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Review

Neuronal signaling and the regulation of bone remodeling

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Abstract. An increasing number of studies suggest that nerve-derived signals play an important role in the regulation of bone remodeling. Neuropeptides and receptors/transporters of adrenergic, glutaminergic, serotoninergic, dopaminergic and sensory nature have been described in osteoblasts in vitro. Downstream signaling pathways and targets genes have been identified, but the in vivo relevance of these findings remained controversial until more recent gene gain and loss of function studies confirmed the role of CGRP and β2-adrenergic receptor signaling in osteoblasts. Tissue and time-conditional mutant mice originally generated for studies unrelated to bone are now available tools to determine the role of neuronal signaling in bone and to dissociate the central and peripheral role of these signals. Lastly, understanding how the central nervous system integrates homeostatic signals with the regulation of bone homeostasis will be the next exciting subject of research in the field.

Key words. Bone; osteoblast; osteoclast; adrenergic receptor; neuropeptide

Introduction

The skeleton is composed of more than 200 bones spread out throughout the body. Bone elements are formed from mesenchymal condensations during embryonic development and progressively get their shape and complex assemblage through patterning. During the process of endochondral ossification in long bones, a cartilaginous template mostly made of chondrocytes is formed and is then progressively replaced by a bony tissue, a process involving vascular invasion and the action of osteoclasts and osteoblasts [1–3]. Osteoclasts are multinucleated cells of monocyte origin whose function is to degrade calcified bone tissue through a proteolytic process [4, 5]. Osteoblasts are cells of mesodermal origin whose main function is to secrete a collagen-rich extracellular matrix that will eventually become mineralized, exclusively in bone [6]. Beyond development, bone is still a physiologically active and reactive tissue responding to hormonal, paracrine/autocrine and mechanical signals necessary to release minerals in the blood stream and to adapt to physiological and mechanical demands such as pregnancy and exercise. This responsiveness of bones is accomplished by the action of both osteoclasts and osteoblasts through the process of bone remodeling, a mechanism that allows the conservation of an appropriate architecture and constant bone mass during adulthood. Defects in bone remodeling, generally induced by the imbalance between osteoblast and osteoclast activity, lead to debilitating conditions such as osteoporosis, a low bone mass disease characterized by increased fracture rates, or osteopetrosis, a congenital high bone mass disease characterized by defective osteoclast function and fractures [7–10].

Bone remodeling is a slow process regulated by several hormonal, paracrine/autocrine, mechanical and transcriptional signals whose targets can be osteoclasts and osteoblasts at various stage of their lifespan [11, 12]. The existence of a bone remodeling regulatory arm with neuronal characteristics was first suggested from clinical observations reporting patients with head trauma and

post-injury increase in osteogenic activity [13], from patients with reflex sympathetic dystrophy who display localized sympathetic hyperactivity and osteopenia, and from the association between patients stroke, spinal cord injury or neurological disorders and osteopenia (low bone mass), bone fragility and poor fracture healing [14–19]. Decades later these first reports, a number of in vitro studies and recent investigations using in vivo experimental models and mouse genetics confirmed the notion that bone homeostasis is under the influence of central and peripheral neuronal control. This review focuses on the influence of the peripheral nervous system on bone cells.

Neuropeptides and neuropeptide receptors in bone

A number of histological studies revealed the existence of neuropeptides in bones, including enzymes and neuropeptides of sensory, sympathetic and glutaminergic types. Substance P (SP), α-calcitonin gene-related peptide (CGRP), vasointestinal peptide P (VIP), pituitary adenylate cyclase activating peptide (PACAP), neuropeptide Y (NPY), serotonin, glutamate, tyrosine hydroxylase (TH) and norepinephrine are among the neuronal products that have been detected in bone [23–32]. Other studies have characterized the expression of the receptors for these neuropeptides to specific bone cell lineages, including osteoblasts, osteocytes and osteoclasts (table 1). Detailed examination revealed a regional distribution of these neuropeptides within bone elements. For instance, NPY and TH-containing fibers are mostly distributed close to blood vessels, while VIP, SP and CGRP-containing fibers form abundant varicosities in the vicinity of epiphyseal trabecular bones and in the periosteum, where osteogenesis is active [23, 24, 33]. NPY, VIP and TH are localized in the periosteum as non-vascular fibers. Neurons containing SP and CGRP usually enter bones in association with blood vessels but dissociate from the vessels and terminate as free endings in the marrow, whereas cortical innervation by these neurons is sparse [27, 28, 33–36]. Differences of bone innervation among various elements of the skeleton have not been assessed so far but may correlate with preferential response to loading or hormonal signals at specific anatomical sites. Interestingly, the 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 (TH and NE-positive) when they reach the periosteal region of the sternum, but switch their properties to cholinergic/peptidergic traits (acetylcholine transporter and VIP-positive) after contact with the sternum tissue during the first two post-natal weeks. These data suggest that the targeted bony tissue plays a role in determining neurotransmitter type in innervating neurons [37]. Using osteoblast/neuron co-culture assays to search for the bone-derived mediator(s) triggering these effects, Asmus et al. demonstrated that immature osteoblasts release soluble factor(s) of the neuropoietic cytokine family to induce choline acetyltransferase activity in sympathetic neurons [38].

Beside these anatomical studies, a number of experimental investigations have also addressed the potential role of sensory, bioamine, glutaminergic and adrenergic neuropeptides and downstream intracellular mediators in bone cell biology by in vitro and more recently in vivo analyses.

Sensory neuropeptides

CGRP is a sensory neuropeptide whose function in bone metabolism is now well demonstrated. CGRP is generated by alternative splicing from the *Calca* gene in cells of the central and peripheral nervous system [39]. Osteoblastic cells are equipped with receptors to CGRP and respond to CGRP by an increase in intracellular cyclic AMP (cAMP) and calcium and an acute efflux of potassium accompanied by hyperpolarisation, which leads to changes in cell morphology and function [40 42]. CGRP stimulates osteoblast proliferation [43], the synthesis of growth factors and cytokines (including insulin-like growth factor 1 (IGF-1, which is known to stimulate osteoblast proliferation), collagen synthesis and bone formation [44]. CGRP also increases the number of bone colonies formed from bone marrow stromal cells in vitro and induces *Bmp2* expression in cells isolated from human pulpal explants [45–47]. These actions of CGRP on osteoblasts could be relevant to the extensive remodeling of bone that occurs following fracture, since fracture repair is associated with a rapid proliferation of CGRP immunoreactive nerves [48, 49]. CGRP secreted by nerve terminals may therefore modulate osteoblasts by a direct mechanism under basal or osteo-inductive conditions. Interestingly, CGRP may also function as an autocrine factor since it is expressed by osteoblasts as well [33, 50]. In agreement with this hypothesis, transgenic mice overexpressing CGRP in differentiated osteoblasts display a bone phenotype characterized by an increased bone volume due to an increase in the rate of bone formation, an index of osteoblast activity [51]. In addition, injection of CGRP to rat partially protects them from gonadectomy-induced bone loss [52], while mice deficient for CGRP are mildly osteopenic due to a decrease in bone formation [53]. Regardless of its origin, these results suggest that CGRP acts as an anabolic factor for bone.

CGRP-containing fibers have been shown to contact osteoclasts within the bone microenvironment, suggesting

Table 1. Neuropeptides and neuropeptide receptors in bone.

