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Regulation of bone remodeling by the central and peripheral nervous system

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Abstract

The homeostatic nature of bone remodeling has become a notion further supported lately by the demonstration that neuropeptides and their receptors regulate osteoblast and osteoclast function in vivo. Following initial studies reporting the presence of nerves and nerve-derived products within the bone micro-environment and the expression of receptors for these neuropeptides in bone cells, new experimental and mechanistic evidence based on in vivo murine genetic and pharmacologic models recently demonstrated that inputs from the central and peripheral nervous system feed into the already complex regulatory machinery controlling bone remodeling. The function of a number of “osteo-neuromediators” has been characterized, including norepinephrine and the beta 2-adrenergic receptor, Neuropeptide Y and the Y1 and Y2 receptors, endocannabinoids and the CB1 and CB2 receptors, as well as dopamine, serotonin and their receptors and transporters, Calcitonin gene-related peptide, and neuronal NOS. This new body of evidence suggests that neurons in the central nervous system integrate clues from the internal and external milieus, such as energy homeostasis, glycemia or reproductive signals, with the regulation of bone remodeling. The next major tasks in this new area of bone biology will be to understand, at the molecular level, the mechanisms by which common central neural systems regulate and integrate these major physiological functions, the relative importance of the central and peripheral actions of neuropeptides present in both compartments and their relationship, and how bone cells signal back to central centers, because the definition of a homeostatic function implies the existence of feedback signals. Together, these findings shed a new light on the complexity of the mechanisms regulating bone remodeling and uncovered new potential therapeutic strategies for the design of bone anabolic treatments. This review summarizes the latest advances in this area, focusing on investigations based on in vivo animal studies.

Keywords

Bone; osteoblast; osteoclast; neuron; sympathetic nervous system; hypothalamus; leptin; neuropeptide; adrenergic receptor; neuropeptide Y; endocannabinoid

The central nervous system restrains bone formation

It is the association between body weight and bone density as well as the association between loss of gonadal function and osteoporosis that initially pointed to the putative existence of a common factor regulating body weight, reproduction and bone mass. Based on the knowledge that the adipocyte-derived hormone leptin regulates major physiological functions, including body weight and reproduction, via the hypothalamus and its receptor ObRb, it was

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hypothesized by Dr Karsenty's group that leptin could be one of such factor. Importantly, this hypothesis implied that bone remodeling could be under the control of central center(s), in agreement with its homeostatic nature, and led to the original concept that the central nervous system (CNS) could regulate bone remodeling.

The finding that leptin-deficient mice (the *ob/ob* mice) have a high bone mass in spite of their hypogonadism and hypercorticosteronemia was the first evidence supporting this concept, and suggested that leptin is a potent inhibitor of bone formation in vivo, possibly acting via a central relay. However, the existence of multiple metabolic abnormalities in this mouse model made it difficult, if not impossible experimentally, to dissect the exact mechanism whereby bone mass was increased [1-3]. The similar high bone mass phenotype observed in several lipodystrophic mouse models, characterized by absent or low serum leptin level and normal body weight, however further supported the predominant inhibitory effect of leptin on bone formation [4,5].

One of the most convincing data demonstrating the existence of a central locus for the regulation of bone formation at that point was the rescue of the high bone mass of the *ob/ob* mice by intra-cerebro-ventricular (icv) infusion of leptin. Indeed, providing minute amounts of leptin in the hypothalamic region of these mice via micro-osmotic pumps and without detectable leakage in the blood stream for 28 days resulted in a significant decrease in bone mass and bone formation, to a level similar to their wild type (WT) *ob/ob* littermates [6]. This result was further strengthened by the observation that a) destruction of ObRb-positive hypothalamic neurons in WT mice by gold thio-glucose recapitulated the high bone mass phenotype of the leptin-deficient mice and b) that these lesioned mice were no longer responsive to the anti-osteogenic effect of icv leptin, [7]. GTG-lesioned mice were however still responsive to leptin's anorexigenic effect, in agreement with the integrity of arcuate neurons in this model, indicating that leptin-induced bone loss was not due to starvation. Leptin's use of a central relay for regulating bone mass was then demonstrated in rats and sheep by similar means [6,8,9].

Leptin inhibits the expression of the orexigenic peptide Neuropeptide Y (NPY) in arcuate neurons, however icv infusion of NPY to WT mice inhibited bone formation in a similar manner to leptin, suggesting that NPY's induction in *ob/ob* mice does not contribute to their high bone mass phenotype [6]. NPY acts through at least 5 Y-receptors (Y1, Y2, Y4, Y5 and Y6). All of these receptors are expressed in the hypothalamus and several respond to other ligands, including peptide YY and pancreatic polypeptide (PP), which makes the mechanistic dissection of NPY-ergic signaling difficult. A high percentage of arcuate NPY-positive neurons however co-express the Y2 receptor, whose germline deletion leads to increased osteoblast activity and a high bone mass phenotype, suggesting that central Y2 receptor inhibits bone formation [10]. Confirming the existence of a tonic inhibition of bone formation exerted by hypothalamic neurons, conditional hypothalamic Y2 receptor deletion recapitulated the bone phenotype of germline mutant mice. In contrast to Y2 receptor-deficient mice, Y4 receptor deletion and overexpression of the Y4 receptor ligand PP in transgenic mice did not affect bone mass [11].

Neuromedin U (NMU) is another neuropeptide expressed in hypothalamic neurons and in the small intestine, whose expression is regulated by leptin centrally. Its functions include the regulation of appetite and sympathetic activation [12], as demonstrated by the obesity of NMU-deficient mice [13] but also the regulation of bone mass. Mice deficient for NMU indeed display a high bone mass phenotype caused by an increase in bone formation [14], and several observations concurred to demonstrate that NMU acts via a central relay: 1) the receptors for NMU, NMU1R and NMU2R, are not detectable in bone, 2) NMU treatment has no direct effect on osteoblast differentiation in vitro, 3) NMU2R is expressed in hypothalamic neurons and most importantly 4) the high bone mass of NMU-deficient mice can be rescued by NMU icv

infusion. The fact that NMU icv infusion could decrease the high bone mass of leptin-deficient mice indicated that NMU acts downstream of leptin to regulate bone formation. Most interestingly, NMU-deficient mice were resistant to the anti-osteogenic effect of leptin and beta-adrenergic receptor agonists. Furthermore, osteoblast number paradoxically increased in NMU-deficient mice treated by leptin icv, as observed in mice deficient for several circadian clock genes, suggesting that NMU regulates circadian gene function in osteoblasts (see below). In support of this hypothesis, expression of *Per* genes was arrhythmic and down-regulated in NMU-deficient bones compared to WT bones. These findings, all based on mutant animal models and in vivo studies, thus identified the Y2-receptor and NMUR2 as two major central neuronal system, both regulated by leptin, inhibiting bone formation (Figure 1).

