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Review

Neuronal control of bone and muscle

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ABSTRACT

The functional interplay between bone and muscle that enables locomotion is a fundamental aspect of daily life. However, other interactions between bone and muscle continue to attract attention as our understanding of the breath and importance of this inter-relationship continues to expand. Of particular interest is the regulatory connection between bone and muscle, which adds a new insight to the coordination of the bone/muscle unit. We have appreciated the importance of neuronal signaling to the control of bone turnover and muscle contraction, but recent data indicate that neuronal inputs control a far wider range of bone and muscle physiology than previously appreciated. This review outlines the role of the sympathetic nervous system and neuronal/neuropeptide inputs upon the regulation of bone and muscle tissue, and the potential for co-regulatory actions, particularly involving the sympathetic nervous system.

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Neuronal control of bone remodeling

Bone is remodeled, repaired and replaced throughout life by means of basic multicellular units, consisting of osteoclasts and osteoblasts acting in a coordinated fashion to resorb existing bone and form new bone in its place. Remodeling allows bone tissue to adapt its structure and mass to mechanical demands to ensure maximal strength with minimal bone mass [1]. The prevailing paradigm of skeletal homeostasis is that bone remodeling is controlled in a predominantly endocrine manner, while simultaneously responding to local mechanical stimuli driven by load and in response to skeletal muscle mass, strength and contraction. However, the recent discovery of bone regulation by the central nervous system represents an emergent area of study that is identifying novel regulatory axes between the nervous system and

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bone. A direct role of the nervous system in bone cells is strongly supported by immunocytochemistry studies which revealed the presence of innervation and receptors for neuropeptides in bone cells [2]. Moreover, retrograde trans-synaptic tracing has identified neuronal tracts from the femoral bone marrow linked direct to the central nervous system [3]. Taken together, these findings suggest the existence of neuronal signaling between the brain and the cells of the bone. Studies in mouse mutant models have provided the most powerful evidence for direct central control of bone mass, which will form the basis of the review.

Leptin deficiency and bone

Leptin, an adipokine, is primarily secreted into the circulation by white adipocytes at levels of positively correlated with percentage body fat, which facilitates its role as an adipostat [4]. The initial identification of a direct neuronal output from the hypothalamus to the bone came about from study of leptin. Leptin signaling deficiency in the mutant (*ob*/*ob*) mice or inactivation of the leptin receptor Ob-Rb (*db*/*db*) produces complex, multi-system phenotypes, consistent with the loss of leptin signaling paralleling a starvation signal. As a result, the most characteristic change in *ob*/*ob* or *db*/*db* mice is extremely obesity, however, these mice also display skeletal alterations. Cancellous bone volume is increased in *ob*/*ob*, associated with increased bone turnover favoring bone formation [5]. At the same time, another group reported leptin as a potent stimulator of bone growth in *ob*/*ob* mice; leptin treatment in 4-week-old *ob*/*ob* mice increased cortical mineral content of the femora [6]. The leptin treatment also increased femur length, total body bone area, bone mineral content and density compared to vehicle-treated controls. Thus, it appears that leptin has opposing effects on cancellous and cortical bone in *ob*/*ob* mice. Further analysis supported the envelope specific effects in leptin-deficient mice. Consistent with both initial studies, cancellous bone volume was greater in *ob*/*ob*, associated with elevated turnover [7], while total body bone mineral content, cortical area and mineralizing surface of the femur were reduced compared to normal mice [6,8–11]. Leptin treatment increased whole body BMC and endosteal bone formation in the *ob*/*ob* mice but not in wild type mice [12]. These data indicate that the largest effect of leptin deficiency on the skeleton is a reduction in cortical bone formation. Moreover, the mechanism of this action has revealed a new regulatory axis to the bone, the sympathetic nervous system.