Neuropeptide	Osteoblats	Osteocytes	Osteoclasts
CGRP	OHS-4, MG-63, Saos-2, HOS-TE85, primary osteoblasts [32, 42, 50]		
CGRP receptor	primary osteoblasts MC3T3-E1 UMR106 Saos2 [32, 41, 43, 44] [40, 119]		primary osteoclasts [55, 58]
Substance P receptor	primary osteoblasts [40]		primary osteoclasts [120]
VIP receptor	MC3T3-E1 UMR106 Saos2 [32, 40, 121]		primary osteoclasts [60, 61]
PACAP receptor	primary osteoblasts [121]		primary osteoclasts [60, 61]
Serotonin receptors	primary osteoblasts, ROS 17/2.8, UMR 106-H5, and Py1a [68, 69]	bone osteocytes [68]	primary osteoclasts
Serotonin transporter (5-HTT)	primary osteoblasts, ROS 17/2.8, UMR 106-H5 and Py1a [69]		primary osteoclasts RAW264.7 [73]
Glutamate	bone and primary osteoblasts MG63, TE85, SaOS-2 [74]		no
Glutamate receptors	bone and primary osteoblasts MG63, SaOS-2 [77, 78, 81, 83, 85]	bone osteocytes [77]	Bone, primary osteoclasts, Raw264.7 [77, 81, 89, 92–94, 122]
Glutamate transporter	bone osteoblasts, SaOS-2[79]	bone osteocytes, MLO-Y4 [79]	
NPY receptor	UMR106 [32] Not detected in bone [123]		
β-adrenergic receptors	β1: SaOS-2, OHS-4, and TE-85 β2: primary osteoblasts, UMR106, MG63, ROS 17/2.8, Saos2 [40, 31, 32] α1: MC3T3-E1 [124, 125]		

that CGRP may also control osteoclast differentiation or function [34, 54]. Supporting this hypothesis, CGRP inhibits bone resorption in vitro [55 58]. However, no bone resorption abnormalities have been observed in *CGRP*-deficient mice [53].

Two other sensory neuropeptides, VIP and SP, may have a role in bone biology and osteoclast biology more specifically. VIP belongs to a family of structurally related peptides including secretin, glucagons, gastric inhibitory peptide, growth hormone releasing factor and PACAP. It is a neuromodulator and a neurorotrophic factor involved in neuronal growth, differentiation, survival and transmitter synthesis. VIP may play an important role in the local control of osteoclast formation, because destruction of nerves expressing VIP by guanethidine treatment induces a concomitant 50% increase in osteoclast-covered surface in the mandible and calvariae [27]. In agreement with this result, VIP binds osteoclasts and inhibits osteoclastogenesis induced by $1,25(OH)_2$-vitamin D3 [59–61]. VIP may also control bone resorption by indirectly stimulating PGE2 expression in osteoblasts [62, 63]. Substance P is another neuropeptide richly expressed in small sensory neurons that innervate bones. Lesioning studies using capsaicin led to the conclusion that capsaicin-sensitive sensory neurons contribute to the maintenance of trabecular bone integrity. Capsaicin-treated rats displayed bone loss, increased bone resorption and decreased bone formation associated with destruction of SP and CGRP-positive unmyelinated sensory neurons [64]. These results are in agreement with the human familial dysautomia disease characterized by the loss of unmyelinated sensory neuron, reduced bone mineral density and frequent fractures [17, 65].

A more classical role of sensory nerves in bones has been investigated by testing pain perception in a mouse model deficient for TRPV1 (transient receptor potential vanilloid subtype 1). Bones receive a rich innervation by sensory fibers that express TRPV1, a receptor that can be activated by acidosis. Interestingly, acidosis is known to occur in osteolytic and osteoblastic cancer bone lesions. Administration of a TRPV1 antagonist or disruption of

the TRPV1 gene resulted in a significant attenuation of nocifensive behaviors [66], demonstrating that sensory innervation of bone is also involved in pain perception.

Bioactive amines

The central serotoninergic system is well known to modulate mood, emotion, sleep and appetite, while the dopaminergic system is involved in processing reward information and learning. In this family of neuropeptides, dopamine but more likely serotonin (5-HT) appears to play a role in bone biology.

The dopamine transporter (DAT) is an important determinant of dopamine signaling activity since it is responsible for the rapid uptake of released dopamine into presynaptic terminals, and therefore for efficient clearance of extracellular dopamine and termination of dopamine signaling. *Dat*-deficient mice have been generated and displayed a low bone mass phenotype, which suggested that dopamine signaling is involved in the regulation of bone mass [67]. However, there is no evidence so far that DAT is expressed in osteoblasts or in the bone micro-environment; therefore, it is unknown whether this bone phenotype originates from a peripheral or possibly a central defect.

In contrast, another member of the family of neurotransmitter transporters for bioactive amines, the serotonin transporter (5-HTT), is expressed in bones along with most of the 5-HT receptors [68]. 5-HTT uptakes 5-HT from the extracellular space and therefore downregulates serotoninergic activity. Binding and uptake studies have confirmed the functionality of these receptors and transporters in osteoblasts [69]. In vitro mechanistic studies allowed the identification of protein kinase C (PKC) as a kinase involved in 5-HTT phosphorylation and the downregulation of 5-HTT activity in osteoblastic cells. They also demonstrated an influence of 5-HT signaling on AP1 transcription factor binding activity regulated by PTH, suggesting that 5-HT signaling is a functional component involved in osteoblast differentiation [69]. As observed in patients treated with selective serotonin-reuptake inhibitors (SSRIs) for depression [70], blocking 5-HTT activity by SSRIs in mice led to a significant decrease in bone mass due to a decrease in bone formation [71]. In agreement with these pharmacological interventions, null mutation of the gene coding for 5-HTT induced a low bone mass phenotype due to decreased bone formation [71]. Among the different 5-HT receptors expressed in bones, the 5-HT receptor 2B subtype is of particular interest since it appears to be involved in mechanical sensing. This receptor subtype is expressed in osteoblasts, but stronger expression was detected in osteocytes, the bone-embedded cells considered to act as sensor cells that translate mechanical stimuli resulting from gravitational and muscular forces on the skeleton into biochemical signals [72]. Reinforcing a role of this receptor in the mechano-sensing function of osteocytes, a 5-HT2 receptor serotonin analog decreased nitric oxide release by mechanically stimulated osteoblasts [68]. Because nitric oxide is a signaling molecule released upon mechanical stimulation of osteoblasts and osteocytes, these data suggest that osteocytes are under the control of neurogenic signals for their response to mechanical stimuli. However, the origin of 5-HT within the bone micro-environment, i.e. nerve release or serum diffusion, will need to be addressed. Finally, 5-HT signaling could be involved in osteoblast proliferation during bone fracture repair. This notion is based on two observations: (i) serotonin is released by coagulated platelets that are present within the bone environment following fractures, and (ii) treatment of osteoblast precursors with a 5-HT2B agonist or antagonist increased and decreased proliferation, respectively [68]. Therefore, the existence of functional serotoninergic signaling in osteoblasts, the in vivo bone phenotype of mice characterized by increased serotonergic signaling and the results of in vivo pharmacological studies all strongly support a role of serotonin in the regulation of bone formation and bone repair.

Serotoninergic signaling could also participate in the regulation osteoclastogenesis. Battaglino et al. detected functional 5-HTT in RANKL-treated immature osteoclasts by gene micro-arrays and an increase in 5-HTT expression in differentiated osteoclasts [73]. Moreover, the 5-HTT inhibitor fluoxetine (Prozac) inhibited osteoclast differentiation, while inhibition of 5-HT intracellular transport or the addition of 5HT stimulated osteoclast differentiation [73]. Several receptor types for 5-HT (5-HT1B, 5-HT2B and 5-HT4) are expressed by osteoclasts as well and could be involved in 5-HT signaling. Supporting this contention, specific antagonists of receptor 1B and 4 inhibited the formation of differentiated osteoclasts in vitro, suggesting that these receptors are required for the early steps of osteoclast differentiation. Interestingly, nuclear factor kappa B (NF-kB) activation in osteoclasts treated with RANKL and fluoxetine was inhibited by 80% compared with cells treated by RANKL alone [73], suggesting that elevations in cytoplasmic levels of 5-HT may be required to enhance NF-kB activation through mechanisms to be characterized. The absence of a bone resorption phenotype in mice deficient for 5-HTT, however, suggests that the net effect of the serotonergic system on bone mass is likely to be complex in vivo [71].