The sympathetic nervous system links leptin-responsive hypothalamic neurons to osteoblasts

How can neurons located in the hypothalamus regulate the activity of osteoblasts residing in the bone microenvironment? Obviously, two main pathways come to mind: a humoral and/or a neuronal one. The existence of a humoral pathway was partly ruled out by parabiosis experiments performed between *ob/ob* mouse pairs [7]. In this experimental system, the blood circulation of two *ob/ob* mice was linked and one mouse of each pair received either PBS or leptin icv. It was found that only in the mouse receiving leptin icv, not the contra-lateral partner, lost bone, suggesting that the link between leptin-responsive hypothalamic neurons and osteoblasts was neuronal rather than humoral. A number of additional observations strengthened this conclusion: first, *ob/ob* mice have a high bone mass phenotype that is accompanied by a low sympathetic tone, which may contribute to it [6]; second, electrical stimulation of ventromedial hypothalamic (VMH) neurons [15] and leptin injection in the VMH area results in enhanced sympathetic tone [16]; additionally, bones are innervated, as evidenced by immunological reactivity to various neuropeptides ([17] for review), and linked by nerves to the CNS as evidenced by retrograde neuronal labeling [18]. Finally, primary osteoblasts express a functional beta2-adrenergic receptor (β 2AR) but none of the other post-synaptic adrenergic receptors [7], suggesting that osteoblasts are receptive to sympathetic tone in vivo.

The availability of various genetic mouse models with autonomic dysfunctions as well as the use of pharmacological approaches have been instrumental to demonstrate the existence of a neuronal pathway between hypothalamic neurons and osteoblasts in vivo. Lack of *Dopamine beta-hydroxylase* (*Dbh*), the enzyme generating norepinephrine (NE), induced a late onset increase in bone mass [7]. In agreement with this result, mice and rats treated with the non-selective beta-adrenergic blocker propranolol exhibited an increased bone mass, whereas mice treated with the non-selective beta-agonist isoproterenol or the β 2AR selective agonists clenbuterol or salbutamol exhibited a low bone mass [7,19]. Together, these results demonstrated that the sympathetic nervous system controls bone formation via β AR in osteoblasts in vivo. The presence of multiple types of nerves within the bone microenvironment and periosteum however suggested that additional neuropeptides and receptors might be involved in the regulation of bone remodeling. Similarly, the expression of neuropeptide receptors in other cells than osteoblasts within the bone microenvironment suggested that neuronal signaling may regulate bone remodeling indirectly, via cell lineages such as osteoclasts, adipocytes or immune cells. The generation of conditional cell-specific knock-out mice for these receptors, including the β 2AR, will be critical to address these questions.

The growing family of peripheral osteo-neuromediators

The expression of the β 2AR on osteoblasts and the effect of β AR agonists and antagonists on bone formation in vivo strongly suggested that sympathetic signaling targeted β AR on

osteoblasts (57). Supporting these results, genetic disruption of the β 2AR gene (*Adrb2*), (but not *Adrb1*), as well as lack of adenylyl cyclase 5, a downstream mediator of β 2AR signaling, both led to a high bone mass phenotype [20,21]. Importantly, this high bone mass was observed in absence of endocrine and metabolic abnormalities, as opposed to what was observed in the *ob/ob* or *Dbh*^{-/-} mice. These results thus demonstrated that sympathetic signaling inhibits bone formation via β 2AR on osteoblasts, and that the high bone mass of the *ob/ob* mice is at least in part caused by their low sympathetic tone. Furthermore, the fact that leptin icv infusion failed to decrease bone mass in β 2AR-deficient mice demonstrated not only that the sympathetic nervous system (SNS), via β 2AR, mediates leptin inhibition of bone formation, but also that there is no other mediator of this function. The mechanism whereby this anti-osteogenic function of the SNS occurs does not seem to involve osteoblast apoptosis but rather an inhibition of osteoblast proliferation and function, since bone formation rate, osteoblast number and mineralized surfaces were decreased in isoproterenol-treated mice and increased in β 2AR-deficient and propranolol-treated mice [7,20]. Further studies demonstrated that peripheral circadian genes that make up the molecular clock, including *Per* and *Cry* genes, mediate the effect of the SNS on osteoblast proliferation [22]. Although originally motivated by the predominant role of central circadian genes on multiple homeostatic functions (potentially including the regulation of bone remodeling), these studies revealed that osteoblasts express peripheral subordinate circadian genes that mediate leptin-dependent sympathetic inhibition of bone formation, by suppressing the expression of *G1 cyclins* and thereby proliferation. The studies also revealed that leptin-dependent sympathetic signaling exerts a countervailing stimulatory effect on osteoblast proliferation through the AP-1 family of transcription factors [22], possibly providing a mechanism to restrict proliferative activity to specific times of the day (Figure 1). The involvement of circadian genes in the regulation of bone remodeling is in agreement with the known daily variation in bone marrow cell proliferation, collagen synthesis and turnover markers [23,24].

Recent independent studies indicated that endocannabinoids, such as 2-arachidonoylglycerol (2-AG), regulate of bone homeostasis by modulating adrenergic signaling. Endocannabinoids signal via the seven-transmembrane domain CB1 and CB2 receptors, are highly expressed in the CNS, and their production is promoted by Traumatic Brain Injury (TBI) [25-27]. CB1 is expressed in the CNS and the peripheral nervous system (PNS) and accounts for most of the CNS actions of cannabinoid drugs and endocannabinoids. Two lines of CB1-deficient mice have been generated on different mouse genetic backgrounds, with different gene deletion strategies. Mice on a C57BL6 background displayed a low bone mass phenotype caused by a decrease in bone formation [28], whereas mice on a CD1 background displayed the opposite phenotype [29]. Although the reason for these apparent opposite phenotypes is unresolved, the fact that CB1 signaling inhibits NE release by sympathetic neurons [30,31] suggested that the low bone mass of CB1-deficient mice on a C57BL6 background may at least in part be due to increased NE signaling, caused by the absence of CB1 on pre-synaptic nerve terminals. This hypothesis was verified by the increase in 2-AG and decrease in NE level measured in peripheral bone formation sites induced by TBI [32]. This effect was blunted by CB1 deficiency and mimicked by 2-AG administration in WT mice. These studies strongly suggest that activation of CB1 by 2-AG regulates sympathetic signaling at the pre-synaptic level in bone nerve endings by reducing NE release and inhibiting the anti-osteogenic function of the SNS (Figure 1).

