Crosstalk between the brain and bone

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Summary

Bone alters its metabolic and anabolic activities in response to the variety of systemic and local factors such as hormones and growth factors. The responsiveness of bone is accomplished by the action of osteoblasts, osteoclasts and osteocytes through the process of bone remodeling. The importance of the nervous system on body homeostasis systems has been described (1) and has been suggested that organogenesis and tissue repair are under neuronal control. The first documentation of an anatomic relationship between nerves and bone was made via woodcut, by Charles Estienne in Paris in 1545, which demonstrated nerves entering and leaving the bones of a skeleton (2). Later, several authors showed that cortical bone is densely innervated (3) and differentiated myelinated and non-myelinated fibers are associated with the arterial vessels and venous sinusoids in bone (4). This began a steady flow of studies of various nerve types in bone by a number of different groups (2). The field has recently been reinvigorated by the observation of an important role of neural control of many aspects of bone metabolism (2).

KEY WORDS: bone nerves; neuropeptides; beta-adrenergic signaling; serotonin; leptin.

Anatomy and physiology

The distribution of nerves in bone, specifically those with neuropeptide-containing fibers, has been extensively studied. A number of histological studies revealed the existence of neuropeptides in bones including enzymes, neuropeptides of sensory, sympathetic and glutaminergic types (5). The distribution of nerves in bone is most frequently found in metabolically active area and the majority of nerves are localized along blood vessels. To date, no classical synapses have been found to involve osteoblasts, osteoclast, or osteocytes (2). However, nerve fibers with active expression of various neural transmission ligands have been demonstrated to be in close spatial association with bone cells and receptors for these neural ligands have been found expressed by bone cells. In addition administration of these neural transmission molecules has potent effects on bone cells (2). Although there are few nerve fibers in bone, their presence may represent sophisticated and specialized regulatory elements able to deliver time- and site-specific stimuli according to demand (6, 7). Essentially, bone nerves have been implicated in two different roles: a) as regulators of bone mechanical forces and b) as a source of trophic factors essential for structure and bone function. Bone nerves may represent the ‘forgotten’ ability to perceive mechanical strain and stresses, process the information and then transform this physical signal into cellular and biochemical responses (7). The perception of stretch, pressure, and position of the bone nerves may contribute to the overall mechanism of coordinated movement of the limbs and bone modeling (6-8). Chemical nature of bone innervation appears to change along developmental stages, suggesting the existence of a bidirectional signaling system between nerves and bone cells and a possible influence of bone cells on nerve behavior, survival and signaling. For instance, rat thoracic sympathetic axons mostly display catecholaminergic properties [tyrosine hydroxylase (TH) and norepinephrine (NE)-positive] when they reach the periosteal region of the sternum, but switch their properties to cholinergic/predominantly nerve (acetylcholine transporter and VIP-positive) after contact with the sternal tissue during the first two post-natal weeks suggesting that the targeted bony tissue plays a role in determining neurotransmitter type in innervating neurons (9). Bone is richly innervated by sensory, sympathetic and glutaminergic fibers. Substance P (SP), calcitonin gene-related peptide (CGRP), vasoconstrictor peptide (VIP), neuropeptide Y (NPY), serotonin, glutamate, TH and NE are among the neuronal products that have been detected in bone. Finally, together with neuronal products act on bone there are evidence for crosstalk between the brain and bone through two different 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 effenter neuronal discharges originating from the hypothalamus and processed through the brainstem (10).

