Hypothalamic regulation of bone

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Abstract

On initial inspection, bone remodeling, the process whereby the skeleton adapts through time, appears to be relatively simple. Two cell types, the bone-forming osteoblasts and the bone-resorbing osteoclasts, interact to keep bone mass relatively stable throughout adult life. However, the complexity of the regulatory influences on these cells is continuing to expand our understanding of the intricacy of skeletal physiology and also the interactions between other organ systems and bone. One such example of the broadening of understanding in this field has occurred in the last decade with study of the central, neural regulation of bone mass. Initial studies of an adipose-derived hormone, leptin, helped define a direct, sympathetic pathway involving efferent neural signals from the hypothalamus to receptors on the osteoblast. Since the leptin-mediated pathway has been continuously modified to reveal a complex system involving neuromedin U, cocaine-and amphetamine-related transcript and serotonin interacting within the hypothalamus and brainstem to regulate both bone formation and resorption in cancellous bone, a number of other systems have also been identified. Neuropeptide Y, acting through hypothalamic Y2 receptors, is capable of skeleton-wide modulation of osteoblast activity, with important coordination between body weight and bone mass. Cannabinoids, acting through central cannabinoid receptor 1 and bone cell cannabinoid receptor 2 receptors, modulate osteoclast activity, thereby identifying pathways active on both aspects of the bone remodeling process. This review explores the key central pathways to bone and explores the complexity of the interactions being revealed by this emergent field of research.

Journal of Molecular Endocrinology (2010) 45, 175–181

Function of hypothalamus

The brain has long been appreciated as a pivotal regulator of homeostasis in peripheral tissues, including the skeleton. There is now clear evidence for crosstalk between the brain and bone through two distinct routes. The first pathway comprises well-defined hormonal signals arising from neuroendocrine neurons of the hypothalamus and subsequently processed within the pituitary. The second pathway consists of efferent neuronal discharges originating from the hypothalamus and processed through the brainstem. The hypothalamus, with its semipermeable blood–brain barrier, is thus one of the most powerful regulatory regions within the body, integrating signals not only from peripheral tissues but also from within the brain itself. These direct, neural pathways represent an emergent area of study that is identifying novel regulatory axes between the brain and the cells of bone. Moreover, this work is also providing insights into regulatory connections involving skeletal tissue, which are proving to be unexpected, thereby outlining a level of interconnectedness that has been previously unappreciated. This review examines the expanded understanding of the central, neural outputs to bone metabolism and remodeling.

An adipocyte hormone represses bone formation: leptin actions in cancellous bone

The first model to define a central, neural pathway to bone involved the action of an endocrine signal not from bone, but from fat cells. Leptin, a peptide hormone secreted by adipocytes, signals within the hypothalamus to control body weight. Circulating leptin acts within the hypothalamus as an adipostat, altering appetite and energy expenditure (Boden et al. 1996). Interestingly, leptin-deficient ob/ob mice exhibit a phenotype with varied skeletal abnormalities. Cancellous bone mass is increased despite hypogonadism and hypercortisolism, while, in contrast, bone length and mass are reduced despite markedly greater fat mass compared to wild-type mice (Ducy et al. 2000,

DOI: 10.1677/JME-10-0015

Online version via http://www.endocrinology-journals.org
Hamrick et al., 2005, Baldock et al., 2006, Iwaniec et al., 2007). Additional models with altered serum leptin levels confirmed the effect of leptin on bone; cancellous bone mass was reduced in leptin transgenic mice and increased in hypoleptinemic A-ZIP lipodystrophic mice (Elefteriou et al., 2004). Moreover, leptin replacement corrected the skeletal phenotype of ob/ob mice by either peripheral or central routes (Ducy et al., 1996, Elefteriou et al., 2004, Wolf 2008). Importantly, centrally administered leptin was able to correct the ob/ob changes in cancellous bone at inactive doses when administered peripherally, indicating a pathway confined to the brain (Ducy et al., 2000). Chemical lesion of neurons in the ventromedial hypothalamus recapitulated the ob/ob phenotype, and abrogated the effect of central leptin administration, localizing the pathway to this nucleus of the hypothalamus (Takeda et al., 2002). This central afferent pathway to bone was subsequently shown to regulate sympathetic tone and thereby modulate adrenergic signaling locally via osteoblastic β2 adrenergic receptor (β2 AR; Takeda et al., 2002). Mice that are null for β2 AR displayed increased cancellous bone mass and formation, similar to ob/ob mice, and moreover, they were resistant to central leptin administration (Takeda et al., 2002). For the first time, this series of experiments defined a central, neural pathway acting from the hypothalamus directly to the cells of bone. Moreover, the adipose origin of the circulating mediator of this pathway identified a novel regulation between bone and energy homeostasis.