Glutaminergic signaling

L-Glutamate is a major excitatory amino acid neurotransmitter in the central nervous system, but it appears in the light of recent studies to play a role in skin, pancreas and bone as well. Bone cells, including osteob-

lasts, osteoclasts and osteocytes, are equipped with the molecular machinery necessary for glutamate release, extracellular recovery and glutamate response. Osteoblasts are in close association with glutamatergic nerve endings, contain glutamate-filled vesicles [74], express the glutamate transporter GLAST-1 and the glutamate receptors iGluR (NMDA, AMPA and kainite ionotropic-type glutamate receptor) as well as mGluR (metabotropic-type glutamate receptor 1, 4 and 8) [75–80]. A number of signaling molecules known to associate or colocalize with iGluRs have been detected in osteoblasts as well, including Yotio, PSD95, GRIP and SHANK [81, 82], but their role in glutamate signaling and bone biology is still speculative. The functional role of glutamate signaling in bone biology has been assessed mainly by in vitro cell-based analyses. Stimulation of osteoblast post-synaptic glutamatergic proteins by L-glutamate or N-methyl-D-aspartate (NMDA) triggers membrane depolarization, influx of calcium and a rapid increase in membrane current [83]. Blockade of iGluR in osteoblasts inhibits their differentiation; however, the downstream target gene(s) activated in response to glutamate signaling have not yet been identified [84–87]. Results from in vivo studies and from mutant animal models supporting a role for this signaling system in vivo are still scarce, which is partly due to the fact that available glutamate receptor antagonists quite severely affect neurological functions precluding or interfering with bone analyses. It is also due to the diversity of receptors and transporters mediating glutamate signaling and redundancy issues. Still, a few glutaminergic signaling-deficient mouse models have been analyzed for their potential bone phenotype. Mice that underexpress *NMDAR1* are smaller than littermates expressing normal levels of *NMDAR1*, which may reflect a disruption in skeletal development [88]. In contrast, NMDA subunit *NR1*-deficient mice did not show any obvious bone phenotype [89], and no significant bone phenotype has been detected in *Glast*-deficient mice [90]. More detailed studies assessing more accurately bone formation, bone resorption, bone mechanical properties and bone architecture parameters may reveal bone defects not detected in these studies. Interestingly, however, *Glast* expression is downregulated by mechanical loading, which suggests that this glutamate transporter may be involved in coupling mechanical signals to skeletal modeling [79, 91].

Like osteoblasts, osteoclasts express functional iGluR and mGluR as well as Glu transporters with characteristics similar to neuronal cells [92, 93, 89]. Inhibition of NMDAR by specific antagonists or antibodies in vitro inhibited osteoclast differentiation and bone resorption [77, 89, 93, 94]. It is not clear whether the mechanism involves inhibition of osteoclast activity or differentiation. Activation of NMDAR in RAW264.7 osteoclastic cells by specific agonists induced nuclear translocation of NF-kB, a pivotal factor for osteoclast differentiation [93], which suggests that NF-kB is involved in glutamate regulation of osteoclast formation.

In conclusion, glutaminergic signaling in bone is a very exciting concept supported by a number of cell-based studies, but so far the in vivo relevance of this system is still not well demonstrated and its function not well understood.

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 [29, 31, 32, 95]. Adrenergic receptors belong to a large family of membrane proteins that transduce signals through heterodimeric guanine-nucleotide-binding proteins (G-proteins). Activated βARs couple to Gsα proteins to activate adenylyl 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. This action of catecholamines on osteoblasts is specifically mediated by β2AR. Indeed, β2AR-specific agonists stimulate cAMP formation in osteoblasts as opposed to α and β1-specific agonists, and this effect is blocked by the β-blocker propranolol and *Adr*β2 deficiency [31, 40, 96, 97, 95].

β2AR is mostly known for its role in the regulation of cardiovascular, uterine and airway smooth muscle functions. However, recent studies demonstrated the essential role of this receptor in bone biology. These studies were initiated following the discovery that leptin, a pleiotrophic adipocyte-derived hormone, is an inhibitor of bone formation acting via a hypothalamic relay [98]. These studies were the first to uncover the role of hypothalamic centers in the control of bone mass and supported the notion that osteoblasts could be under the control of neuronal signals originating from the central nervous system. In the search of the mechanisms whereby hypothalamic neurons control osteoblast function and bone mass, the sympathetic nervous system (SNS) was identified as the downstream mediator of leptin antiosteogenic function, linking central hypothalamic neuron signaling to bone cells [31]. The involvement of the 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 characterized by low sympathetic tone, such as mice treated with the β-blocker propranolol, mice deficient for *dopamine*

β-*hydroxylase* (*Dbh*), the step-limiting enzyme responsible of catecholamine synthesis, and leptin-deficient *ob/ob* mice. Conversely, mice treated with the beta-agonist isoproterenol, used in these studies as a surrogate of SNS hyperactivity, displayed a marked decrease in osteoblast number, activity and bone mass [31, 98]. More recently, the analysis of mice deficient for *Adr*β2 confirmed the involvement and sole causal origin of β2AR signaling in the high bone mass phenotype of *Adr*β2–/–, *ob/ob*, *Dbh–/–* and propranolol-treated mice. *Adr*β2–/– mice displayed a high bone mass phenotype similar to the one observed in *ob/ob* mice, without any of their endocrine abnormalities, while *Adr*β*1–/–* mice had a normal bone mass (table 2) [96]. Altogether, these results indicate that (i) β2AR activation decreases bone formation by osteoblasts, (ii) β2AR is the main mediator of sympathetic signaling in osteoblasts, and (iii) the endocrine abnormalities plaguing *ob/ob* and *Dbh-/-* mice are not responsible for their increased bone formation and bone mass.

The downstream target genes of β2AR signaling in osteoblasts are not well characterized. One of them could be *c-fos*, which binds to AP1-responsive bone genes such as *Alkaline phosphatase, Osteocalcin* and *alpha 1 type I collagen* as a dimer with c-jun [29, 99]. Genes involved in osteoblast proliferation or apoptosis may be a target of β2AR signaling because osteoblast number and surface are increased in propranolol-treated mice, in *Adr*β2–/– mice and in *Dbh–/–* mice and are decreased in wild-type mice receiving isoproterenol or intracerebroventricular infusion of leptin (which activates sympathetic signaling). However, the signaling mechanisms and the precise genes involved remain to be characterized.

Table 2 Correlation sympathetic tone/bone mass in various mouse models.

