required to ascertain exactly how CART is regulating bone resorption and whether that regulation directly involves CART neurons.

Other pathways implicated in the central control of bone include nitric oxide signalling (van't Hof et al., 2004), Neuromedin U (NMU) signalling (Sato et al., 2007), the cannabinoid system (Idris et al., 2005; Tam et al., 2006), and, most importantly, the NPY system. Peripheral nitric oxide signalling is known to have important regulatory effects on bone cell function. Furthermore, the demonstration that mice deficient in the neuronal isoform of nitric oxide synthase have a suppressed rate of bone turnover resulting in a high bone mass phenotype suggests that nitric oxide signalling may also regulate bone homeostasis via central relays (van't Hof et al., 2004). NMU, an anorexigenic neuropeptide, has been implicated as a central inhibitor of bone formation possibly via the modulation of leptin-mediated regulation of bone mass (Sato et al., 2007). The central effects of the cannabinoid system

on the regulation of bone are mediated via the cannabinoid type 1 (CB1) receptor which is primarily expressed within the central nervous system (Mackie, 2008). Although the bone phenotype induced by the lack of CB1 receptors in mice is dependent on strain background (Tam et al., 2006), a synthetic CB1 antagonist has been shown to prevent ovariectomy-induced bone loss *in vivo* (Idris et al., 2005). Furthermore, it has been shown that CB1 signalling may be important in mediating the bone response to traumatic injury by stimulating bone formation via the modulation of adrenergic signalling (Tam et al., 2008). The focus of this review, however, will be on the NPY system, where most progress has been made in the last years.

4. The NPY family

In addition to NPY, the NPY family also includes the endocrine hormones peptide YY (PYY) and pancreatic polypeptide (PP), all of which exert their effects through activation of G-protein coupled receptors termed the Y receptors. The NPY family members are structurally similar, all being 36-amino acid peptides with a carboxy-terminal amide and a common hairpin-like three-dimensional structure called the PP-fold (Gehlert, 2004). They have arisen through successive duplications of an ancestral gene (Cerda-Reverter and Larhammar, 2000) and still share a high degree of sequence homology (Tatemoto, 1982). Most mammals have identical NPY sequences making NPY one of the most evolutionary conserved peptides known (Gehlert, 2004; Larhammar et al., 1993).

Five Y receptors have so far been cloned in mammals and are termed the Y1, Y2, Y4, Y5 and y6 receptors. The y6 receptor has the lower case designation as, although it is functional in mice and rabbits (Gregor et al., 1996; Weinberg et al., 1996), it has accumulated mutations that lead to a truncated form of the receptor in most mammals including humans (Gregor et al., 1996; Matsumoto et al., 1996). Despite a high degree of sequence divergence within the Y receptor family, they all use similar signal transduction pathways and mediate their response through pertussis toxin-sensitive G_i or G_o proteins, inhibiting the accumulation of cyclic adenosine monophosphate (cAMP) (Blomqvist and Herzog, 1997). In addition, Y receptors can influence intracellular calcium concentrations by mobilising intracellular calcium stores and activating or blocking calcium channels (Gerald et al., 1995; Herzog et al., 1992; Motulsky and Michel, 1988). Potassium channels can also be either activated or inhibited by signalling through Y receptors (Xiong and Cheung, 1995). Furthermore, Y receptor signalling has been shown to stimulate mitogen-activated protein kinase (MAPK) pathways by inducing the phosphorylation of extracellularly regulated kinase (ERK) (Nakamura et al., 1995; Nie and Selbie, 1998). This effect has been shown to be dependent on phosphatidylinositol (PI)-3-kinase (Keffel et al., 1999) and also to involve intermediary protein kinase C (PKC) (Cho and Kim, 2004; Mannon and Mele, 2000).