Involvement of the brainstem: brain-derived serotonin

Recent studies have further expanded our understanding of the leptin pathway to bone, elucidating an important role for serotonin production in the brainstem. Leptin inhibition of bone mass accrual requires the integrity of specific hypothalamic neurons (Takeda et al., 2002). However, loss of the leptin receptors from these neurons did not affect leptin action (Balthasar et al., 2004), suggesting that direct leptin signaling acts elsewhere in the brain to achieve these functions. Yadav et al. (2009) have shown that serotonin, produced locally within the brainstem (brain-derived serotonin, BDS) in an isolated circuit from that of the circulatory serotonin, is able to modulate leptin’s effects on bone mass.

Serotonin acts via a multiple receptor (HTR) of receptors to modulate numerous processes. These actions have now been expanded to include the regulation of bone mass (Yadav et al., 2009). The marked effects of leptin deficiency on the bone and energy homeostasis were corrected by specific inactivation of serotonin production in the brainstem, while loss of the leptin receptor in serotonergic neurons in the brainstem recapitulates them. Specifically, central serotonin stimulates bone mass accrual through binding to HTR2C receptors on ventromedial hypothalamic neurons and appetite via HTR1A and 2B receptors on arcuate neurons. Thus, leptin inhibits these functions by reducing serotonin synthesis and firing of serotonergic neurons. Thus, leptin and serotonin have opposing actions on bone mass, with leptin’s influence on bone mass, at least in cancellous bone, involving the modulation of central serotonin signaling, with a common mechanism regulating energy homeostasis (Fig. 1).

Another step in the pathway: neuromedin U

Subsequent to the initial studies outlining the central effects of leptin on bone mass, a number of studies have identified additional aspects of the pathway that have expanded our appreciation of the breadth and complexity of central osteo-modulatory signaling. One such discovery involved the actions of neuromedin U (NMU). NMU is a neuropeptide expressed in hypothalamic neurons and in the small intestine, and is regulated by sympathetic activation (Brighton et al., 2004). However, it has also been shown to regulate bone mass. NMU null mice display increased bone formation and bone mass via a central, hypothalamic pathway (Sato et al., 2007). NMU2 receptor is expressed in the paraventricular nucleus, and central infusion of NMU rescued the high bone mass of NMU null mice. Moreover, NMU and its receptors are not detectable in bone, and osteoblast activity is not altered by in vitro NMU treatment. Interestingly, NMU treatment was...
found to decrease the high cancellous bone mass of leptin-deficient mice, suggesting a role for NMU downstream of leptin to regulate bone formation. Consistent with this hypothesis, NMU-deficient mice were resistant to the skeletal effects of leptin and β2 AR agonists. NMU signaling thus begins to outline the mechanistic framework involved in the transmission of the regulatory signals beginning with circulatory leptin and ending with altered bone cell activity (Fig. 2).

**Leptin regulation of bone resorption: cocaine- and amphetamine-regulated transcript**

In addition to signals regulating the activity of bone formation, central neural signaling has been shown to alter bone resorption. Cocaine- and amphetamine-regulated transcript (CART), a neuropeptide precursor protein involved in the regulation of food intake and energy expenditure, is broadly expressed in the hypothalamus and the peripheral organs such as the pancreas and adrenal glands (Elefteriou et al. 2005). Interestingly, the phenotype of ob/ob mice suggested that leptin could be affecting bone resorption via central effects on CART. ob/ob mice can be distinguished from the β2 AR null mice by their decrease in hypothalamic CART expression and their increased resorption, thereby implicating CART as a potential regulator of bone resorption. Furthermore, the decreased CART expression in ob/ob mice can be restored by i.p. treatment with leptin (Kristensen et al. 1998). Consistent with this hypothesis, CART knockout mice are osteoporotic due to an increase in bone resorption (Elefteriou et al. 2005). Moreover, CART-deficient mice express higher levels of RANKL in bone than wild-type mice, with in vitro osteoclast differentiation experiments indicating that the effect of CART on bone is not cell autonomous, suggesting a local mechanism for the central CART changes

Figure 2 Model of neuromedin U signaling in the regulation of bone formation. As a central regulator of a leptin-dependent regulation of bone mass, neuromedin U acts in the central nervous system downstream of leptin. Efferent sympathetic signaling through the sympathetic nervous system (SNS) affects the molecular clock in bone to regulate bone remodeling.

Figure 3 Model of CART signaling in the regulation of bone remodeling. CART expression is altered in proportion to serum leptin levels. Low CART expression induces an increase in bone resorption through higher levels of RANKL, while increased hypothalamic CART expression produces a higher bone mass phenotype.

