

*Forum Minireview***Pharmacological Topics of Bone Metabolism:
The Physiological Function of the Sympathetic Nervous System
in Modulating Bone Resorption**Akifumi Togari^{1,*} and Michitsugu Arai¹¹Department of Pharmacology, School of Dentistry, Aichi-Gakuin University, Nagoya 464-8650, Japan

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Abstract. The vertebrate skeleton is richly innervated with adrenergic and peptidergic nerve terminals, and these play important roles in bone remodeling. Recent studies have generally shown that increased sympathetic nervous activity causes bone loss via an increase in bone resorption and a decrease in bone formation. Increased bone resorption is based on the stimulation of both osteoclast formation and osteoclast activity. These effects are associated with β_2 -adrenergic activity toward both osteoblastic and osteoclastic cells. Such findings indicate that β -blockers may be effective against osteoporosis, in which case there is increased sympathetic activity. This review summarizes evidence obtained both in vitro and in vivo implicating sympathetic neuron action in bone resorption.

Keywords: sympathetic nervous system, osteoclastogenesis, bone resorption, osteoporosis, β -blocker, bone metabolism

Introduction

Bone remodeling is the physiological process consisting of two balanced opposing activities: the formation of new bone by osteoblasts and the resorption of old bone by osteoclasts. Osteoblastic and osteoclastic activities are regulated by several systemic hormones, including parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), calcitonin, glucocorticoids, sex steroids, and thyroid hormones; and pathological disturbances in the production or activities of these hormones lead to pathological conditions in the skeleton. Moreover, various factors are produced by the bone cells themselves and others are released by non-bone cells in the vicinity. Such autocrine and paracrine factors include cytokines, growth factors, and prostaglandins (PGs). To some extent, the release of these local factors is controlled by the systemic hormones. In addition to endocrine and paracrine/autocrine mechanisms, bone remodelling, like most other homeostatic functions, has also been revealed to be under sympathetic control.

In recent studies (1 – 5), it has become evident that bone cells are equipped with functional receptors for several neuro-osteogenic factors; and, therefore, it has been proposed that signaling molecules in the nervous system may participate in the control of bone metabolism and that consequently a neuro-osteogenic network may exist, similar to the previously proposed neuro-immune and neuro-immune-endocrine interactions (6, 7).

The present article reviews our current understanding of the role of adrenergic innervation in bone resorption, an understanding based on the findings made by a variety of in vitro and in vivo studies. This article also reviews the role of β -blockers, used in clinical studies, for the prevention of bone fractures.

Nerve-bone cell interplay

In recent years, it has been demonstrated that human osteoblastic as well as osteoclastic cells are equipped with adrenergic receptors (ARs) and neuropeptide receptors (1, 2) and that they constitutively express diffusible axon guidance molecules known to function as a chemoattractant and/or chemorepellent for growing nerve fibers (2, 8). These findings suggest that the extension of axons of sympathetic and peripheral

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sensory neurons to osteoblastic and osteoclastic cells is required for the dynamic neural regulation of local bone metabolism. However, while several studies have shown a functional nerve-bone cell interplay, whether both osteoblastic and osteoclastic cells activation occurs as a direct response to neuronal activation or requires an intermediary cell is unclear. Therefore, we examined direct nerve-osteoblastic cell communication by using an in vitro co-culture model comprising mouse osteoblastic cells, MC3T3-E1, and neurite-spouting mouse superior cervical ganglia. Following loading with the calcium fluorophore Fluo-3, neurite-osteoblastic cell units were examined by confocal laser scanning microscopy. The addition of scorpion venom (SV) elicited neurite activation (i.e., Ca^{2+} mobilization) and, after a lag period, osteoblastic Ca^{2+} mobilization. The SV had no direct effect on the MC3T3-E1 cells in the absence of neurites. The addition of an α 1-AR antagonist, prazosin, concentration-dependently prevented the osteoblastic activation that resulted as a consequence of the neural activation by SV. Thus, our recent findings demonstrate that MC3T3-E1 activation, as judged by Ca^{2+} mobilization, can be a direct consequence of contact with a specific activated nerve fiber. This evidence obtained in vitro demonstrates that nerve-osteoblastic cell cross-talk can occur in the absence of an intermediary transducing cell and that noradrenaline is an important mediator of this communication. Several recent in vivo and in vitro studies have demonstrated a sympathomimetic action on bone formation and resorption via osteoblastic and osteoclastic cells, respectively, expressing α - and β -ARs.