On the other hand, CB2 is mainly expressed in peripheral tissues, including osteoblasts, osteocytes and osteoclasts. Mice lacking CB2 display an accelerated age-related bone loss caused by a high turnover state. These results are supported by human genetic association studies linking *CNR2* and reduced bone mass in women [33,34]. In vitro pharmacological experiments have demonstrated a direct activation of CB2 in osteoclasts. The fact that CB2

agonists, that are not psycho-active, attenuate ovariectomy-induced bone loss in mice suggests that these compounds might be adapted for the treatment of low bone mass diseases [35].

Data regarding NPY-ergic inhibition of bone formation pointed to a mechanism by which neural Y2 receptor signaling inhibits bone formation by reducing the pool of bone mesenchymal progenitors, which may be mediated by the Y1 receptor and NPY. Indeed, the Y2 receptor was not detected in osteoblast progenitors, but the pool of osteoblast progenitors was increased in the bone marrow from Y2 receptor-deficient mice compared to WT littermates, leading to greater bone nodule formation in vitro and likely the cause of the increase in bone mass in vivo [36]. The presence of NPY within the bone micro-environment and the various effects of NPY on osteoblastic cells in vitro suggested the existence of Y receptors on these cells [37,38]. The Y1 receptor, but not Y2, was found highly expressed in bone marrow stromal cells and most importantly its expression was down-regulated in Y2 receptor-deficient mice. Together with the increase in bone mineral density observed in Y1 receptor-deficient mice, the absence of a bone phenotype in mice lacking the Y1 receptor specifically in the hypothalamus, and the reduction in bone marrow cell number following in vitro treatment by NPY, these observations led to the conclusion that central Y2 receptor signaling down-regulates peripheral Y1 receptor expression or signaling in bone marrow stromal cells via a NPY-ergic sympathetic neuronal pathway (Figure 1).

Do hypothalamic neurons regulate bone resorption?

A closer look at the β 2AR-deficient mice revealed a defect of osteoclastogenesis, suggesting that the other arm of bone remodeling, i.e. bone resorption, is also regulated by the SNS and possibly hypothalamic centers. Although osteoclasts express β 2AR [39], the in vivo effect of the SNS on osteoclastogenesis was demonstrated to be mostly indirect and mediated by osteoblasts, via β 2AR and stimulation of the expression of the main osteoclast-differentiating factor, Receptor Activator of NF- κ B Ligand (RANKL) [20]. In these studies, a crucial determinant of osteoblast differentiation, namely the transcription factor ATF4 [40], was identified as a transcriptional target of β 2AR signaling in osteoblasts and as a necessary factor for the β 2AR-mediated increase in *Rankl* expression [20]. ATF4 is indeed phosphorylated by PKA following β 2AR stimulation by NE or isoproterenol, and directly binds to the *Rankl* promoter to activate *Rankl* gene transcription. Accordingly, in an osteoblast/osteoclast co-culture assay, isoproterenol treatment of WT osteoblasts stimulated the differentiation of osteoclast precursors isolated from both WT and *Adrb2*-deficient mice. This effect was blocked when *Adrb2*-deficient osteoblasts were used, indicating that the main effect of the SNS on osteoclastogenesis requires osteoblasts and the β 2AR. In vivo, leptin icv infusion did not rescue the bone resorption defect of the β 2AR-deficient mice, indicating that leptin regulates osteoclastogenesis via the SNS and β 2AR [20].

Another observation from the β 2AR-deficient mice led to the identification of an additional central pathway regulating bone resorption. Absence of leptin in *ob/ob* mice increases osteoblast proliferation and function and thus bone formation, but also leads to an increase in osteoclast number, which was assumed to be secondary to the hypogonadism caused by the absence of this hormone. When β 2AR-deficient mice were subjected to gonadectomy, it was expected that these mice should “phenocopy” the *ob/ob* mice in terms of bone remodeling because they lack both sympathetic tone and gonadal function. This procedure that increases osteoclast number and decreases bone mass in WT mice did not have this effect in β 2AR-deficient mice. This indicated that gonadal failure-induced bone loss is blunted by the absence of sympathetic responsiveness and that the increase in bone resorption observed in the *ob/ob* mice is not only due to their hypogonadism, otherwise β 2AR-deficient mice would have a similar increase in bone resorption parameters. The *ob/ob* mice can be distinguished from β 2AR-deficient mice by their obesity and by a decrease in hypothalamic *Cart* expression,

pointing to CART as a potential CNS candidate that might regulate bone resorption. CART is a neuropeptide broadly expressed in the CNS, including hypothalamic neurons, as well as in peripheral tissues such as the pancreas [41]. Supporting a crucial role of CART in the regulation of bone resorption, animal models characterized by absent, low or high hypothalamic *Cart* expression showed significant bone phenotypes: eugonadal *Cart*-deficient mice displayed a low bone mass phenotype caused by increased osteoclastogenesis and bone resorption [20], low *Cart* expression in *ob/ob* mice accompanied the increased bone resorption observed in these mice, whereas increased hypothalamic *Cart* expression in obese and hyper-leptinemic mice deficient for melanocortin receptor 4 (*Mcr4*) correlated with their low bone resorption and high bone mass [20]. Furthermore, lack of one copy of *Cart* in *Mc4r*-deficient mice rescued their resorption phenotype, indicating that *Cart* increased hypothalamic level in *Mc4r*-deficient mice causes their high bone mass phenotype [42]. Importantly, this CART-mediated regulatory loop of bone resorption may be conserved in humans as individuals lacking *MC4R* have increased CART serum levels and increased bone mineral density (BMD), associated with decreased bone resorption [20,42,43]. The molecular mode of action of CART on bone resorption remains to be defined and may involve the SNS or an independent neuroendocrine pathway, but ultimately involves RANKL since *Cart*-deficient bone expressed a higher level of *Rankl* than WT bones (Figure 1) [20]. In conclusion, osteoclastogenesis and bone resorption appear to be under the control of neural mediators like bone formation, but in each case identified so far is dependent on osteoblasts and RANKL expression.

Correlation with human diseases and treatments

Several clinical observations support the existence of a central and neuronal control of bone formation in humans. As mentioned above, the lower risk of fracture and increased BMD in obese patients, as well as the bone loss that invariably follows gonadal deficiency, were the first clues suggesting the existence of a common link between the regulation of reproduction, body weight and bone remodeling. This original observation implied from its inception that the regulation of bone remodeling, like the regulation of reproduction and body weight, could be orchestrated by the central nervous system. The work summarized above demonstrated this concept in various animal models [6,7,10,14,20]. However, the increased BMD and decreased fracture risk associated with obesity in patients is counter-intuitive based on these results that demonstrated that high leptin level reduces bone mass. This paradoxical observation may be explained by the notion of leptin resistance, which invokes that leptin level above a critical threshold creates a state of resistance to the hormone. This explanation has been used to explain why obesity is associated with high leptin serum levels and may hold true to explain the increased bone density in obese patients, who may be in a state of low leptin responsiveness centrally.