The understanding of the roles that the individual Y receptors play in physiology and disease has been greatly enhanced by the use of transgenic and knockout mouse models. Specific functions can now even be pinpointed to an individual Y receptor in a particular location through the use of conditional knockout models, allowing a better understanding of the involvement of the NPY system in numerous processes.

5. NPY and bone

NPY has long been known to be important for vasoconstriction. Therefore, as NPY-immunoreactive fibres in bone tissue are commonly associated with blood vessels, their presence in bone tissue was long presumed to be solely for vasoregulation (Ahmed et al., 1993; Bjurholm et al., 1988b; Sisask et al., 1996). However, NPY-immunoreactive fibres have also been shown located around bone lining and marrow cells (Hill and Elde, 1991; Sisask et al., 1996) and, more recently, analysis of Y receptor knockout models has revealed a novel and important role for NPY signalling in the regulation of bone metabolism. Importantly, although Y receptors are co-expressed with leptin receptors on NPY neurons in the arcuate nucleus and have been shown to interact in mediating leptin's effects on energy homeostasis, this novel anabolic bone pathway appears to be distinct from that of leptin (Baldock et al., 2006; Baldock et al., 2005). A summary of the current knowledge of the major bone phenotypes found in Y receptor knockout models is shown in Table 1 with a more detailed description of the findings following below.

5.1. Y2 receptor involvement

The first *in vivo* indication for a role for NPY in the regulation of bone metabolism came from the observation that germline Y2 receptor knockout ($Y2^{-/-}$) mice have a twofold increase in cancellous bone volume (Baldock et al., 2002). This effect was attributed to hypothalamic Y2 receptors as selective deletion of hypothalamic Y2 receptors in adult mice resulted in an identical increase in cancellous bone volume 5 weeks after initiating the deletion (Baldock et al., 2002). The increase in bone volume was due to a stimulation of osteoblast activity and an increased rate of bone formation with no indication of changes in bone resorption (Baldock et al., 2002). Interestingly, even when deprived of neural input, cultured bone marrow stromal cells (BMSCs) isolated from $Y2^{-/-}$ mice displayed increased mineralisation *in vitro* (Lundberg et al., 2007). Analysis of mesenchymal progenitor cells within the bone tissue of these mice revealed an increase in the number of osteoprogenitor cells in $Y2^{-/-}$ compared to wildtype mice (Lundberg et al., 2007). However, $Y2^{-/-}$ osteoprogenitor cells were comparable to wildtype cells in their colony-forming ability *in vitro*. This finding suggests that an increase in the size of the osteoprogenitor population may be responsible for the increase in bone formation in the absence of the Y2 receptor both *in vivo* as well as *in vitro*.

A major cause of remodelling imbalance is oestrogen deficiency after menopause, which results in increased osteoclastogenesis and a subsequent loss of cancellous bone mass. Importantly, the osteoblastic bone formation response mediated by hypothalamic

Table 1
Bone phenotypes observed in Y receptor knockout mice.

	Y1 (G)	Y1 (Hyp)	Y2 (G)	Y2 (Hyp)	Y4	Y2Y4	Y1Y2
Cancellous bone volume	⇑	⇔	⇑	⇑	⇔	⇑⇑	⇑
Cortical bone mass	⇑	⇔	⇑	⇑	⇔	⇓	⇑
Osteoblast activity	⇑	⇔	⇑	⇑	⇔	⇑	⇑
Resorption (osteoclast surface)	⇑	⇔	⇔	⇔	⇔	⇑	⇔
Protection against ovariectomy-induced osteopenia	⇑	–	⇑	⇑	–	–	–
Protection against orchidectomy-induced osteopenia	⇔	–	⇑	⇑	–	–	–
Bone marrow stromal cell mineralisation *in vitro*	–	–	⇑	–	–	–	–
Osteoprogenitor cell number	–	–	⇑	–	–	–	–

G = germline knockout, Hyp = hypothalamus-specific knockout; ⇑ = increased, ⇔ = unaltered, ⇓ = decreased.