Effect of adrenergic agonists on osteoclastogenesis

Osteoclasts have a hematopoietic origin, and thus bone marrow culture techniques have been successfully employed to study the development of the osteoclasts from their precursor cells. Such cultures provide an appropriate system to investigate osteotropic hormones, cytokines, and other bone-active factors that may be involved in the generation of osteoclasts. In this culture system, receptor activator of nuclear factor kappa B (NF- κ B) (RANKL) and osteoprotegerin (OPG) were reported to play an essential role in osteoclast differentiation. The expression of both proteins was shown to be regulated by several osteotropic factors including $1,25(\text{OH})_2\text{D}_3$, interleukin (IL)- 1α , IL-11, PGE_2 , transforming growth factor (TGF)- β 1, and PTH (9–13). β -AR agonists, adrenaline and isoprenaline, have been also demonstrated to modulate osteoclastogenesis. The involvement of RANKL and/or OPG in adrenaline-induced bone resorption was shown by determining the effect of adrenaline on the mRNA expression of RANKL and OPG in MC3T3-E1 and on the formation of tartrate-resistant acid phosphatase (TRAP)-positive multinuclear cells (MNCs) in mouse bone marrow cultures, thus providing us a better understanding of the bone resorption induced by the sympathetic system (14). By use of the RT-PCR procedure, it was shown that the expression of RANKL and OPG mRNAs in osteoblastic cells was regulated by adrenergic stimulation, without the mediation of IL-11 and PGE_2 produced in response to adrenaline (14). The expression of RANKL and OPG elicited by adrenaline appeared to be mediated by β -adrenergic and α -adrenergic stimulation, respectively.

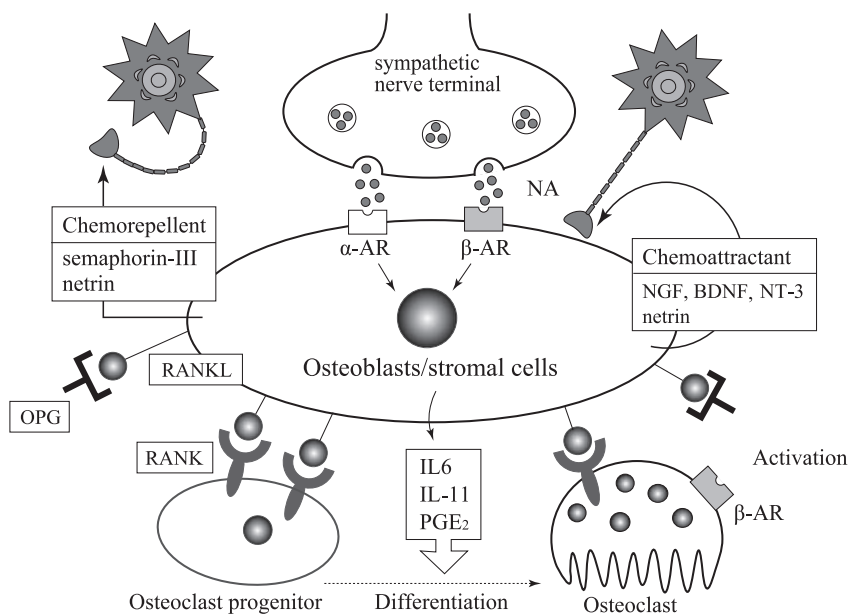


Fig. 1. Sympathomimetic action on osteoclastogenesis and osteoclastic activity. Both osteoblasts and osteoclasts constitutively express α 1B-, α 2B-, and β 2-ARs. Stimulation of β 2-ARs increases osteoclast formation and the expression of RANKL, IL-6, IL-11, and PGE_2 in bone marrow or clonal osteoblastic cells. The increase in osteoclast formation is inhibited by OPG treatment, suggesting the involvement of the RANKL-RANK system in osteoclastogenesis caused by stimulating β 2-ARs. The expression of RANKL and OPG in MC3T3-E1 cells is increased by stimulating β 2-ARs and α -ARs, respectively. In human osteoclasts, stimulation of β -ARs activates osteoclastic activity. Thus, increased sympathetic activity stimulates osteoclast formation as well as osteoclastic activity through β 2-ARs.