Mouse model	Defect	Bone phenotype
WT+isoproterenol	pharmacological adrenergic stimulation	low bone mass
Ob/ob	low SNS activity	high bone mass
WT+propranolol	pharmacological adrenergic blockade	high bone mass
Dbh–/–	absence of catecholamines	high bone mass
*Adr*β2*–/–*	genetic adrenergic blockade	high bone mass
*Adr*β*1–/–*	genetic adrenergic blockade	normal bone mass

Beta-adrenergic signaling in osteoblasts increases bone resorption

Rankl is so far the only well-characterized gene whose expression has been shown to be regulated by β2AR signaling in osteoblasts. RANKL is a critical factor inducing osteoclast differentiation. It is secreted by osteoblasts and its action on osteoclasts is antagonized by a soluble receptor called osteoprotegerin (OPG) [100, 101]. Mice deficient for *Rankl* are severely osteopetrotic due to the lack of osteoclasts and the absence of bone resorption [102]. While pharmacologic treatment of wild-type mice with propranolol did not significantly affect bone resorption at the dose and regimen shown to increase bone formation [31], complete genetic β2AR signaling blockade in *Adr*β2*–/–* mice led to a decrease in bone resorption, suggesting that the SNS does regulate osteoclast differentiation or function [96]. Using osteoblast/osteoclast co-culture experiments and combinations of wild-type and *Adr*β*2–/–* cells, we could demonstrate that adrenergic signaling stimulates osteoclast differentiation indirectly by increasing *Rankl* expression in osteoblasts, via β2AR [96]. Both parathyroid hormone (PTH) [103] and β-adrenergic agonists can therefore upregulate *Rankl* expression in osteoblasts and subsequently bone resorption by osteoclasts. However, although both signals use G-coupled-protein receptors, increase cAMP intracellular levels and activate PKA, they employ different signaling distal pathways: PTH activates cAMP response element-binding protein (CREB), while β_2-adrenergic stimulation leads to the phosphorylation of ATF4, a CREB family member previously shown to control osteoblast terminal differentiation and collagen synthesis [104]. ATF4 phosphorylation by PKA in response to isoproterenol induces ATF4 binding to a CRE-like site within the *Rankl* promoter, which activates the transcriptional mechanisms leading to *Rankl* expression (fig. 1). The fact that isoproterenol could not induce *Rankl* expression in *Atf4-/-* osteoblasts is a strong evidence that β2AR signaling requires ATF4 to induce *Rankl* expression [96]. Finally, *Adr*β2 and *Atf4* are expressed in immature osteoblasts, while *PTH receptor* expression peaks in more differentiated osteoblasts. The distinct signaling pathways leading to the stimulation of *Rankl* expression and the expression of β2AR and PTHR at different stages of osteoblast differentiation therefore suggest that adrenergic and PTH signaling regulate *Rankl* expression and bone resorption by acting on different populations of osteoblasts (fig. 2).

In independent studies, in vitro treatment of osteoblasts with epinephrine increased interlenkin (IL)-6 and IL11 expression, two cytokines stimulating osteoclast differentiation during inflammatory conditions in vivo. This effect of epinephrine appeared to involve PKA and P38 MAPK [105]. As opposed to β1AR, β2AR exhibits a higher selectivity for norepinephrine than epinephrine. Moreover, in vivo adrenalectomy, aiming at decreasing epinephrine serum levels, did not significantly affect bone mass. And, lastly, *Adr*β*1–/–* mice did not have a

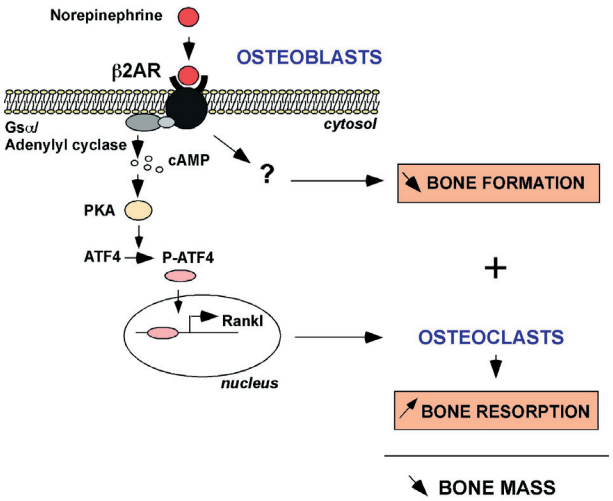

Figure 1. Adrenergic signaling in osteoblasts decreases bone mass. Following binding to β2AR, norepinephrine increases *Rankl* expression and bone resorption via a signaling pathway involving Gsα, adenylyl cyclase, the phosphorylation of ATF4 by activated PKA and the binding of ATF4 to a CRE-like site in the *Rankl* promoter. The signaling pathway leading to reduced bone formation has not yet been characterized.

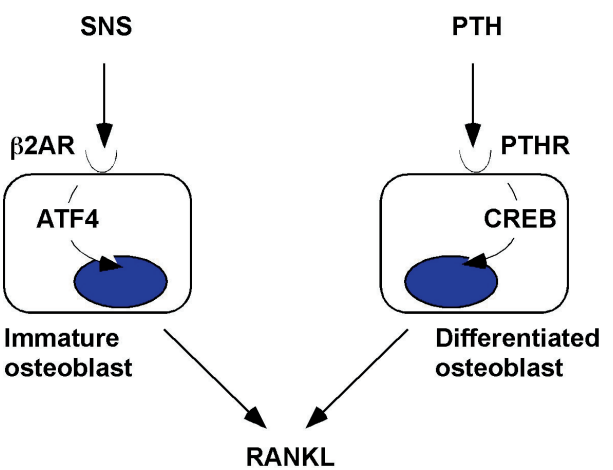

Figure 2. Adrenergic and PTH signaling may stimulate *Rankl* expression in different osteoblast populations. Adrenergic signaling requires β2AR and ATF4, which are both mainly expressed in immature osteoblasts. PTH signaling requires PTHR1 and CREB, which are expressed in differentiated osteoblasts.

high bone mass phenotype [96, 31]. Altogether these observations suggest that norepinephrine, mainly released from nerve endings, rather than epinephrine from the blood circulation, regulates bone resorption and bone formation via β2AR in vivo under normal conditions.

cAMP-specific phosphodiesterases (PDEs) may be additional intracellular mediators of β2AR signaling in osteoblasts based on their role in hydrolyzing intracellular cAMP generated by adenylate cyclase. Supporting this hypothesis, phosphodiesterase inhibitors such as IBMX applied to osteoblast/osteoclast co-cultures increased osteoclast formation by inducing *Rankl* expression by osteoblasts, a process inhibited by a PKA inhibitor [106, 107]. Among all PDEs, PDE4 was demonstrated to be responsible for the induction of *Rankl* expression in osteoblasts. However, PDE inhibitors injected daily in vivo had an anabolic effect [108], induced ERK1/2 and P38 MAPK and increased osteoblast differentiation, but did not significantly affect resorption parameters [109].

Regardless of the nature of the intracellular signaling pathways activated in osteoblasts by β2AR stimulation, these studies indicate that increased sympathetic signaling in vivo influence both arms of bone remodeling, i. e. it decreases bone formation and increases bone resorption, by acting on one cell type, the osteoblast. From a clinical point of view, these findings suggest that β-blockers could be used as bone anabolic and anti-catabolic agents for the treatment or prevention of bone remodeling diseases. Supporting this hypothesis, retrospective clinical studies of patients treated with β-blockers revealed a lower incidence of fractures and higher bone mineral density, while other studies did not detect any influence [110 113]. Additional prospective and well-controlled studies will be necessary to confirm these results.

Signal transmission from nerve to bone cells and signal propagation

Even though immunocytochemistry and electronic microscopy images suggest the existence of close contacts between osteoblasts and nerve endings, the overall pattern of nerve distribution within the bone microenvironment suggests that only a limited number of osteoblasts are in direct contact with nerve terminals [80, 31]. This raises the hypothesis that signal transduction by neuropeptides could be non-synaptic and/or that neurotransmitter primary signals are transduced to groups of osteoblasts via intercellular junctions. The possibility of non-synaptic secretion of neuropeptides that diffuse through the volume of extracellular space and not only act within a synaptic cleft before encountering their receptors is supported by the observation that CGRP-containing fibers innervating epiphyseal trabeculae are scarcely covered by Schwann cell sheaths, as opposed to fibers innervating non-skeletal elements that use fast synaptic transmission [34]. Propagation of neuronal signals via intercellular junctions is likely since osteoblasts and osteocytes are well known to communicate and exchange molecules and signals through GAP junctions. Interestingly, increased levels of cAMP in osteoblastic cells in vitro (as observed following β2AR stimulation) led to the phosphorylation of connexin 43, an oligomeric protein composing intercellular channels and linking adjacent osteoblasts and osteocytes, and increased its targeting to the membrane, which

favors intercellular diffusion [114]. The physiological role of gap junctions in osteoblasts is supported by the delayed ossification and osteoblast dysfunction of *Cx43*-null mice [115]. Resistance of *Cx43–/–* mice to neuronal signaling would support the hypothesis that nerves signal to osteoblast units via gap junctions, but so far no data supporting this hypothesis are available. The pattern of nerve distribution in bones also raises questions about how nerves transmit signals to osteoclasts. Based on the fact that osteoclasts are quite mobile and isolated cells attached to bone trabeculae (as opposed to units of functional osteoblasts), it is likely that nerves would use a diffusion mechanism to transmit signals to osteoclasts rather than direct synaptic contacts.