Neuropeptides involved in bone metabolism

Glutamate

Glutamatergic synaptic transmission dominates interneuronal signaling in the central and peripheral nervous systems. Glutamate has been identified in bone both in association with other nerve markers in proximity to bone cells and blood vessels, and as a product released by osteoblasts themselves (2, 11, 12). Osteoblasts and osteoclast like cells actively release glutamate at concentrations that are sufficient to activate receptors expressed on bone cell surfaces, providing convincing evidence for an intrinsic osteo-glutamatergic signaling mechanism (13). In addition, glutamate signaling in osteoblasts is regulated by the cytokines TNF-α and IFN-γ, which are known to have potent effects on bone remodeling. These cytokines have been shown to induce apoptosis in osteoblasts, and this may be mediated at least in part through decreased glutamate release (13). Osteoblasts, osteoclasts, and osteocytes express the NMDA (ionotropic glutamate receptors: NMDAR1) and other glutamate recep-
tors and induce patch-clamp-demonstrable currents (the standard measure of ion channel controlled currents in nerves) in response to glutamate signaling. Mice under express NMDAR1 are smaller in comparison with wild type, which may reflect a disruption in skeletal development (5). Glutamate has also a paracrine function on bone cells and it is suspected to be at work when expression of transporters of glutamate is down regulating in response to mechanical loading of osteocytes (14).

Calcitonin Gene-Related Protein (CGRP)
CGRP is a 37 amino acid neuropeptide generated by alternative splicing of the calcitonin gene. In bone, nerve fibers immunostaining for CGRP are found in the periostum, bone marrow, and preferentially in the epiphyseal trabecular bone (2, 15, 16). CGRP-immunoreactive fibers have been shown to serve three functions. Acting on the vasculature, CGRP is the most potent vasodilator among sensory neuropeptides. CGRP acts also in skeletal muscle where the primary effects are the inhibition of glycogen synthesis and stimulation of glycogenolysis with a reduction of glucose uptake and increased glycolysis. In the liver they stimulate the glucose output as a result of gluconeogenesis. By these ways CGRP act as noncompetitive antagonist of insulin and produce insulin resistance (17). On bone cells, CGRP has been reported to stimulate osteogenesis, either by activating stem cell mitosis or osteogenor cell differentiation, or both. In addition, CGRP increased the number and size of bone colonies in vitro (6, 18).

Nerve fibers staining for substance P, a well-known nociceptive signaling molecule typically associated with sensory nerves, enter the bone marrow in association with vessels (23). Substance P has been shown to increase osteoblast differentiation, bone colony formation, and osteoblast cyclic AMP production (2). Table 1 shows the neuropeptide receptors on bone.

### Beta-adrenergic signaling
Among all post-synaptic beta-adrenergic receptors (β1AR, β2AR and β3AR), β2AR is the main, if not the only adrenergic receptor expressed in osteoblasts (5). Activated βARs couple to Gs proteins to activate adenyl cyclase, which increases cAMP intracellular levels. Increased cAMP levels then activate protein kinase A (PKA), which can phosphorylate various protein targets, including transcription factors, kinases and cell surface receptors, including β2AR. This signaling system is fully functional in osteoblasts, as demonstrated by the increase in intracellular cAMP following osteoblast stimulation with norepinephrine or with βAR pharmacological agonists such as isoproterenol (5).

The involvement of the sympathetic nervous system (SNS) in the regulation of bone mass has been demonstrated both pharmacologically and genetically by an increase in osteoblast number and activity and a subsequent increase in bone mass in mice charac-

### Table 1 - Receptors for neuropeptides on bone cells.

<table>
<thead>
<tr>
<th>Neuropeptide</th>
<th>Osteoblast receptors</th>
<th>Osteoclast receptors</th>
<th>Bone formation</th>
<th>Bone resorption</th>
<th>Osteoclast formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGRP</td>
<td>+</td>
<td>+</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>SP</td>
<td>+</td>
<td>+</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>VIP</td>
<td>+</td>
<td>+</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>NMDA</td>
<td>+</td>
<td>+</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

CGRP: calcitonin gene-related peptide; SP: substance P; VIP: vasoactive intestinal peptide; NMDA: glutamate receptors.
Crossstalk between the brain and bone

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 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 (10). 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 is a peptide secreted by adipocytes acting on hypothalamic control body weight (26). Leptin-deficient ob/ob mice exhibit a phenotype with marked increases in body weight, also evident in ob/ob mice (10). In this manner, as calorie restriction reduces body weight, central NPY levels rise (37) and bone formation is inhibited, conserving limited energy resources. In addition to the central actions in the hypothalamus, NPY appears to provide a local circuit in the osteoblast. A direct effect of NPY on osteoblastic activity is not altered by NPY receptor null mice by their decrease in hypothalamic CART expression and their increased resorption, thereby implicating CART as a potential regulator of bone resorption.