Y2 receptors has been shown to be independent of sex steroid signalling (Allison et al., 2006). In both male and female mice, hypothalamus-specific deletion of Y2 receptors protected against gonadectomy-induced bone loss (Allison et al., 2006). This effect was attributable to enhanced bone formation and was even evident despite coincident increases in bone resorption. These results highlight the potential of this promising new avenue for the treatment of osteoporosis.

5.2. Y4 receptor involvement

Although bone mass was unaltered by germline deletion of Y4 receptors, a synergistic relationship exists between Y2 and Y4 receptor pathways in the regulation of bone metabolism (Sainsbury et al., 2003). Deletion of both Y2 and Y4 receptors increased cancellous bone volume in male mice to an even greater extent than that observed in $Y2^{-/-}$ mice (Sainsbury et al., 2003). This increase in Y2, Y4 double knockout ($Y2^{-/-}Y4^{-/-}$) mice was associated with a general increase in bone turnover. However, they showed significant reductions in cortical bone mass that were not evident in single receptor knockout mice (Sainsbury et al., 2003). The synergistic interaction between Y2 and Y4 receptors also had implications for obesity as the male $Y2^{-/-}Y4^{-/-}$ mice displayed a lean phenotype despite hyperphagia with reduced body weight, white adipose tissue mass, leptinemia and insulinemia (Sainsbury et al., 2003). In fact, the reduced leptin levels seen in these mice are the probable cause for the additive effects on bone metabolism observed above that of the single $Y2^{-/-}$ mice. Indeed, female $Y2^{-/-}Y4^{-/-}$ mice, which do not have a reduction in leptin levels, have no additional increase in cancellous bone volume (Baldock et al., 2005).

5.3. Y1 receptor involvement

More recently, an investigation of the bone phenotype of germline Y1 knockout ($Y1^{-/-}$) mice revealed a similar high bone mass phenotype to $Y2^{-/-}$ mice due to a generalised increase in bone formation on both cancellous and cortical surfaces (Baldock et al., 2007). Interestingly, bone resorption was also altered in $Y1^{-/-}$ mice as indicated by an increase in osteoclast surface (Baldock et al., 2007). However, in contrast to Y2 receptors, specific deletion of Y1 receptors from the hypothalamus of adult mice had no effect on bone volume or bone cell activity suggesting that the effects of Y1 receptors on bone metabolism are mediated, at least in part, by non-hypothalamic receptors (Baldock et al., 2007). It is possible that Y1 receptors in regions of the brain other than the hypothalamus may be involved in mediating the actions of NPY on bone. However, Y1 receptor expression has been detected by *in situ* hybridisation in osteoblasts on endocortical and trabecular bone surfaces within femur bone tissue (Lundberg et al., 2007). This raises the possibility that there could be a direct effect of NPY on osteoblastic cells acting via Y1 receptors. In support of this possibility, NPY-immunoreactive fibres have been identified within bone marrow and around bone lining cells (Ahmed et al., 1993; Bjurholm et al., 1988b; Hill and Elde, 1991; Sisask et al., 1996). Peripherally, NPY is generally co-localised with noradrenaline in sympathetic nerve terminals (Ekblad et al., 1984; Lundberg et al., 1982) and is released together with noradrenaline during sympathetic nerve stimulation (Lundberg et al., 1985). Sympathetic signalling has been shown to regulate bone formation and resorption via β2 adrenergic receptors present on osteoblasts (Elefteriou et al., 2005; Takeda et al., 2002) and thus may also regulate bone formation via NPY signalling on osteoblastic Y1 receptors.