Conclusion

A growing amount of evidence has accumulated over the last 20 years that demonstrates the involvement of neuronal signaling in the process of bone remodeling. The increasing availability of mutant animal models and receptor-selective pharmacological drugs now allows us to dissect the role of specific neuronal systems in this process both in vitro and within the complex physiological environment of living mammals, which should further provide solid experimental proofs of the role of these neuronal components in bone. The broad expression of neuropeptides in the central and peripheral nervous systems as well as in diverse peripheral tissues such as bone raised doubts about the peripheral or central origin of these signals in the control of bone remodeling. The use of inducible cell-type conditional and time conditional mutant mice should allow a better characterization of these signaling systems by blocking neuronal signals in specific cells and at specific time points during development.

This area of bone research which overlaps with neuroscience and obesity research is exciting for several reasons. From a fundamental point of view, understanding the complex homeostatic systems involved in the regulation of bone mass is still in the early stages, and much more remains to be discovered to explain how a complex array of signals of autocrine/paracrine, hormonal, mechanical and neuronal nature leads to a healthy and reactive skeleton. The concept that bone remodeling is controlled by the central nervous system and in particular the hypothalamus also raises a number of questions. Several hormones such as leptin and amylin are involved in the regulation of energy homeostasis, reproduction, immune defense and bone mass [116–118]. How the hypothalamus integrates energy availability, environmental changes and signals from the internal hormonal milieu to the regulation of bone remodeling is far from being understood. Next challenge of this area of research will also be the characterization of feedback loops originating either from bones or from other organs, which modulate brain center(s) involved in the control of bone mass. From a therapeutic point of view, identification of the central and peripheral components of these neuronal signaling systems will allow the characterization of additional targets of potential interest for the generation of bone anabolic and/or anti-catabolic drugs. Because of the complexity of hypothalamic regulations, however, it is likely that phamacological manipulation of peripheral targets will be more successful for the treatment of low bone mass diseases such as osteoporosis.

1 Karsenty G. and Wagner E.F. (2002) Reaching a genetic and molecular understanding of skeletal development. Dev. Cell **2**(4): 389 406
2 Martin T. J., Ng K. W. and Suda T. (1989) Bone cell physiology. Endocrinol. Metab. Clin. North Am. **18**(4): 833 858
3 Wagner E. F. and Karsenty G. (2001) Genetic control of skeletal development. Curr. Opin. Genet. Dev. **11**(5): 527 532
4 Suda T., Udagawa N. and Takahashi N. (1996) Cells of bone: osteoclast generation. In: Principles of Bone Biology, 87 102. Bilezikian J. P., Raisz L. G. and Rodan G. A. (eds), Academic Press, San Diego
5 Teitelbaum S. L. (2000) Bone resorption by osteoclasts. Science **289:** 1504 1508
6 Manolagas S. C. (2000) Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr. Rev. **21**(2): 115 137
7 Kleerekoper M. and Avioli L. V. (1998) Osteoporosis pathogenesis and therapy. In: Metabolic Bone Disease, L.V. Avioli and Krane S. M. (eds), Academic Press, San Diego
8 Melton L. J. 3rd (1993) Hip fractures: a worldwide problem today and tomorrow. Bone **14**(Suppl. 1): S1 8
9 Riggs B. L., Khosla S. and Melton L. J. 3rd (1998) A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. J. Bone Miner. Res. **13**(5): 763 773
10 Statement N. C. (2001) Osteoporosis prevention, diagnosis, and therapy. JAMA **285**(6): 785 795
11 Karsenty G. (1999) The genetic transformation of bone biology. Genes Dev. **13**(23): 3037 3051
12 Mundy G. R., Boyce B., Yoneda T., Bonewald L. F. and Roodman G. D. (1996) Cytokines and bone remodeling. In: Osteoporosis, vol. 1, 302313, Marcus R., Feldman D. and Kelsey J. (eds), Academic Press, San Diego
13 Perkins R. and Skirving A. P. (1987) Callus formation and the rate of healing of femoral fractures in patients with head injuries. J. Bone Joint Surg. Br. **69**(4): 521 524
14 Aro H. (1985) Effect of nerve injury on fracture healing. Callus formation studied in the rat. Acta Orthop. Scand. **56**(3): 233 237
15 Dauty M., Perrouin Verbe B., Maugars Y., Dubois C. and Mathe J. F. (2000) Supralesional and sublesional bone mineral density in spinal cord-injured patients. Bone **27**(2): 305 309
16 Freehafer A. A. and Mast W. A. (1965) Lower extremity fractures in patients with spinal-cord injury. J. Bone Joint Surg. Am. **47:** 683 694
17 Pearson J., Dancis J., Axelrod F. and Grover N. (1975) The sural nerve in familial dysautonomia. J. Neuropathol. Exp. Neurol. **34**(5): 413 424
18 Poole K. E., Reeve J. and Warburton E. A. (2002) Falls, fractures, and osteoporosis after stroke: time to think about protection? Stroke **33**(5): 1432 1436

19 Ramnemark A., Nyberg L., Lorentzon R., Englund U. and Gustafson Y. (1999) Progressive hemiosteoporosis on the paretic side and increased bone mineral density in the nonparetic arm the first year after severe stroke. Osteoporos Int. **9**(3): 269 275

20 Felten D. L., Ackerman K. D., Wiegand S. J. and Felten S. Y. (1987) Noradrenergic sympathetic innervation of the spleen: I. Nerve fibers associate with lymphocytes and macrophages in specific compartments of the splenic white pulp. J. Neurosci. Res. **18**(1): 28 36, 118 121

21 Felten D. L., Felten S. Y., Bellinger D. L., Carlson S. L., Ackerman K. D., Madden K. S. et al. (1987) Noradrenergic sympathetic neural interactions with the immune system: structure and function. Immunol. Rev. **100:** 225 260

22 Livnat S., Madden K. S., Felten D. L. and Felten S. Y. (1987) Regulation of the immune system by sympathetic neural mechanisms. Prog. Neuropsychopharmacol. Biol. Psychiatry **11**(23): 145 152

23 Bjurholm A. (1991) Neuroendocrine peptides in bone. Int. Orthop. **15**(4): 325 329

24. Bjurholm A., Kreicbergs A., Brodin E. and Schultzberg M. (1988) Substance P- and CGRP-immunoreactive nerves in bone. Peptides **9**(1): 165 171

25 Bjurholm A., Kreicbergs A., Terenius L., Goldstein M. and Schultzberg M. (1988) Neuropeptide Y-, tyrosine hydroxylase- and vasoactive intestinal polypeptide-immunoreactive nerves in bone and surrounding tissues. J. Auton. Nerv. Syst. **25**(2 3): 119 125

26 Goto T., Yamaza T., Kido M. A. and Tanaka T. (1998) Light- and electron-microscopic study of the distribution of axons containing substance P and the localization of neurokinin-1 receptor in bone. Cell Tissue Res. **293**(1): 87 93

27 Hill E. L. and Elde R. (1991) Distribution of CGRP-, VIP-, D beta H-, SP- and NPY-immunoreactive nerves in the periosteum of the rat. Cell Tissue Res. **264**(3): 469 480