Activation of sympathetic signaling by stress is associated with low BMD in humans and animal models [44-47]. Furthermore, reflex sympathetic dystrophy is characterized by a localized increase in sympathetic tone and bone loss [48]. These clinical observations suggest that the anti-osteogenic function of sympathetic signaling is conserved from mice to humans, and the obvious implication of these results is that beta-blockers might be used as bone anabolic and anti-catabolic drugs. This hypothesis has been verified with propranolol-treated and β 2AR-deficient mice that displayed resistance to ovariectomy-induced bone loss [7,20]. The availability of millions of women treated with beta-blockers for hypertension allowed a number of retrospective studies to be performed. Overall, the majority of these studies uncovered a lower risk of fracture associated with the use of beta-blockers [49-53]; however a few of them did not detect any effect, and the predominance of β 1-selective antagonists used point to a β 1AR mediated effect in contrast to the β 2AR-mediated effect in mice [54-57]. Recent prospective studies supported a reduced fracture risk and increased BMD in beta-blocker users

[58,59], but human data are still unclear and more efforts are required before beta-blockers can be considered for the treatment of low bone mass diseases.

More complexity to come

A number of additional nerve-derived peptides are likely to augment the growing list of the molecules regulating bone remodeling. For instance, unlike eNOS and iNOS, which are both expressed in and modulate osteoblasts, neuronal NOS (nNOS) is not detected in osteoblasts but is highly expressed in the CNS [60]. Osteoblasts purified from nNOS-deficient mice do not show any proliferation and differentiation defects, however nNOS-deficient mice display a high bone mass phenotype [61]. These results thus identify central nNOS as another potential negative regulator of bone remodeling.

Similarly, a number of studies suggested that glutamate signaling might be involved in the regulation of bone homeostasis, based on the expression of the glutamate signaling machinery in bone cells and on the presence of glutaminergic nerves in bones [62-67]. Conditional KO mouse models should soon bring further support to the numerous in vitro results accumulated in this area.

Bioactive amines such as dopamine and serotonin may be involved as well. Indeed, dopamine transporter (*Dat*)-deficient mice display a low bone mass phenotype [68,69], however the mechanism whereby dopamine reuptake affects bone mass is unclear so far. On the other hand, rats treated with serotonin have increased BMD [70] and lack of the serotonin transporter (5-HTT) [71] or serotonin receptor (5-HTR) [72] in mice induces a low bone mass phenotype due to a decrease in bone formation. Pharmacological inhibition of serotonin reuptake by selective serotonin reuptake inhibitors (SSRIs) had the same effect with the notable exception of fluoxetine that induced opposite phenotype in two independent studies [71,73]. Available data point to a cell autonomous effect of serotonin signaling in osteoclasts and osteoblast [72,74, 75].

Calcitonin gene-related peptide (CGRP) secreted by nerve terminals is an additional neural factor that may modulate osteoblasts biology by a direct mechanism. CGRP is made by cells of the central and peripheral nervous system and binds a receptor expressed by osteoblasts [76]. CGRP provokes intracellular signaling events in osteoblasts [77-79] and stimulates their proliferation [80], the synthesis of growth factors and cytokines, collagen synthesis and bone formation [81]. CGRP also increases the number of bone colonies formed from bone marrow stromal cells in vitro and induces bone morphogenetic protein 2 (*Bmp2*) expression in cells isolated from human pulpal explants [82-84]. CGRP may also function as an autocrine factor since it is expressed by osteoblasts as well [85,86]. In agreement with this hypothesis, transgenic mice overexpressing CGRP in differentiated osteoblasts display an increase in bone volume due to an increase in the rate of bone formation [87]. In addition, injection of CGRP to rats partially protects them from gonadectomy-induced bone loss [88], while mice deficient for *Cgrp* are mildly osteopenic due to a decrease in bone formation [89]. Regardless of its origin, these results suggest that CGRP acts as an anabolic factor for bone. Whether the elevated autonomic tone of *Cgrp*-deficient mice contributes to their osteopenia is yet unknown [90]. CGRP-containing fibers have also been shown to contact osteoclasts within the bone micro-environment, suggesting that CGRP may also control osteoclast differentiation or function [91,92]. Supporting this hypothesis, CGRP inhibits bone resorption in vitro [93-96]. However, no bone resorption abnormalities have been observed in *Cgrp*-deficient mice [89].

The density of bone innervation is an anatomical feature that may also contribute to the regional differences of the mode of action of neuropeptides. This is illustrated by the increased vertebral trabecular bone mass but reduced cortical thickness and femoral trabecular bone mass of the *ob/ob* mice, which may be explained by a differential innervation of axial and appendicular

bones, and/or by a differential bone marrow composition, as suggested by the high adiposity of *ob/ob* femurs versus *ob/ob* vertebrae [97,98].

Lastly, it is interesting to note that molecules known to be important for neuron biology, including ephrins, semaphorins and neurotrophins have recently been shown to be involved in bone cell biology [99-102]. Experimental manipulations of gene gain- and loss-of-function, specifically in the CNS versus bone cells, will be essential to further demonstrate the *in vivo* relevance of these nerve-derived factors on bone remodeling.

Conclusions

The existence of a neural arm regulating bone remodeling has now been demonstrated by a number of *in vivo* animal studies. The neuronal circuitry regulating this process, especially in the CNS, is however still quite obscure and characterizing it lies as a major challenge ahead of us. The complexity of this regulatory system obviously stems from its homeostatic and central nature and dissecting out the intricate mechanisms whereby the many homeostatic functions controlled by the hypothalamus are integrated with bone remodeling will take some more time and efforts. The use of conditional mouse mutants with cell-specific gene inactivation and inducible systems, as well as multi-disciplinary investigations, will be required to elucidate in greater details the mechanisms in play. Importantly, conservation of function between murine models and human pathophysiology will have to be demonstrated, using either clinical studies with pharmacological approaches or errors from Nature, i.e. genetic diseases involving neuromediators and bone abnormalities. From a therapeutic point of view, these studies uncovered novel targets from which pharmacological approaches will be based on to treat low bone mass diseases. The complexity of this hypothalamic regulatory arm of bone remodeling and its central and homeostatic nature will make it quite difficult to intervene centrally, but the identification of downstream peripheral osteo-neuromediators will likely be more appropriate for therapeutic interventions. Lastly, like in every physiological system, there must exist a negative feedback loop between bone cells and CNS centers, which can be direct or indirect. Osteoblasts and osteocytes are already known to release factors in the circulation (e.g. Osteocalcin, Fibroblast Growth Factor 23) that affect the function of distant organs such as the pancreas, adipose tissue and kidney [103,104]. A link, direct or indirect, to the CNS is still lacking, and the path to characterizing it is exciting, but challenging.