Recent studies have elucidated an important role for serotonin production in the brainstem. Leptin inhibition of bone mass growth requires the integrity of specific hypothalamic neurons (10, 26). However, loss of leptin receptors from these neurons did not affect leptin action, suggesting that direct leptin signaling acts elsewhere in the brain to achieve these functions (10). Yadav et al. (29) have shown that 5-hydroxytryptamine (5-HT) or serotonin, is able to modulate leptin’s effects on bone mass. Recent studies have allowed the role of leptin pathway to bone, elucidating an important role for serotonin production in the brainstem. Serotonin acts via a multiple receptors (5-HT) to modulate numerous processes. These actions have now been expanded to include the regulation of bone mass (29). 5-HT receptors have been identified in all the major bone cell types (osteoblasts, osteocytes and osteoclasts), and stimulation of these receptors influences bone cell activities (30, 31). A number of in vitro studies have confirmed the functionality of 5-HT signaling in bone cells, but offer contrasting evidence as to its effects. Some suggest a direct stimulatory effect on bone formation pathways with 5-HT increasing prostaglandin E2 release from osteocyte-like (MOLO) cells and enhancing proliferation of MC3T3-E1 cells and primary human osteoblasts (32). In contrast, other investigations suggest an inhibitory effect of 5-HT on bone formation with 5-HT reducing proliferation of primary osteonecrotic mice (33) and inhibiting nitric oxide release from mouse-derived osteoblasts (34). Interestingly, gut-derived 5-HT acted as a downstream mediator for the entire skeletal effects of LRP5. To establish a more compelling link between LRP5, 5-HT and bone, transgenic mice with either gut- or osteoblast-specific loss- or gain-of-function of Lp5 were generated. This was achieved by crossing mice harboring either a fixed loss- or gain-of-function allele of Lp5 with either Villin (gut-specific) or collagen type I (osteoblast-specific) promoter-driven Cre transgenic mice. Gut-specific deletion of Lp5 recapitulated the high circulating 5-HT and skeletal phenotype of Lp5-/- mice, whereas osteoblast-specific deletion did not (33). In addition, 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 serotoninergic neurons in the brainstem recapitulates them. Specifically, central serotonin stimulates bone mass accrual (through binding to HTR2C receptors on ventromedial hypothalamic neurons and apetite via HTR1A and 2B receptors on anorexic neurons). One of the molecule involved in the leptin-bone pathway is neuromedin U (NMU). NMU is a neuropeptide expressed in hypothalamic neurons and in the small intestine, and is regulated by sympathetic activation (10, 35). However, it has also been shown to regulate bone mass. NMU 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 receptor are not detectable in bone, and osteoblast activity is not altered by in vitro (10). In hypothalamicus is also broadly expressed in several other systems (10).

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cellular bone loss and cortical expansion due to increased bone turnover (42). In vitro studies indicate that CB2 signaling contributes to the maintenance of bone mass by two mechanisms: i) stimulating stromal cells/osteoclasts directly; and ii) inhibiting monocytes/osteoclasts RANKL expression both directly and indirectly (10).

Conclusions

In conclusion, the simultaneous maintenance of bone mass, mechanical integrity, and mineral homeostasis by the process of bone remodeling requires a complex regulatory milieu. These neural signals convey rapid and often marked effects on osteoblast and osteoclast activity, and thus present tempting therapeutic potential. Further research in this field will allow a better understanding of the basic mechanisms of neural control on skeletal cells, and could provide new pathways for the study of skeletal development and growth, fracture healing, osteoporosis, arthropathies or even neoplasias. Experimental manipulation 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.

References