Sympathetic nervous system regulation of bone mass

The existence of hypothalamic-mediated effects on the bone through endocrine hormone release from the pituitary has long been appreciated. In recent years, the discovery of a central locus for the regulation of bone mass has been one of the most exciting developments in the area of bone metabolism, and has revealed regulatory associations between the bone that were previously unknown. Although, leptin deficiency is characterized by numerous endocrine changes, a humoral pathway was ruled out by parabiosis experiments in *ob*/*ob* mice [13]. This indicated that leptin signaling in the brain (supplied by brain-only supplementation, icv) was responsible for the skeletal changes, without the necessity of a humoral signal. The ventro-medial hypothalamus (VMH) was identified as the source of the leptin signaling and origin of the anti-osteogenic signal to cancellous bone. Thus sympathetic activity was likely the important downstream of central leptin. Subsequent studies support the involvement of sympathetic nervous system (SNS) in central leptin signaling on bone regulation. Osteoblasts and osteoclasts were found to express functional β2-adrenergic receptors (β2AR) [13]. Consistent with a role for SNS signaling in the bone, mice deficient in β2AR showed increased cancellous bone volume [14] while deletion of a downstream mediator of β2AR signaling, adenylyl cyclase 5, protects against age-related bone loss [15]. When β2AR were blocked with non-selective antagonist propranolol, cancellous bone mass was increased in wild type mice and disruption of dopamine β-hydroxylase, an enzyme generating adrenaline and noradrenaline, exhibited greater cancellous bone mass in mice. Importantly, reducing adrenergic signaling protected the bone from the cancellous bone loss following icv leptin; through loss of dopamine β-hydroxylase in wild type mice and propranolol treatment in *ob*/*ob* [13]. β2AR remained unchanged after icv leptin infusion [13]. This novel role of β adrenergic signaling in the bone completed the central leptin signaling pathway to the bone.

Neuropeptide Y system and bone

The role of leptin in the hypothalamus was followed by the identification of a number of central pathways to the bone. One neuronal system of particular importance to the bone is the neuropeptide Y (NPY) system. The NPY system consists of 3 ligands; NPY, peptide YY (PYY), and pancreatic polypeptide (PP) mediating its effects via G protein-coupled receptors, of which five have been identified to date: Y1, Y2, Y4, Y5 and y6 [16,17]. NPY, a 36-amino acid peptide, is widely expressed in the central and peripheral nervous systems, and is present in both sympathetic and parasympathetic nerve fibers, often co-released with noradrenaline during nerve stimulation; it also circulates in the blood. Very early studies demonstrated that NPY treatment in osteoblastic cell lines inhibited the cAMP response to parathyroid hormone and norepinephrine [18,19], suggesting the presence of functional Y receptors on bone cells and a possible regulatory role for NPY in the bone.

Similar to leptin, NPY-mediated effects in the bone was confirmed in mutant mouse models. Two Y receptors, Y1 and Y2, have been connected with bone homeostasis. These receptors are abundant in the hypothalamus as well as in peripheral nerves [20–22]. The first Y receptor model evaluated for skeletal activity was $Y2^{-/-}$, due to the known co-expression of Y2 and leptin receptors on neurons within the arcuate nucleus [23,24]. Initial analysis of germline $Y2^{-/-}$ mice were similar to those following conditional deletion of Y2 receptors in the hypothalamus, demonstrating a role for central Y2 receptors in this pathway and the first specific gene deletion in the hypothalamus to alter bone homeostasis. They revealed a greater cortical and cancellous bone volume associated with a greater bone formation rate [8,25]. Bone resorption parameters were unchanged except for a modest increase in osteoclast number. Importantly, the skeletal changes observed in germline $Y2^{-/-}$ mice and hypothalamic $Y2^{-/-}$ mice occurred in the absence of any measurable changes in bone active endocrine factors. Thus these findings suggest that the bone anabolic effects after Y2 receptor deletion are mediated by a neuronal mechanism and not by endocrine effectors of bone turnover.

Similar to the phenotype resulting from Y2 receptor deletion, germline Y1 receptor deficiency resulted in a generalized anabolic phenotype with greater cortical bone and cancellous accrual, although with an additional increase in bone resorption [26]. However, unlike the anabolic effects in hypothalamus-specific Y2 deletion [49], loss of hypothalamic Y1 receptors had no effect on bone homeostasis indicating a non-central mechanism for Y1 action in the bone. The existence of a direct Y1-mediated effect on bone anabolism was further suggested following identification of Y1 expression in osteoblastic cells in vivo [26]. NPY treatment of calvarial osteoblast cultures markedly decreased cell numbers, an effect absent in cultures from Y1 receptor knockout mice, indicating functional osteoblastic Y1 receptors. The direct regulation of osteoblasts by NPY was confirmed in osteoblast-specific Y1 receptor knockout mice, which displayed an increase in bone formation similar to germline Y1 null mice [27]. Loss of Y1 receptor has also been demonstrated to regulate mesenchymal stem cell activity and mineralization of osteoblastic cultures in vitro [28]. In addition, NPY is produced by osteoblasts and elevation of NPY production in osteoblast-specific NPY transgenic mice shown an opposing phenotype