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References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;372:425–432. [PubMed: 7984236]
2. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, et al. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995;83:1263–1271. [PubMed: 8548812]
3. Ahima RS, Flier JS. Leptin. *Annu Rev Physiol* 2000;62:413–437. [PubMed: 10845097]
4. Cock TA, Back J, Elefteriou F, Karsenty G, Kastner P, Chan S, Auwerx J. Enhanced bone formation in lipodystrophic PPARgamma(hyp/hyp) mice relocates haematopoiesis to the spleen. *EMBO Rep* 2004;5:1007–1012. [PubMed: 15359271]
5. Elefteriou F, Takeda S, Ebihara K, Magre J, Patano N, Kim CA, Ogawa Y, Liu X, Ware SM, Craigien WJ, Robert JJ, Vinson C, Nakao K, Capeau J, Karsenty G. Serum leptin level is a regulator of bone mass. *Proc Natl Acad Sci U S A* 2004;101:3258–3263. [PubMed: 14978271]

6. Ducy P, Amling M, Takeda S, Priemel M, Schilling AF, Beil T, Shen J, Vinson C, Rueger JM, Karsenty G. Leptin inhibits bone formation through a hypothalamic relay: A central control of bone mass. *Cell* 2000;100:197–207. [PubMed: 10660043]
7. Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell* 2002;111:305–317. [PubMed: 12419242]
8. Guidobono F, Pagani F, Sibilia V, Netti C, Lattuada N, Rapetti D, Mrak E, Villa I, Cavani F, Bertoni L, Palumbo C, Ferretti M, Marotti G, Rubinacci A. Different skeletal regional response to continuous brain infusion of leptin in the rat. *Peptides* 2006;27:1426–1433. [PubMed: 16321456]
9. Pogoda P, Egermann M, Schnell JC, Priemel M, Schilling AF, Alini M, Schinke T, Rueger JM, Schneider E, Clarke I, Amling M. Leptin inhibits bone formation not only in rodents, but also in sheep. *J Bone Miner Res* 2006;21:1591–1599. [PubMed: 16995814]
10. Baldock PA, Sainsbury A, Couzens M, Enriquez RF, Thomas GP, Gardiner EM, Herzog H. Hypothalamic Y2 receptors regulate bone formation. *J Clin Invest* 2002;109:915–921. [PubMed: 11927618]
11. Sainsbury A, Baldock PA, Schwarzer C, Ueno N, Enriquez RF, Couzens M, Inui A, Herzog H, Gardiner EM. Synergistic effects of Y2 and Y4 receptors on adiposity and bone mass revealed in double knockout mice. *Mol Cell Biol* 2003;23:5225–5233. [PubMed: 12861009]
12. Brighton PJ, Szekeres PG, Willars GB. Neuromedin U and its receptors: structure, function, and physiological roles. *Pharmacol Rev* 2004;56:231–248. [PubMed: 15169928]
13. Hanada R, Teranishi H, Pearson JT, Kurokawa M, Hosoda H, Fukushima N, Fukue Y, Serino R, Fujihara H, Ueta Y, Ikawa M, Okabe M, Murakami N, Shirai M, Yoshimatsu H, Kangawa K, Kojima M. Neuromedin U has a novel anorexigenic effect independent of the leptin signaling pathway. *Nat Med* 2004;10:1067–1073. [PubMed: 15448684]
14. Sato S, Hanada R, Kimura A, Abe T, Matsumoto T, Iwasaki M, Inose H, Ida T, Mieda M, Takeuchi Y, Fukumoto S, Fujita T, Kato S, Kangawa K, Kojima M, Shinomiya KI, Takeda S. Central control of bone remodeling by neuromedin U. *Nat Med*. 2007
15. Perkins MN, Rothwell NJ, Stock MJ, Stone TW. Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. *Nature* 1981;289:401–402. [PubMed: 7464907]
16. Satoh N, Ogawa Y, Katsuura G, Numata Y, Tsuji T, Hayase M, Ebihara K, Masuzaki H, Hosoda K, Yoshimasa Y, Nakao K. Sympathetic activation of leptin via the ventromedial hypothalamus: leptin-induced increase in catecholamine secretion. *Diabetes* 1999;48:1787–1793. [PubMed: 10480609]
17. Elefteriou F. Neuronal signaling and the regulation of bone remodeling. *Cell Mol Life Sci* 2005;62:2339–2349. [PubMed: 16132233]
18. Denes A, Boldogkoi Z, Uhreczky G, Hornyak A, Rusvai M, Palkovits M, Kovacs KJ. Central autonomic control of the bone marrow: multisynaptic tract tracing by recombinant pseudorabies virus. *Neuroscience* 2005;134:947–963. [PubMed: 15994021]
19. Bonnet N, Brunet-Imbault B, Arlettaz A, Horcjada MN, Collomp K, Benhamou CL, Courteix D. Alteration of trabecular bone under chronic beta2 agonists treatment. *Med Sci Sports Exerc* 2005;37:1493–1501. [PubMed: 16177600]
20. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, Kondo H, Richards WG, Bannon TW, Noda M, Clement K, Vaisse C, Karsenty G. Leptin regulation of bone resorption by the sympathetic nervous system and CART. *Nature* 2005;434:514–520. [PubMed: 15724149]
21. Yan L, Vatner DE, O'Connor JP, Ivessa A, Ge H, Chen W, Hirotani S, Ishikawa Y, Sadoshima J, Vatner SF. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* 2007;130:247–258. [PubMed: 17662940]
22. Fu L, Patel MS, Bradley A, Wagner EF, Karsenty G. The molecular clock mediates leptin-regulated bone formation. *Cell* 2005;122:803–815. [PubMed: 16143109]
23. Simmons DJ, Nichols G Jr. Diurnal periodicity in the metabolic activity of bone tissue. *Am J Physiol* 1966;210:411–418. [PubMed: 5901786]
24. Gundberg CM, Markowitz ME, Mizuchi M, Rosen JF. Osteocalcin in human serum: a circadian rhythm. *J Clin Endocrinol Metab* 1985;60:736–739. [PubMed: 3871789]
25. Orzel JA, Rudd TG. Heterotopic bone formation: clinical, laboratory, and imaging correlation. *J Nucl Med* 1985;26:125–132. [PubMed: 3918147]