As no additive effects on bone metabolism were observed in mice lacking both the Y1 and the Y2 receptors (Baldock et al., 2007), we suggest that Y1 and Y2 receptors may act at different points along a common signalling pathway with respect to the control of bone formation as shown in Fig. 1. In support of this hypothesis, Y1 receptor expression was virtually abolished in BMSC cultures from $Y2^{-/-}$ mice suggesting that reduced Y1 receptor signalling in the bone microenvironment is important for the high bone mass phenotype of the $Y2^{-/-}$ model (Lundberg et al., 2007). Furthermore, a recent study has shown that, consistent with these findings, osteoblast phenotype markers in cultured osteoprogenitor cells were enhanced by NPY treatment most likely due to a down-regulation of Y1 receptor expression (Teixeira et al., 2009), supporting a direct role for Y1 receptor signalling in the control of osteoblast activity.

However, there may also be additional, discrete effects of the individual Y receptors to take into consideration as, for example, the $Y1^{-/-}$ mice are the only knockout mice thus far shown to have an alteration in osteoclast surface. In addition, the hypothalamic Y2 receptor associated anabolic response has been shown to persist in the absence of sex hormones in both male and female mice (Allison et al., 2006). In contrast, gonadectomy studies suggest that the Y1 mediated bone anabolic response may be dependent upon the presence of intact androgen signalling. In female mice, Y1 receptor deficiency partially protected against ovariectomy-induced osteopenia due to preservation of the Y1 receptor mediated elevation in mineral apposition rate (Allison et al., 2008). However, in male mice, the increase in mineral apposition rate due to lack of

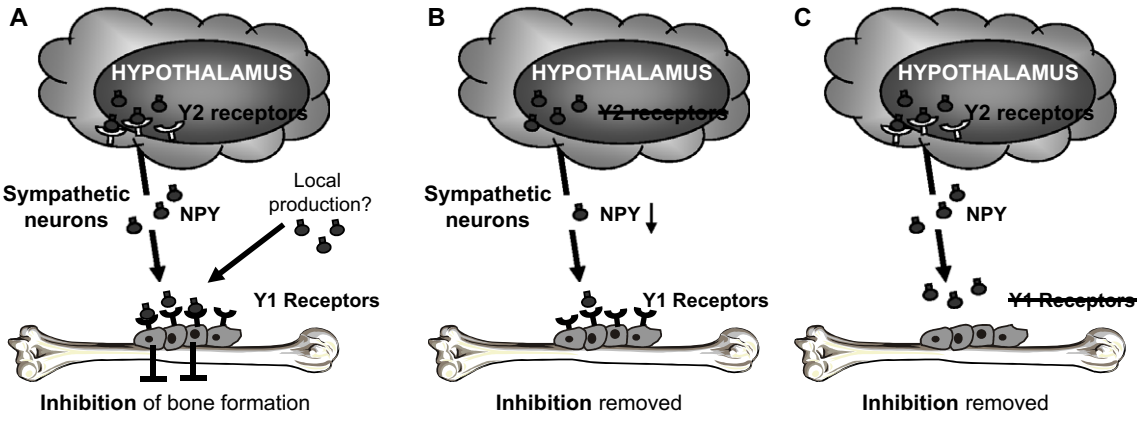

Fig. 1. Proposed pathways for the regulation of bone metabolism via central Y2 receptors and osteoblastic Y1 receptors. Under normal conditions, hypothalamic Y2 receptors mediate NPY signalling though sympathetic neurons to inhibit bone formation via osteoblastic Y1 receptors or beta-adrenergic pathways (A). This inhibition of bone formation can be removed by either the deletion of hypothalamic Y2 receptors (B) thereby reducing NPY levels and sympathetic neuron activity or by removal of Y1 receptors in bone tissue again blocking NPY mediated down-regulation of osteoblast activity (C).

Y1 receptor signalling was abolished following gonadectomy (Allison et al., 2008). Nevertheless, Y1 receptors act powerfully to inhibit bone production by non-hypothalamic pathways, with potentially direct effects on bone tissue through a single pathway with hypothalamic Y2 receptors.