(Elefteriou et al. 2005). Conversely, increased hypothalamic CART expression in melanocortin 4 receptor null mice has been shown to be necessary for their reduced bone resorption (Elefteriou et al. 2005, Ahn et al. 2006). Thus, in addition to neuro-osteoblastic effects, osteoclastogenesis and bone resorption also appear to be under the control of central neural mediators. At this stage, many studies have produced information about the regulation, the location, processing, and functions of the CART peptide. However, additional studies are needed at elucidating the physiological role and importantly at characterizing the receptor of CART (Fig. 3).

**A second central pathway to bone: the neuropeptide Y system**

Another major contributor to the neural output from the hypothalamus to bone is the neuropeptide Y (NPY) system. The NPY system involves three ligands, NPY and two ligands expressed in the periphery, peptide Y2, and pancreatic polypeptide. These ligands signal through five receptor subtypes, Y1, Y2, Y4, Y5, and Y6 in mice, expressed widely in central and peripheral tissues (Blomqvist & Herzog 1997, Lin et al. 2005). NPY is one of the most common neuropeptides in the brain, with strong expression in the hypothalamus. NPY is synthesized in neurons whose cell bodies lie in the arcuate nucleus with projections into surrounding hypothalamic structures. NPY is also co-secreted with norepinephrine in peripheral sympathetic neurons (Bernet et al. 1998). To date, analysis of brain-specific NPY overexpression and Y receptor knockout models has revealed a powerful anti-anabolic pathway involving hypothalamic Y2 receptors and nonhypothalamic Y1 receptors.

The first in vivo indication for the role of NPY in regulation of bone metabolism was demonstrated
in a murine Y2 receptor null model. These mice displayed a generalized increase in osteoblast activity in cortical and cancellous bone, with no indication of changes in bone resorption (Baldock et al. 2002). Importantly, this effect was recapitulated by conditional deletion of Y2 receptors from the arcuate nucleus in mature mice, with osteoblast activity elevated up to sevenfold compared to Y2-intact mice (Baldock et al. 2002). While Y2 deletion altered some aspects of endocrine function, such as increasing serum corticosterone levels (Allison et al. 2006), none of these changes was consistent with the increase in bone formation. This finding therefore indicated that the changes in bone homeostasis observed in hypothalamus-specific Y2 receptor-deficient mice were the result of an efferent neural pathway from the hypothalamus, rather than through altered pituitary outflow.

Central involvement in the body weight bone mass relationship?

Despite early reports of no effect (Elefteriou et al. 2003), a recent study has confirmed the role of NPY in skeletal metabolism. NPY null mice demonstrated a generalized bone anabolic phenotype (Baldock et al. 2009), without significant changes in body weight, similar to that reported for Y2 receptor null mice. The negative relationship between hypothalamic NPY and bone formation is consistent with previous reports of reduced bone formation following overexpression of NPY in the hypothalamus of wild-type mice (Baldock et al. 2005). Interestingly, central NPY overexpression, a model of forced central starvation, similar to that evident in ob/ob mice (Sainsbury et al. 2002), resulted in a reduction in bone mass despite marked increases in body weight, also evident in ob/ob. In this manner, as caloric restriction reduces body weight, central NPY levels rise (Lauzurica et al. 2010) and bone formation is inhibited, conserving limited energy resources. Conversely, under excessive caloric intake, body weight increases, NPY expression is reduced, and bone formation is stimulated, thereby matching bone mass to body mass, one of the most fundamental relationships in skeletal physiology. Thus, the central perception of body weight, as evident by alterations in central NPY, may match body weight to bone mass in concert with the well-described mechanical responses.

In addition to the central actions in the hypothalamus, NPY appears to provide a local circuit in the osteoblast. Analysis of Y1 receptor null mice revealed a similar bone phenotype to the high bone mass evident in NPY and Y2 null mice, although with an additional increase in bone resorption (Baldock et al. 2007). However, in contrast to the hypothalamic-specific action of Y2 receptors on bone, deletion of Y1 receptors from the hypothalamus had no effect on bone cell activity or bone volume, indicating that the effects of Y1 receptors on bone metabolism are mediated by nonhypothalamic receptors (Baldock et al. 2007). While it is possible that Y1 receptors in regions of the brain other than the hypothalamus may be involved in mediating actions of NPY on bone, a direct effect of NPY on osteoblastic cells acting via locally expressed Y1 receptors is more likely. This is supported by in situ hybridization revealing the presence of Y1 receptor expression in osteoblasts on endocortical and cancellous surfaces within the femoral bone tissue (Lundberg et al. 2007). NPY is also expressed in osteoblasts and osteocytes, and expression is reduced in vitro by mechanical loading (Baldock et al. 2009, Igwe et al. 2009). Moreover, NPY replacement in the hypothalamus of NPY null mice was only able to partially correct the bone anabolic phenotype (Baldock et al. 2009). No additive effects on bone metabolism were observed in mice deficient for both the Y1 and Y2 receptors, indicating that Y1 and Y2 receptors may act at different points along a common signaling pathway to control bone formation (Lundberg et al. 2007). In this manner, local NPY actions, via Y1 receptors in the osteoblast, may enable load-appropriate fine tuning of efferent NPY signals involving hypothalamic Y2 receptors. Thus, the NPY system represents a coordinated system, which is not only acting from the brain to the cells of bone, but also interacting with powerful energy homeostatic processes to modulate the relationship between body weight and bone mass (Fig. 4).