26. Wildburger R, Zarkovic N, Tonkovic G, Skoric T, Frech S, Hartleb M, Loncaric I, Zarkovic K. Post-traumatic hormonal disturbances: prolactin as a link between head injury and enhanced osteogenesis. *J Endocrinol Invest* 1998;21:78–86. [PubMed: 9585380]
27. Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, Shohami E. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature* 2001;413:527–531. [PubMed: 11586361]
28. Tam J, Ofek O, Fride E, Ledent C, Gabet Y, Muller R, Zimmer A, Mackie K, Mechoulam R, Shohami E, Bab I. Involvement of neuronal cannabinoid receptor CB1 in regulation of bone mass and bone remodeling. *Mol Pharmacol* 2006;70:786–792. [PubMed: 16772520]
29. Idris AI, van 't Hof RJ, Greig IR, Ridge SA, Baker D, Ross RA, Ralston SH. Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. *Nat Med* 2005;11:774–779. [PubMed: 15908955]
30. Ishac EJ, Jiang L, Lake KD, Varga K, Abood ME, Kunos G. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br J Pharmacol* 1996;118:2023–2028. [PubMed: 8864538]
31. Niederhoffer N, Schmid K, Szabo B. The peripheral sympathetic nervous system is the major target of cannabinoids in eliciting cardiovascular depression. *Naunyn Schmiedebergs Arch Pharmacol* 2003;367:434–443. [PubMed: 12709782]
32. Tam J, Trembovler V, Di Marzo V, Petrosino S, Leo G, Alexandrovich A, Regev E, Casap N, Shteyer A, Ledent C, Karsak M, Zimmer A, Mechoulam R, Yirmiya R, Shohami E, Bab I. The cannabinoid CB1 receptor regulates bone formation by modulating adrenergic signaling. *Faseb J*. 2007
33. Karsak M, Cohen-Solal M, Freudenberg J, Ostertag A, Morieux C, Kornak U, Essig J, Erxleben E, Bab I, Kubisch C, de Vernejoul MC, Zimmer A. Cannabinoid receptor type 2 gene is associated with human osteoporosis. *Hum Mol Genet* 2005;14:3389–3396. [PubMed: 16204352]
34. Yamada Y, Ando F, Shimokata H. Association of candidate gene polymorphisms with bone mineral density in community-dwelling Japanese women and men. *Int J Mol Med* 2007;19:791–801. [PubMed: 17390085]
35. Ofek O, Karsak M, Leclerc N, Fogel M, Frenkel B, Wright K, Tam J, Attar-Namdar M, Kram V, Shohami E, Mechoulam R, Zimmer A, Bab I. Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proc Natl Acad Sci U S A* 2006;103:696–701. [PubMed: 16407142]
36. Lundberg P, Allison SJ, Lee NJ, Baldock PA, Brouard N, Rost S, Enriquez RF, Sainsbury A, Lamghari M, Simmons P, Eisman JA, Gardiner EM, Herzog H. Greater bone formation of Y2 knockout mice is associated with increased osteoprogenitor numbers and altered Y1 receptor expression. *J Biol Chem* 2007;282:19082–19091. [PubMed: 17491022]
37. Bjurholm A, Kreicbergs A, Terenius L, Goldstein M, Schultzberg M. Neuropeptide Y-, tyrosine hydroxylase- and vasoactive intestinal polypeptide-immunoreactive nerves in bone and surrounding tissues. *J Auton Nerv Syst* 1988;25:119–125. [PubMed: 2906951]
38. Bjurholm A. Neuroendocrine peptides in bone. *Int Orthop* 1991;15:325–329. [PubMed: 1725676]
39. Arai M, Nagasawa T, Koshihara Y, Yamamoto S, Togari A. Effects of beta-adrenergic agonists on bone-resorbing activity in human osteoclast-like cells. *Biochim Biophys Acta* 2003;1640:137–142. [PubMed: 12729923]
40. Yang X, Matsuda K, Bialek P, Jacquot S, Masuoka HC, Schinke T, Li L, Brancorsini S, Sassone-Corsi P, Townes TM, Hanauer A, Karsenty G. ATF4 is a substrate of RSK2 and an essential regulator of osteoblast biology; implication for Coffin-Lowry Syndrome. *Cell* 2004;117:387–398. [PubMed: 15109498]
41. Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 1998;393:72–76. [PubMed: 9590691]
42. Ahn JD, Dubern B, Lubrano-Berthelier C, Clement K, Karsenty G. Cart overexpression is the only identifiable cause of high bone mass in melanocortin 4 receptor deficiency. *Endocrinology* 2006;147:3196–3202. [PubMed: 16614075]
43. Orwoll, B.; Bouxsein, ML.; Marks, DL.; Cone, RD.; Klein, RF. ORS/AAOS Presentations 2003, 71st Annual Meeting of the AAOS; 2004; San Francisco, CA.