28 Hohmann E. L., Elde R. P., Rysavy J. A., Einzig S. and Gebhard R. L. (1986) Innervation of periosteum and bone by sympathetic vasoactive intestinal peptide-containing nerve fibers. Science **232**(4752): 868 871

29 Kellenberger S., Muller K., Richener H. and Bilbe G. (1998) Formoterol and isoproterenol induce c-fos gene expression in osteoblast-like cells by activating beta2-adrenergic receptors. Bone **22**(5): 471 478

30 Sisask G., Bjurholm A., Ahmed M. and Kreicbergs A. (1996) The development of autonomic innervation in bone and joints of the rat. J. Auton. Nerv. Syst. **59**(1 2): 27 33

31 Takeda S., Elefteriou F., Levasseur R., Liu X., Zhao L., Parker K. L. et al. (2002) Leptin regulates bone formation via the sympathetic nervous system. Cell **111**(3): 305 317

32 Togari A., Arai M., Mizutani S., Mizutani S., Koshihara Y. and Nagatsu T. (1997) Expression of mRNAs for neuropeptide receptors and beta-adrenergic receptors in human osteoblasts and human osteogenic sarcoma cells. Neurosci. Lett. **233**(2 3): 125 128

33 Imai S. and Matsusue Y. (2002) Neuronal regulation of bone metabolism and anabolism: calcitonin gene-related peptide-, substance P- and tyrosine hydroxylase-containing nerves and the bone. Microsc. Res. Tech. **58**(2): 61 69

34 Imai S., Tokunaga Y., Maeda T., Kikkawa M. and Hukuda S. (1997) 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. **15**(1): 133 140

35 Irie K., Hara-Irie F., Ozawa H. and Yajima T. (2002) Calcitonin gene-related peptide (CGRP)-containing nerve fibers in bone tissue and their involvement in bone remodeling. Microsc. Res. Tech. **58**(2): 85 90

36 Mach D. B., Rogers S. D., Sabino M. C., Luger N. M., Schwei M. J., Pomonis J. D. et al. (2002) Origins of skeletal pain: sensory and sympathetic innervation of the mouse femur. Neuroscience **113**(1): 155 166

37 Asmus S. E., Parsons S. and Landis S. C. (2000) Developmental changes in the transmitter properties of sympathetic neurons that innervate the periosteum. J. Neurosci. **20**(4): 1495 1504

38 Asmus S. E., Tian H. and Landis S. C. (2001) Induction of cholinergic function in cultured sympathetic neurons by periosteal cells: cellular mechanisms. Dev. Biol. **235**(1): 1 11

39 Amara S. G., Jonas V., Rosenfeld M. G., Ong E. S. and Evans R. M. (1982) Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature **298**(5871): 240 244

40 Bjurholm A., Kreicbergs A., Schultzberg M. and Lerner U. H. (1992) 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. **7**(9): 1011 1019

41 Kawase T. and Burns D. M. (1998) Calcitonin gene-related peptide stimulates potassium efflux through adenosine triphosphate-sensitive potassium channels and produces membrane hyperpolarization in osteoblastic UMR106 cells. Endocrinology **139**(8): 3492 3502

42 Michelangeli V. P., Fletcher A. E., Allan E. H., Nicholson G. C. and Martin T. J. (1989) Effects of calcitonin gene-related peptide on cyclic AMP formation in chicken, rat, and mouse bone cells. J. Bone Miner. Res. **4**(2): 269 272

43 Cornish J., Callon K. E., Lin C. Q., Xiao C. L., Gamble G. D., Cooper G. J. et al. (1999) Comparison of the effects of calcitonin gene-related peptide and amylin on osteoblasts. J. Bone Miner. Res. **14**(8): 1302 1309

44 Vignery A. and McCarthy T. L. (1996) The neuropeptide calcitonin gene-related peptide stimulates insulin-like growth factor I production by primary fetal rat osteoblasts. Bone **18**(4): 331 335

45 Bernard G. W. and Shih C. (1990) The osteogenic stimulating effect of neuroactive calcitonin gene-related peptide. Peptides **11**(4): 625 632

46 Calland J. W., Harris S. E. and Carnes D. L. J. (1997) Human pulp cells respond to calcitonin gene-related peptide in vitro. J. Endod. **23**(8): 485 489

47 Shih C. and Bernard G. W. (1997) Calcitonin gene related peptide enhances bone colony development in vitro. Clin. Orthop. Relat. Res. **334:** 335 344

48 Hukkanen M., Konttinen Y. T., Santavirta S., Paavolainen P., Gu X. H., Terenghi G. et al. (1993) Rapid proliferation of calcitonin gene-related peptide-immunoreactive nerves during healing of rat tibial fracture suggests neural involvement in bone growth and remodelling. Neuroscience **54**(4): 969 979

49 Li J., Ahmad T., Spetea T., Ahmed M. and Kreicbergs A. (2001) Bone reinnervation after fracture: a study in the rat. J. Bone Miner. Res. **16**(8): 1505 1510

50 Drissi H., Hott M., Marie P. J. and Lasmoles F. (1997) Expression of the CT/CGRP gene and its regulation by dibutyryl cyclic adenosine monophosphate in human osteoblastic cells. J. Bone Miner. Res. **12**(11): 1805 1814

51 Ballica R., Valentijn K., Khachatryan A., Guerder S., Kapadia S., Gundberg C. et al. (1999) Targeted expression of calcitonin gene-related peptide to osteoblasts increases bone density in mice. J. Bone Miner. Res. **14**(7): 1067 1074

52 Valentijn K., Gutow A. P., Troiano N., Gundberg C., Gilligan J. P. and Vignery A. (1997) Effects of calcitonin gene-related peptide on bone turnover in ovariectomized rats. Bone **21**(3): 269 274

53 Schinke T., Liese S., Priemel M., Haberland M., Schilling A. F., Catala-Lehnen P. et al. (2004) Decreased bone formation and osteopenia in mice lacking alpha-calcitonin gene-related peptide. J. Bone Miner. Res. **19**(12): 2049 2056

54 Hara-Irie F., Amizuka N. and Ozawa H. (1996) Immunohistochemical and ultrastructural localization of CGRP-positive nerve fibers at the epiphyseal trabecules facing the growth plate of rat femurs. Bone **18**(1): 29 39

55 Akopian A., Demulder A., Ouriaghli F., Corazza F., Fondu P. and Bergmann P. (2000) Effects of CGRP on human osteoclast-