The cannabinoid receptors

Indicating a diversity of central pathways to bone, yet another system, involving endocannabinoid signaling has been elucidated. The endocannabinoid system mediates its actions via two cannabinoid receptors, CB1 and CB2 (Howlett et al. 2002). CB1 is primarily...
found within the central nervous system (CNS) and accounts for most of the CNS actions of cannabinoid drugs and endocannabinoids (Mackie 2008), while CB2 is predominantly expressed in peripheral tissues (Tam et al. 2008). The main CB1 and CB2 endogenous ligands are 2-arachidonoylglycerol and N-arachidonoylthanolamine (Devane et al. 1992, Mechoulam et al. 1995).

Recent independent studies indicated that endocannabinoids regulate bone homeostasis by modulating adrenergic signaling. CB1 receptor signaling activation on presynaptic nerve terminals in bone inhibits norepinephrine release by sympathetic neurons, thus balancing the tonic sympathetic restrain of bone formation (Ishac et al. 1996, Niederhoffer et al. 2003). However, inactivating CB1 receptor increased bone mineral density and additionally provided protection against ovariectomy-induced bone loss (Idris et al. 2005). Furthermore, CB1 receptor antagonism inhibited osteoclast formation and bone resorption, while CB1 knockout mice were resistant to these effects. Thus, cannabinoid signaling, acting via the CB1 receptor, regulates osteoclasts (Idris et al. 2005).

CB2 receptor is expressed in osteoblasts, osteocytes, and osteoclasts. CB2 null mice have accelerated age-related cancellous bone loss and cortical expansion due to increased bone turnover (Ofek et al. 2006). These results are supported by human genetic association studies linking CNR2 gene (encoding CB2) and reduced bone mass in women (Karsak et al. 2005, Yamada et al. 2007) and in vitro pharmacological studies demonstrating a direct activation of CB2 in osteoclasts. These in vitro studies indicate that CB2 signaling contributes to the maintenance of bone mass by two mechanisms: i) stimulating stromal cells/osteoblasts directly; and ii) inhibiting monocytes/osteoclasts osteoblast/stromal cell RANKL expression both directly and indirectly. The fact that CB2 agonists, which are not psychoactive, attenuate ovariectomy-induced bone loss in mice also suggests that these compounds might be adapted for the treatment of low bone mass diseases (Ofek et al. 2006). Jointly, these data suggest that the cannabinoid system plays an important role in the regulation and maintenance of bone mass through the signaling of both the central and skeletal cannabinoid receptors. This system, in keeping with the NPY system above, highlights the importance of centrally expressed receptors, but also the locally expressed receptors in bone. Importantly, the notion of integrated central and local aspects of these pathways indicates the requirement for feedback. The identification of such agents remains one of the most critical aspects of this work (Fig. 5).

**Conclusion**

The simultaneous maintenance of bone mass, mechanical integrity, and mineral homeostasis by the process of bone remodeling requires a complex regulatory milieu. It is now well appreciated that efferent neural signals, involving for the most part of the hypothalamus, play an important role in this regulation. These neural signals convey rapid and often marked effects on osteoblast and osteoclast activity, and thus present tempting therapeutic potential. In addition, these pathways are also highlighting a level of interaction between skeletal tissue and other organ systems, which has, until now, remained unappreciated. Interestingly, this work is revealing crosstalk between other metabolic processes, including energy regulation, but also has the potential to involve higher brain functions. The future promises to reveal a skeletal system far more complex than simply a structural entity, but rather a system of organs integrated with many others throughout the body.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

**Funding**

This review was funded in part by the National Heath and Medical Research Program Grants 276412, 376028, and 427632. PAB is supported by a fellowship from the NHMRC.

**Acknowledgements**

The authors would like to thank Prof. Patrick Bradley for his insight during the production of this manuscript.
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Received in final form 6 July 2010
Accepted 26 July 2010
Made available online as an Accepted Preprint 26 July 2010