5.4. Y5 and y6 receptor involvement

To date, little is known about the involvement of Y5 and y6 receptor signalling in the control of bone metabolism. The gene for the Y5 receptor most likely evolved as a gene duplication event from the Y1 receptor gene and overlaps with the Y1 receptor gene on human chromosome 4q31. The two genes are transcribed in opposite directions but from a common promotor region suggesting that there may be coordinated transcriptional regulation (Herzog et al., 1997). Therefore, as the Y1 receptor has been shown to play an important role in bone formation, it is conceivable that the Y5 receptor may also play a role. Finally, although the y6 receptor is truncated and non-functional in humans, it is fully functional in mice and, thus, it is important to determine whether or not it also plays a role in the control of bone mass in these animals.

6. The NPY ligands and bone

Studies using Y receptor knockout mice have shown that both Y1 and Y2, but not Y4 receptors are important for the control of bone metabolism. Consistent with this, PP, which preferentially binds to Y4 receptors, is the only NPY ligand that does not appear to have a major influence on bone mass *in vivo*. This has been demonstrated using PP transgenic mice (Sainsbury et al., 2003) and independently using PP knockout mice (Wortley et al., 2007). Early *in vitro* studies suggested a possible role for NPY in the regulation of osteoblast activity by the demonstration that NPY treatment inhibited the cAMP response to PTH and noradrenaline in osteoblastic cell lines (Bjurholm et al., 1988a; Bjurholm et al., 1992). Recently, *in vitro* and *in vivo* studies have sought to elucidate the role of NPY and PYY in the regulation of bone metabolism.

6.1. NPY

Consistent with the findings from Y1 and Y2 receptor knockout mice, intracerebroventricular infusion of NPY into wildtype mice led to a significant reduction in cancellous bone volume (Ducy et al., 2000). Similarly, increased NPY expression confined to the hypothalamus by viral vector-mediated delivery led to a reduction in osteoblast activity (Allison et al., 2008; Baldock et al., 2005). These findings confirm a central, hypothalamic role for NPY in the regulation of bone formation. However, NPY has a wide distribution in both the central and peripheral nervous systems and the involvement of NPY derived from non-hypothalamic sources in the control of osteoblast activity remains to be determined.

As well as effects on bone formation, there is some evidence that the NPY system may also be able to regulate bone resorption. Despite no change in osteoclast number, germline $Y1^{-/-}$ mice exhibit an increase in osteoclast surface (Baldock et al., 2007). NPY has also been shown to selectively inhibit isoprenaline and parathyroid hormone (PTH)-induced osteoclastogenesis in mouse bone marrow cells via the Y1 receptor by blocking the production of cAMP and RANK-L (Amano et al., 2007). Isoprenaline acts via β-adrenergic receptors on marrow stromal cells stimulating them to produce RANK-L, which in turn acts to induce osteoclast differentiation (Ishizuka et al., 2005). The stimulation of RANK-L production by both isoprenaline and PTH is mediated via the cAMP/protein kinase A (PKA) pathway (Amano et al., 2007). In that study, NPY alone did not affect the formation of osteoclasts nor did it affect osteoclastogenesis induced by the addition of either $1,25(OH)_2$ vitamin D, which utilises a different signal transduction pathway, or soluble RANK-L (Amano et al., 2007). 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 over a short time-course directly inhibited RANK-L expression by osteoblastic cells (Teixeira et al., 2009). In addition, it showed that NPY treatment on BMSCs resulted in a transient increase in osteoprotegerin (OPG) expression, the decoy receptor that inhibits RANK-L activation of osteoclastogenesis (Teixeira et al., 2009). Therefore, the effects of NPY on the regulation of bone resorption appear to be at least partially mediated by alterations in osteoblast behaviour.