like cell formation: a possible connection with the bone loss in neurological disorders? Peptides **21**(4): 559 564
56 D'Souza S. M., MacIntyre I., Girgis S. I. and Mundy G. R. (1986) Human synthetic calcitonin gene-related peptide inhibits bone resorption in vitro. Endocrinology **119**(1): 58 61
57 Roos B. A., Fischer J. A., Pignat W., Alander C. B. and Raisz L. G. (1986) Evaluation of the in vivo and in vitro calcium-regulating actions of noncalcitonin peptides produced via calcitonin gene expression. Endocrinology **118**(1): 46 51
58 Zaidi M., Fuller K., Bevis J., GainesDas R. E., Chambers T. J. and MacIntyre I. (1987) Calcitonin gene-related peptide inhibits osteoclastic bone resorption: a comparative study. Calcif. Tissue Int. **40**(3): 149 154
59 Lundberg A., Lie A., Bjurholm A., Lehenkari P. P., Horton M. A., Lerner U. H. et al. (2000) Vasoactive intestinal peptide regulates osteoclast activity via specific binding sites on both osteoclasts and osteoblasts. Bone **27**(6): 803 810
60 Mukohyama H., Ransjo M., Taniguchi H., Ohyama T. and Lerner U. H. (2000) The inhibitory effects of vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide on osteoclast formation are associated with upregulation of osteoprotegerin and downregulation of RANKL and RANK. Biochem. Biophys. Res. Commun. **271**(1): 158 163
61 Ransjo M., Lie A., Mukohyama H., Lundberg P. and Lerner U. H. (2000) Microisolated mouse osteoclasts express VIP-1 and PACAP receptors. Biochem. Biophys. Res. Commun. **274**(2): 400 404
62 Hohmann E. L., Levine L. and Tashjian A. H. Jr (1983) Vasoactive intestinal peptide stimulates bone resorption via a cyclic adenosine 3',5'-monophosphate-dependent mechanism. Endocrinology **112**(4): 1233 1239
63 Rahman S., Dobson R., Bunning R. A., Russell R. G. and Brown B. L. (1992) The regulation of connective tissue metabolism by vasoactive intestinal polypeptide. Regul. Pept. **37**(2): 111 121
64 Offley S. C., Guo T. Z., Wei T., Clark J. D., Vogel H., Lindsey D. P. et al. (2005) Capsaicin-sensitive sensory neurons contribute to the maintenance of trabecular bone integrity. J. Bone Miner. Res. **20**(2): 257 267
65 Maayan C., Bar-On E., Foldes A. J., Gesundheit B. and Pollak R. D. (2002) Bone mineral density and metabolism in familial dysautonomia. Osteoporos. Int. **13**(5): 429 433
66 Ghilardi J. R., Rohrich H., Lindsay T. H., Sevcik M. A., Schwei M. J., Kubota K. et al. (2005) Selective Blockade of the Capsaicin Receptor TRPV1 Attenuates Bone Cancer Pain. J. Neurosci. **25**(12): 3126 3131
67 Bliziotes M., McLoughlin S., Gunness M., Fumagalli F., Jones S. R. and Caron M. G. (2000) Bone histomorphometric and biomechanical abnormalities in mice homozygous for deletion of the dopamine transporter gene. Bone **26**(1): 15 19
68 Westbroek I., van der Plas A., de Rooij K. E., Klein-Nulend J. and Nijweide J. (2001) Expression of serotonin receptors in bone. J. Biol. Chem. **276**(31): 28961 28968
69 Bliziotes M. M., Eshleman A. J., Zhang X. W. and Wiren K. M. (2001) Neurotransmitter action in osteoblasts: expression of a functional system for serotonin receptor activation and reuptake. Bone **29**(5): 477 486
70 Weintrob N., Cohen D., Klipper-Aurbach Y., Zadik Z. and Dickerman Z. (2002) Decreased growth during therapy with selective serotonin reuptake inhibitors. Arch. Pediatr. Adolesc. Med. **156**(7): 696 701
71 Warden S. J., Robling A. G., Sanders M. S., Bliziotes M. M. and Turner C. H. (2005) Inhibition of the serotonin (5-hydroxytryptamine) transporter reduces bone accrual during growth. Endocrinology **146**(2): 685 693
72 Goodship A. E., Lanyon L. E. and McFie H. (1979) Functional adaptation of bone to increased stress. An experimental study. J. Bone Joint Surg. Am. **61**(4): 539 546
73 Battaglino R., Fu J., Spate U., Ersoy U., Joe M., Sedaghat L. et al. (2004) Serotonin regulates osteoclast differentiation through its transporter. J. Bone Miner. Res. **19**(9): 1420 1431
74 Bhangu S., Genever P. G., Spencer G. J., Grewal T. S. and Skerry T. M. (2001) Evidence for targeted vesicular glutamate exocytosis in osteoblasts. Bone **29**(1): 16 23
75 Chenu C. (2002) Glutamatergic innervation in bone. Microsc. Res. Tech. **58**(2): 70 76
76 Chenu C. (2002) Glutamatergic regulation of bone remodeling. J. Musculoskelet. Neuronal Interact. **2**(3): 282 284
77 Chenu C., Serre C. M., Raynal C., Burt-Pichat B., and Delmas P. D. (1998) Glutamate receptors are expressed by bone cells and are involved in bone resorption. Bone **22**(4): 295 299
78 Gu Y. and Publicover S. J. (2000) Expression of functional metabotropic glutamate receptors in primary cultured rat osteoblasts. Cross-talk with N-methyl-D-aspartate receptors. J. Biol. Chem. **275**(44): 34252 34259
79 Mason D. J., Suva L. J., Genever P. G., Patton A. J., Steuckle S., Hillam R. A. et al. (1997) Mechanically regulated expression of a neural glutamate transporter in bone: a role for excitatory amino acids as osteotropic agents? Bone **20**(3): 199 205
80 Serre, C.M., D. Farlay,D. Delmas, and C. Chenu, 1999, Evidence for a dense and intimate innervation of the bone tissue, including glutamate-containing fibers. Bone **25**(6): 623 629
81 Patton A. J., Genever P. G., Birch M. A., Suva L. J. and Skerry T. M. (1998) Expression of an N-methyl-D-aspartate-type receptor by human and rat osteoblasts and osteoclasts suggests a novel glutamate signaling pathway in bone. Bone **22**(6): 645 649
82 Smart T. G. (1997) Regulation of excitatory and inhibitory neurotransmitter-gated ion channels by protein phosphorylation. Curr. Opin. Neurobiol. **7**(3): 358 367
83 Laketic-Ljubojevic, I., Suva L. J., Maathuis F. J., Sanders D. and Skerry T. M. (1999) Functional characterization of N-methyl-D-aspartic acid-gated channels in bone cells. Bone **25**(6): 631 637
84 Genever G. and Skerry T. M. (2001) Regulation of spontaneous glutamate release activity in osteoblastic cells and its role in differentiation and survival: evidence for intrinsic glutamatergic signaling in bone. FASEB J. **15**(9): 1586 1588
85 Hinoi E., Fujimori S. and Yoneda Y. (2003) Modulation of cellular differentiation by N-methyl-D-aspartate receptors in osteoblasts. FASEB J. **17**(11): 1532 1534
86 Skerry T. M. and Genever P. G. (2001) Glutamate signalling in non-neuronal tissues. Trends Pharmacol. Sci. **22**(4): 174 181
87 Taylor A. F. (2002) Osteoblastic glutamate receptor function regulates bone formation and resorption. J. Musculoskelet. Neuronal Interact. **2**(3): 285 290
88 Mohn A. R., Gainetdinov R. R., Caron M. G. and Koller B. H. (1999) Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell **98**(4): 427 436
89 Peet N. M., Grabowski P. S., Laketic-Ljubojevic I. and Skerry T. M. (1999) The glutamate receptor antagonist MK801 modulates bone resorption in vitro by a mechanism predominantly involving osteoclast differentiation. FASEB J. **13**(15): 2179 2185
90 Gray C., Marie H., Arora M., Tanaka K., Boyde A., Jones S. et al. (2001) Glutamate does not play a major role in controlling bone growth. J. Bone Miner. Res. **16**(4): 742 749
91 Mason D. J. (2004) Glutamate signalling and its potential application to tissue engineering of bone. Eur. Cell Mater. **7:** 12 25; discussion 25 16
92 Espinosa L., Itzstein C., Cheynel H., Delmas D. and Chenu C. (1999) Active NMDA glutamate receptors are expressed by mammalian osteoclasts. J. Physiol. **518 (Pt 1):** 47 53
93 Merle B., Itzstein, C., Delmas D. and Chenu C. (2003) NMDA glutamate receptors are expressed by osteoclast precursors and involved in the regulation of osteoclastogenesis. J. Cell Biochem. **90**(2): 424 436
94 Itzstein C., Espinosa J., Delmas P. D. and Chenu C. (2000) Specific antagonists of NMDA receptors prevent osteoclast