Treatment of mouse bone marrow cells with adrenaline or isoprenaline generated TRAP-positive MNCs capable of excavating dentin to form resorptive pits on dentine slices and caused an increase in RANKL and a decrease in OPG production by the marrow cells (15). The osteoclast formation was significantly inhibited by OPG, suggesting the involvement of the RANKL-RANK system. Since the osteoclastogenesis in mouse bone marrow cells was not stimulated by an α -AR agonist, the osteoclastogenesis may be regulated by the balance between RANKL and OPG production in osteoblasts/stromal cells. A possible mechanism for adrenergic stimulation of osteoclastogenesis is presented schematically in Fig. 1.

Effects of adrenergic agonists on osteoclastic activity

AR agonists stimulated cAMP synthesis in neonatal mouse calvariae and bone resorption in the presence of a phosphodiesterase inhibitor and an antioxidant (4). The cAMP synthesis stimulated by a β -AR agonist was inhibited by propranolol in bone organ cultures (16). The β -adrenergic stimulation of bone resorption might be mediated directly by activated osteoclasts and osteoclastogenesis enhanced by osteotrophic factors released from osteoblasts. In human osteoclast-like multinucleate cells constitutively expressing $\alpha 1B$ -, $\alpha 2B$ -, and $\beta 2$ -ARs, β -AR agonists upregulated the expression of characteristic markers of the mature osteoclast such as integrin, carbonic anhydrase II, and cathepsin K; increased osteoclastic bone-resorbing activity; and clearly caused actin ring formation (17). However, these effects were not obtained by treatment with α -AR agonists. These findings suggest that β -AR agonists directly stimulate bone-resorbing activity in mature osteoclasts. In a clonal cell line of human osteoclast precursors (FLG 29.1 cells), catecholamines were also demonstrated to act as inducers of osteoclast maturation in vitro and as stimulators of osteoclast activity via the binding to $\beta 2$ -ARs (18). As osteoclastogenesis-enhancing osteotrophic factors produced by β -adrenergic stimulation, IL-6, IL-11, and PGE₂ were detected in human and mouse osteoblastic cells (14, 19). The co-induction of IL-6 and IL-11 by activation of β -ARs, which appears to be a common feature in osteoblastic cells, has been shown to be probably mediated via a common signal pathway involving the PKA and p38 MAPK systems, leading to the transcriptional activation of AP-1 in human osteoblastic cells. Thus, the β -adrenergic system could be involved in the catabolic effect of AR agonists on bone metabolism.

Sympathetic activity toward bone resorption in vivo

There are several lines of evidence showing that the sympathetic nervous system modulates bone resorption in vivo. Surgical removal of the superior cervical ganglion increased bone resorption (20), as did chemical treatment with guanethidine in newborn rats (21). However, in adult rats treated with guanethidine, bone resorption was conversely reduced (22). In rats sympathectomized in adulthood, Cherruau et al. (22) found a reduction in bone resorption and assumed that the inhibition reflected the acute effects of sympathectomy on their rats. By using a compartmentalization procedure, they showed that the resorption surface in the osteogenic compartment was reduced significantly in the guanethidine-treated rats, together with a fall in the number of osteoclasts and impaired osteoclast access to the bone surface. The effect on resorption by inhibiting preosteoclast differentiation and disturbing osteoclast activation by the acute sympathectomy suggest that depletion of sympathetic mediators could disturb osteogenic cell-mediated osteoclast differentiation. In addition, a sympathectomy-induced depletion of noradrenaline may be another possible mechanism for the reduction in bone resorption caused by sympathectomy. Such a mechanism is supported by several significant data showing the stimulation of bone resorption in a tissue culture system (4), an increase in preosteoclastic cell activity (18), and the stimulation of the synthesis of osteoclast-like cell formation-stimulating factors in osteoblastic cells by β -adrenergic stimulation (14, 19).