6.2. PYY

PYY is one of many gastrointestinal hormones including ghrelin, amylin and glucagon-like peptide 2 which have now been shown to influence bone metabolism as well as regulating nutrient intake (Cornish et al., 1998; Henriksen et al., 2003; van der Velde et al., 2008). However, there is some conflict in the literature regarding the exact role that PYY plays in the regulation of bone mass. On one side, PYY levels have been shown to be elevated in adolescents with anorexia nervosa, a psychiatric illness that results in significant bone loss (Misra et al., 2006). A recent study has found a correlation between PYY levels in women with anorexia nervosa and BMD, with elevated PYY levels being strongly associated with diminished BMD, particularly at the spine (Utz et al., 2008). This data suggests that high levels of PYY may contribute to bone loss, and is consistent with the studies looking at Y receptor knockout mice. On the other side, an analysis of a PYY knockout ($PYY^{-/-}$) mouse line found that a lack of PYY resulted in an osteopenic phenotype with reductions in vertebral cancellous bone mass and bone strength (Wortley et al., 2007). Their osteopenic phenotype was maintained on a high fat diet despite greater body weight gain than wildtype controls (Wortley et al., 2007). In addition, the loss in BMD following ovariectomy was exaggerated in the $PYY^{-/-}$ mice suggesting that they are more sensitive to oestrogen-induced bone loss (Wortley et al., 2007). However, the investigation was restricted to bone volume and strength parameters with no analysis of either the rate of bone formation and osteoblastic activity or the degree of bone resorption and osteoclastic activity. Therefore it is difficult to correlate these findings with the studies on Y receptor knockout mice at this stage. Nonetheless, it is clear that PYY does have a role in maintaining bone homeostasis.

7. Conclusion

In summary, it is now clearly established that, acting centrally, the NPY system plays a role in the regulation of bone mass. Whilst Y1 and Y2 receptors, but not Y4 receptors, have been shown to be independently involved in the control of bone formation, it remains to be determined whether other Y receptors are also involved. Hypothalamic Y2, but not Y1, receptor signalling has been shown to be able to modulate osteoblast activity, possibly via direct sympathetic neuronal modulation of osteoblastic Y1 receptor signalling. However, exactly how the NPY system acts on the cells in the bone is not yet understood and much work remains to be done to elucidate the specific roles of hypothalamic signalling pathways and the interactions between them in terms of the regulation of bone.

The hypothalamus is part of a complex circuit modulating both body composition and bone turnover. Through the actions of leptin, fat tissue can signal via the hypothalamus to regulate bone turnover and it has also been revealed that the skeleton can act

as an endocrine organ and directly regulate energy metabolism. Osteocalcin, produced by osteoblasts, has been shown to enhance insulin secretion by pancreatic islet cells and to enhance insulin sensitivity by increasing adiponectin expression in adipocytes (Ferron et al., 2008; Lee et al., 2007). NPY is well known to be a key central mediator of energy metabolism however it has also been shown to act directly in the periphery on fat tissue to increase adiposity via peripheral Y1 (Yang et al., 2008) and Y2 (Kuo et al., 2007) receptors. Therefore it is conceivable that Y receptor antagonism could be used to both improve bone mass and reduce adiposity. It remains to be determined whether the NPY system also plays a role in the direct signalling between fat and bone.

Understanding the role of the NPY system in the regulation of bone mass could have powerful therapeutic implications for osteoporosis as well as skeletal disorders caused by diseases such as anorexia nervosa and depression. To avoid the inherent difficulties associated with pharmaceuticals acting in the central nervous system, a more promising therapeutic approach to alter bone formation by targeting the NPY system is through the inactivation of osteoblastic Y1 receptors. However, given the wide distribution of the Y1 receptor both in the central nervous system and in the periphery, further research is needed to develop pharmacological strategies to specifically target Y1 receptor antagonists to bone tissue. Identifying the downstream mechanisms through which the NPY system activates bone cells may also enable the development of effective therapies to modulate bone remodelling whilst reducing their effect on other homeostatic processes controlled by the hypothalamus.

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