44. Schweiger U, Deuschle M, Korner A, Lammers CH, Schmider J, Gotthardt U, Holsboer F, Heuser I. Low lumbar bone mineral density in patients with major depression. *Am J Psychiatry* 1994;151:1691–1693. [PubMed: 7943462]
45. Michelson D, Stratakis C, Hill L, Reynolds J, Galliven E, Chrousos G, Gold P. Bone mineral density in women with depression. *N Engl J Med* 1996;335:1176–1181. [PubMed: 8815939]
46. Cizza G, Ravn P, Chrousos GP, Gold PW. Depression: a major, unrecognized risk factor for osteoporosis? *Trends Endocrinol Metab* 2001;12:198–203. [PubMed: 11397644]
47. Yirmiya R, Goshen I, Bajayo A, Kreisel T, Feldman S, Tam J, Trembovler V, Csernus V, Shohami E, Bab I. Depression induces bone loss through stimulation of the sympathetic nervous system. *Proc Natl Acad Sci U S A* 2006;103:16876–16881. [PubMed: 17075068]
48. Laroche M, Redon-Dumolard A, Mazieres B, Bernard J. An X-ray absorptiometry study of reflex sympathetic dystrophy syndrome. *Rev Rhum Engl Ed* 1997;64:106. [PubMed: 9085445]
49. Pasco JA, Henry MJ, Sanders KM, Kotowicz MA, Seeman E, Nicholson GC. Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. *J Bone Miner Res* 2004;19:19–24. [PubMed: 14753732]
50. Rejnmark L, Vestergaard P, Kassem M, Christoffersen BR, Kolthoff N, Brixen K, Mosekilde L. Fracture risk in perimenopausal women treated with beta-blockers. *Calcif Tissue Int* 2004;75:365–372. [PubMed: 15592792]
51. Schlienger RG, Kraenzlin ME, Jick SS, Meier CR. Use of beta-blockers and risk of fractures. *JAMA* 2004;292:1326–1332. [PubMed: 15367554]
52. Wiens M, Etminan M, Gill SS, Takkouche B. Effects of antihypertensive drug treatments on fracture outcomes: a meta-analysis of observational studies. *J Intern Med* 2006;260:350–362. [PubMed: 16961672]
53. Bonnet N, Gadois C, McCloskey E, Lemineur G, Lespessailles E, Courteix D, Benhamou CL. Protective effect of beta blockers in postmenopausal women: influence on fractures, bone density, micro and macroarchitecture. *Bone* 2007;40:1209–1216. [PubMed: 17324648]
54. Levasseur R, Marcelli C, Sabatier JP, Dargent-Molina P, Breart G. Beta-blocker use, bone mineral density, and fracture risk in older women: results from the Epidemiologie de l'Osteoporose prospective study. *J Am Geriatr Soc* 2005;53:550–552. [PubMed: 15743312]
55. Reid IR, Gamble GD, Grey AB, Black DM, Ensrud KE, Browner WS, Bauer DC. beta-Blocker use, BMD, and fractures in the study of osteoporotic fractures. *J Bone Miner Res* 2005;20:613–618. [PubMed: 15765180]
56. Reid IR, Lucas J, Wattie D, Horne A, Bolland M, Gamble GD, Davidson JS, Grey AB. Effects of a beta-blocker on bone turnover in normal postmenopausal women: a randomized controlled trial. *J Clin Endocrinol Metab* 2005;90:5212–5216. [PubMed: 15998769]
57. Rejnmark L, Vestergaard P, Mosekilde L. Treatment with beta-blockers, ACE inhibitors, and calcium-channel blockers is associated with a reduced fracture risk: a nationwide case-control study. *J Hypertens* 2006;24:581–589. [PubMed: 16467662]
58. Turker S, Karatosun V, Gunal I. Beta-blockers increase bone mineral density. *Clin Orthop Relat Res* 2006;443:73–74. [PubMed: 16462429]
59. Meisinger C, Heier M, Lang O, Doring A. Beta-blocker use and risk of fractures in men and women from the general population: the MONICA/KORA Augsburg cohort study. *Osteoporos Int* 2007;18:1189–1195. [PubMed: 17333446]
60. Helfrich MH, Evans DE, Grabowski PS, Pollock JS, Ohshima H, Ralston SH. Expression of nitric oxide synthase isoforms in bone and bone cell cultures. *J Bone Miner Res* 1997;12:1108–1115. [PubMed: 9200011]
61. van't Hof RJ, Macphee J, Libouban H, Helfrich MH, Ralston SH. Regulation of bone mass and bone turnover by neuronal nitric oxide synthase. *Endocrinology* 2004;145:5068–5074. [PubMed: 15297441]
62. Serre CM, Farlay D, Delmas PD, Chenu C. Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. *Bone* 1999;25:623–629. [PubMed: 10593406]
63. Laketic-Ljubojevic I, Suva LJ, Maathuis FJ, Sanders D, Skerry TM. Functional characterization of N-methyl-D-aspartic acid-gated channels in bone cells. *Bone* 1999;25:631–637. [PubMed: 10593407]

64. Skerry TM, Genever PG. Glutamate signalling in non-neuronal tissues. *Trends Pharmacol Sci* 2001;22:174–181. [PubMed: 11282417]
65. Chenu C. Glutamatergic regulation of bone remodeling. *J Musculoskelet Neuronal Interact* 2002;2:282–284. [PubMed: 15758455]
66. Taylor AF. Osteoblastic glutamate receptor function regulates bone formation and resorption. *J Musculoskelet Neuronal Interact* 2002;2:285–290. [PubMed: 15758456]
67. Merle B, Itzstein C, Delmas PD, Chenu C. NMDA glutamate receptors are expressed by osteoclast precursors and involved in the regulation of osteoclastogenesis. *J Cell Biochem* 2003;90:424–436. [PubMed: 14505357]
68. Bliziotis M, McLoughlin S, Gunness M, Fumagalli F, Jones SR, Caron MG. Bone histomorphometric and biomechanical abnormalities in mice homozygous for deletion of the dopamine transporter gene. *Bone* 2000;26:15–19. [PubMed: 10617152]
69. Bliziotis MM, Eshleman AJ, Zhang XW, Wiren KM. Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. *Bone* 2001;29:477–486. [PubMed: 11704501]
70. Gustafsson BI, Westbroek I, Waarsing JH, Waldum H, Solligard E, Brunsvik A, Dimmen S, van Leeuwen JP, Weinans H, Syversen U. Long-term serotonin administration leads to higher bone mineral density, affects bone architecture, and leads to higher femoral bone stiffness in rats. *J Cell Biochem* 2006;97:1283–1291. [PubMed: 16329113]
71. Warden SJ, Robling AG, Sanders MS, Bliziotis MM, Turner CH. Inhibition of the serotonin (5-hydroxytryptamine) transporter reduces bone accrual during growth. *Endocrinology* 2005;146:685–693. [PubMed: 15539550]
72. Collet C, Schiltz C, Geoffroy V, Maroteaux L, Launay JM, de Vernejoul MC. The serotonin 5-HT_{2B} receptor controls bone mass via osteoblast recruitment and proliferation. *FASEB J*. 2007
73. Battaglini R, Vokes M, Schulze-Spate U, Sharma A, Graves D, Kohler T, Muller R, Yoganathan S, Stashenko P. Fluoxetine treatment increases trabecular bone formation in mice. *J Cell Biochem* 2007;100:1387–1394. [PubMed: 17041947]
74. Westbroek I, van der Plas A, de Rooij KE, Klein-Nulend J, Nijweide PJ. Expression of serotonin receptors in bone. *J Biol Chem* 2001;276:28961–28968. [PubMed: 11387323]
75. Battaglini R, Fu J, Spate U, Ersoy U, Joe M, Sedaghat L, Stashenko P. Serotonin regulates osteoclast differentiation through its transporter. *J Bone Miner Res* 2004;19:1420–1431. [PubMed: 15312242]
76. Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature* 1982;298:240–244. [PubMed: 6283379]
77. Michelangeli VP, Fletcher AE, Allan EH, Nicholson GC, Martin TJ. Effects of calcitonin gene-related peptide on cyclic AMP formation in chicken, rat, and mouse bone cells. *J Bone Miner Res* 1989;4:269–272. [PubMed: 2543186]
78. Bjurholm A, Kreicbergs A, Schultzberg M, Lerner UH. Neuroendocrine regulation of cyclic AMP formation in osteoblastic cell lines (UMR-106-01, ROS 17/2.8, MC3T3-E1, and Saos-2) and primary bone cells. *J Bone Miner Res* 1992;7:1011–1019. [PubMed: 1384276]
79. Kawase T, Burns DM. Calcitonin gene-related peptide stimulates potassium efflux through adenosine triphosphate-sensitive potassium channels and produces membrane hyperpolarization in osteoblastic UMR106 cells. *Endocrinology* 1998;139:3492–3502. [PubMed: 9681500]
80. Cornish J, Callon KE, Lin CQ, Xiao CL, Gamble GD, Cooper GJ, Reid IR. Comparison of the effects of calcitonin gene-related peptide and amylin on osteoblasts. *J Bone Miner Res* 1999;14:1302–1309. [PubMed: 10457262]
81. Vignery A, McCarthy TL. The neuropeptide calcitonin gene-related peptide stimulates insulin-like growth factor I production by primary fetal rat osteoblasts. *Bone* 1996;18:331–335. [PubMed: 8726390]
82. Bernard GW, Shih C. The osteogenic stimulating effect of neuroactive calcitonin gene-related peptide. *Peptides* 1990;11:625–632. [PubMed: 2235673]
83. Shih C, Bernard GW. Calcitonin gene related peptide enhances bone colony development in vitro. *Clin Orthop Relat Res* 1997;335–344. [PubMed: 9005931]