sealing zone formation required for bone resorption. Biochem. Biophys. Res. Commun. **268**(1): 201 209
95 Moore R. E., Smith C. K. 2nd, Bailey C. S., Voelkel E. F. and Tashjian A. H. Jr (1993) Characterization of beta-adrenergic receptors on rat and human osteoblast-like cells and demonstration that beta-receptor agonists can stimulate bone resorption in organ culture. Bone Miner. **23**(3): 301 315
96 Elefteriou F., Ahn J. D., Takeda S., Starbuck M., Yang X., Liu X. et al. (2005) Leptin regulation of bone resorption by the sympathetic nervous system and CART. Nature **434:** 514 520
97 Greenfield E. M., Horowitz M. C. and Lavish S. A. (1996) Stimulation by parathyroid hormone of interleukin-6 and leukemia inhibitory factor expression in osteoblasts is an immediate-early gene response induced by cAMP signal transduction. J. Biol. Chem. **271**(18): 10984 10989
98 Ducy P., Amling M., Takeda S., Priemel M., Schilling A. F., Beil T. et al. (2000) Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. Cell **100:** 197 207
99 Owen T. A., Bortell R., Yocum S. A., Smock S. L., Zhang M., Abate C. et al. (1990) Coordinate occupancy of AP-1 sites in the vitamin D-responsive and CCAAT box elements by Fos-Jun in the osteocalcin gene: model for phenotype suppression of transcription. Proc. Natl. Acad. Sci. USA **87**(24): 9990 9994
100 Chambers T. J. (2000) Regulation of the differentiation and function of osteoclasts. J. Pathol. **192**(1): 4 13
101 Suda T., Takahashi N., Udagawa N., Jimi E., Gillespie M. T. and Martin T. J. (1999) Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr. Rev. **20:** 345 357
102 Lacey D. L., Timms E., Tan H. L., Kelley M. J., Dunstan C. R., Burgess T. et al. (1998) Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell **93**(2): 165 176
103 Ma Y. L., Cain R. L., Halladay D. L., Yang X., Zeng Q., Miles R. R. et al. (2001) Catabolic effects of continuous human PTH (1--38) in vivo is associated with sustained stimulation of RANKL and inhibition of osteoprotegerin and gene-associated bone formation. Endocrinology **142**(9): 4047 4054
104 Yang X., Matsuda K., Bialek P., Jacquot S., Masuoka H. C., Schinke T. et al. (2004) ATF4 is a substrate of RSK2 and an essential regulator of osteoblast biology; implication for Coffin-Lowry Syndrome. Cell **117**(3): 387 398
105 Kondo A., Mogi M., Koshihara Y. and Togari A. (2001) Signal transduction system for interleukin-6 and interleukin-11 synthesis stimulated by epinephrine in human osteoblasts and human osteogenic sarcoma cells. Biochem. Pharmacol. **61**(3): 319 326
106 Cho E. S., Yu J. H., Kim M. S. and Yim M. (2004) Rolipram, a phosphodiesterase 4 inhibitor, stimulates osteoclast formation by ind ucing TRANCE expression in mouse calvarial cells. Arch. Pharm. Res. **27**(12): 1258 1262
107 Takami M., Cho E. S., Lee S. Y., Kamijo R. and Yim M. (2005) Phosphodiesterase inhibitors stimulate osteoclast formation via TRANCE/RANKL expression in osteoblasts: possible involvement of ERK and p38 MAPK pathways. FEBS Lett. **579**(3): 832 838
108 Kinoshita T., Kobayashi S., Ebara S., Yoshimura Y., Horiuchi H., TsutsumimotoT. et al. (2000) Phosphodiesterase inhibitors, pentoxifylline and rolipram, increase bone mass mainly by promoting bone formation in normal mice. Bone **27**(6): 811 817
109 Rawadi G., Ferrer C., Spinella-Jaegle S., Roman-Roman S., Bouali Y. and Baron R. (2001) 1-(5-oxohexyl)-3,7-Dimethylxanthine, a phosphodiesterase inhibitor, activates MAPK cascades and promotes osteoblast differentiation by a mechanism independent of PKA activation (pentoxifylline promotes osteoblast differentiation). Endocrinology **142**(11): 4673 4682
110 Levasseur R., Marcelli C., Sabatier J. P., Dargent-Molina P. and Breart G. (2005) 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. **53**(3): 550 552
111 Pasco J. A., Henry M. J., Sanders K. M., Kotowicz M. A., SeemanE. and Nicholson G. C. (2004) Beta-adrenergic blockers reduce the risk of fracture partly by increasing bone mineral density: Geelong Osteoporosis Study. J. Bone Miner. Res. **19**(1): 19 24
112 Reid I. R., Gamble G. D., Grey A. B., Black D. M., Ensrud K. E., BrownerW. S. et al. (2005) beta-Blocker use, BMD and fractures in the study of osteoporotic fractures. J. Bone Miner. Res. **20**(4): 613 618
113 Schlienger R. G., Kraenzlin M. E., Jick S. S. and Meier C. R. (2004) Use of beta-blockers and risk of fractures. JAMA **292**(11): 1326 1332
114 Romanello M., Moro L., Pirulli D., Crovella S. and D'Andrea P. (2001) Effects of cAMP on intercellular coupling and osteoblast differentiation. Biochem. Biophys. Res. Commun. **282**(5): 1138 1144
115 Lecanda F., Warlow M., Sheikh S., Furlan F., Steinberg T. H. and Civitelli R. (2000) Connexin43 deficiency causes delayed ossification, craniofacial abnormalities, and osteoblast dysfunction. J. Cell Biol. **151**(4): 931 944
116 Cancello R., Tounian A., Poitou C., and Clement K. (2004) Adiposity signals, genetic and body weight regulation in humans. Diabetes Metab. **30**(3): 215 227
117 Dacquin R., Davey R. A., Laplace C., Levasseur R., Morris H. A., Goldring S. R. et al. (2004) Amylin inhibits bone resorption while the calcitonin receptor controls bone formation in vivo. J. Cell Biol. **164**(4): 509 514
118 Takeda S., Elefteriou F. and Karsenty G. (2003) Common Endocrine Control of Body Weight, Reproduction, and Bone Mass. Annu. Rev. Nutr.
119 Millet I. and Vignery A. (1997) The neuropeptide calcitonin gene-related peptide inhibits TNF-alpha but poorly induces IL-6 production by fetal rat osteoblasts. Cytokine **9**(12): 999 1007
120 Mori T., Ogata T., Okumura H., Shibata T., Nakamura Y. and Kataoka K. (1999) Substance P regulates the function of rabbit cultured osteoclast; increase of intracellular free calcium concentration and enhancement of bone resorption. Biochem. Biophys. Res. Commun. **262**(2): 418 422
121 Lundberg P., Lundgren I., Mukohyama H., Lehenkari P. P., Horton M. A. and Lerner U. H. (2001) Vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating peptide receptor subtypes in mouse calvarial osteoblasts: presence of VIP-2 receptors and differentiation-induced expression of VIP-1 receptors. Endocrinology **142**(1): 339 347
122 Chenu C. (2002) Glutamatergic regulation of bone resorption. J. Musculoskelet. Neuronal Interact. **2**(5): 423 431
123 Baldock A., Sainsbury A., Couzens M., Enriquez R. F., Thomas G. P., Gardiner E. M. et al. (2002) Hypothalamic Y2 receptors regulate bone formation. J. Clin. Invest. **109**(7): 915 921
124 Caverzasio J., Palmer G., Suzuki A. and Bonjour J. P. (2000) Evidence for the involvement of two pathways in activation of extracellular signal-regulated kinase (Erk) and cell proliferation by Gi and Gq protein-coupled receptors in osteoblast-like cells. J. Bone Miner. Res. **15**(9): 1697 1706
125 Suzuki A., Palmer G., Bonjour J. P. and Caverzasio J. (1998) Catecholamines stimulate the proliferation and alkaline phosphatase activity of MC3T3-E1 osteoblast-like cells. Bone **23**(3): 197 203