84. Calland JW, Harris SE, Carnes DL Jr. Human pulp cells respond to calcitonin gene-related peptide in vitro. *J Endod* 1997;23:485–489. [PubMed: 9587316]
85. Drissi H, Hott M, Marie PJ, Lasmoles F. Expression of the CT/CGRP gene and its regulation by dibutyryl cyclic adenosine monophosphate in human osteoblastic cells. *J Bone Miner Res* 1997;12:1805–1814. [PubMed: 9383685]
86. Imai S, Matsusue Y. Neuronal regulation of bone metabolism and anabolism: calcitonin gene-related peptide-, substance P-, and tyrosine hydroxylase-containing nerves and the bone. *Microsc Res Tech* 2002;58:61–69. [PubMed: 12203704]
87. Ballica R, Valentijn K, Khachatryan A, Guerder S, Kapadia S, Gundberg C, Gilligan J, Flavell RA, Vignery A. Targeted expression of calcitonin gene-related peptide to osteoblasts increases bone density in mice. *J Bone Miner Res* 1999;14:1067–1074. [PubMed: 10404006]
88. Valentijn K, Gutow AP, Troiano N, Gundberg C, Gilligan JP, Vignery A. Effects of calcitonin gene-related peptide on bone turnover in ovariectomized rats. *Bone* 1997;21:269–274. [PubMed: 9276092]
89. Schinke T, Liese S, Priemel M, Haberland M, Schilling AF, Catala-Lehnen P, Blicharski D, Rueger JM, Gagel RF, Emeson RB, Amling M. Decreased bone formation and osteopenia in mice lacking alpha-calcitonin gene-related peptide. *J Bone Miner Res* 2004;19:2049–2056. [PubMed: 15537449]
90. Oh-hashii Y, Shindo T, Kurihara Y, Imai T, Wang Y, Morita H, Imai Y, Kayaba H, Nishimatsu Y, Suematsu Y, Hirata Y, Yazaki R, Nagai T, Kuwaki T, Kurihara H. Elevated sympathetic nervous activity in mice deficient in alphaCGRP. *Circ Res* 2001;89:983–990. [PubMed: 11717154]
91. Hara-Irie F, Amizuka N, Ozawa H. Immunohistochemical and ultrastructural localization of CGRP-positive nerve fibers at the epiphyseal trabeculae facing the growth plate of rat femurs. *Bone* 1996;18:29–39. [PubMed: 8717534]
92. Imai S, Tokunaga Y, Maeda T, Kikkawa M, Hukuda S. Calcitonin gene-related peptide, substance P, and tyrosine hydroxylase-immunoreactive innervation of rat bone marrows: an immunohistochemical and ultrastructural investigation on possible efferent and afferent mechanisms. *J Orthop Res* 1997;15:133–140. [PubMed: 9066538]
93. D'Souza SM, MacIntyre I, Girgis SI, Mundy GR. Human synthetic calcitonin gene-related peptide inhibits bone resorption in vitro. *Endocrinology* 1986;119:58–61. [PubMed: 3487444]
94. Roos BA, Fischer JA, Pignat W, Alander CB, Raisz LG. Evaluation of the in vivo and in vitro calcium-regulating actions of noncalcitonin peptides produced via calcitonin gene expression. *Endocrinology* 1986;118:46–51. [PubMed: 3484451]
95. Zaidi M, Fuller K, Bevis PJ, GainesDas RE, Chambers TJ, MacIntyre I. Calcitonin gene-related peptide inhibits osteoclastic bone resorption: a comparative study. *Calcif Tissue Int* 1987;40:149–154. [PubMed: 3105845]
96. Akopian A, Demulder A, Ouriaghli F, Corazza F, Fondu P, Bergmann P. Effects of CGRP on human osteoclast-like cell formation: a possible connection with the bone loss in neurological disorders? *Peptides* 2000;21:559–564. [PubMed: 10822112]
97. Hamrick MW, Pennington C, Newton D, Xie D, Isales C. Leptin deficiency produces contrasting phenotypes in bones of the limb and spine. *Bone* 2004;34:376–383. [PubMed: 15003785]
98. Reid IR. Leptin deficiency--lessons in regional differences in the regulation of bone mass. *Bone* 2004;34:369–371. [PubMed: 15003783]
99. Mogi M, Kondo A, Kinpara K, Togari A. Anti-apoptotic action of nerve growth factor in mouse osteoblastic cell line. *Life Sci* 2000;67:1197–1206. [PubMed: 10954053]
100. Harper J, Gerstenfeld LC, Klagsbrun M. Neuropilin-1 expression in osteogenic cells: down-regulation during differentiation of osteoblasts into osteocytes. *J Cell Biochem* 2001;81:82–92. [PubMed: 11180399]
101. Gomez C, Burt-Pichat B, Mallein-Gerin F, Merle B, Delmas PD, Skerry TM, Vico L, Malaval L, Chenu C. Expression of Semaphorin-3A and its receptors in endochondral ossification: potential role in skeletal development and innervation. *Dev Dyn* 2005;234:393–403. [PubMed: 16145665]
102. Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T, Suda T, Matsuo K. Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab* 2006;4:111–121. [PubMed: 16890539]
103. Quarles LD. Evidence for a bone-kidney axis regulating phosphate homeostasis. *J Clin Invest* 2003;112:642–646. [PubMed: 12952909]

104. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007;130:456–469. [PubMed: 17693256]

Figure 1. Neuropeptides in the CNS regulate peripheral bone cell biology via the SNS and likely other neuroendocrine mechanisms

Simplified illustration of the major neuropeptides, and their connections, demonstrated to regulate bone remodeling. MSC: mesenchymal stem cell. ?: unknown.