The intracerebroventricular (i.c.v.) injection of lipopolysaccharide (LPS), which is an inflammatory stimulus in the brain, was demonstrated to increase the output from the peripheral sympathetic nervous system (23, 24). To prove the physiological role of the sympathetic nervous system in bone metabolism in vivo, we performed RT-PCR to examine the effect of an i.c.v. injection of LPS on cyclooxygenase (COX)-2 and IL-6 mRNA expression in mouse calvaria (23, 24). The expression of both was increased by the injection. Both increases were inhibited by treatment with the neurotoxin 6-hydroxydopamine (6-OHDA) or by a β -blocker. Similarly, restraint stress induced the expression of IL-6 mRNA in mouse calvaria (24). This induction was not influenced by 6-OHDA, but was inhibited by propranolol. In addition, the treatment of calvaria with isoprenaline or noradrenaline increased PGE₂ and IL-6 synthesis in an organ culture system. These findings show that the increase in gene expression caused by a restraint stress or i.c.v. injection of LPS was mediated by the activation of sympathetic nerve fibers and β -ARs in mouse calvaria and suggest that in vivo activation

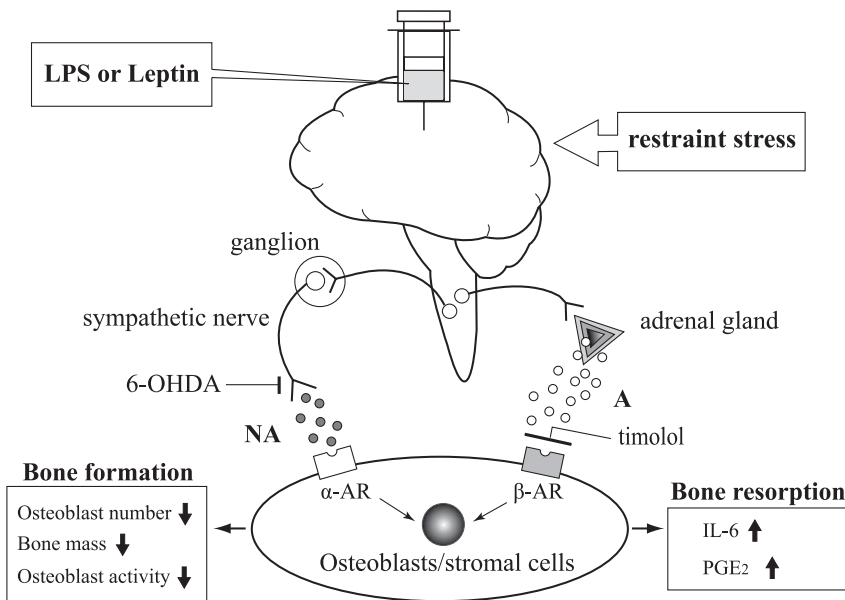


Fig. 2. Bone metabolism modulated by sympathetic nervous system. Central activation of the peripheral sympathetic nervous system caused by the intracerebroventricular (i.c.v.) injection of lipopolysaccharide (LPS) or leptin or by restraint stress alters bone formation and bone resorption. Centrally mediated activation of the sympathetic nervous system is associated with increased catecholamine levels. Leptin-induced sympathetic activation has been reported to decrease bone formation via osteoblastic activity. On the other hand, either LPS- or stress-induced activation increases the synthesis of osteoclastogenic factors such as IL-6, and PGE₂, which are factors that promote bone resorption.

of the sympathetic nervous system modulates bone resorption. The above-mentioned findings are strongly supported by recent experiments (25) showing that β 2-AR-deficient (*Adrb2*^{-/-}) mice had a more severe high bone mass phenotype than ob/ob or wild-type mice receiving β -blockers and that long-term leptin i.c.v. infusion did not reduce the bone mass of *Adrb2*^{-/-} mice. Furthermore, the group just cited showed that the sympathetic nervous system favored bone resorption by increasing the expression of RANKL and that isoprenaline enhanced the generation of osteoclasts when wild-type, but not *Adrb2*^{-/-}, osteoblasts were co-cultured with wild-type bone marrow macrophages.

Thus, these *in vivo* experiments modulating peripheral sympathetic nervous activity suggest that increased sympathetic nervous system activity leads to increased bone resorption through β 2-ARs (Fig. 2).

Concluding remarks

Both *in vitro* and *in vivo* experimental studies indicate that β -blockers may be effective toward osteoporosis attributed to increased sympathetic nervous activity. Inhibition of sympathetic nervous activity by β -blockers to inhibit bone resorption and/or to stimulate bone formation could, therefore, be an important new therapeutic avenue to cure osteoporosis. In a population-based, case-control study generating data on adult women (26), men, and young women (27), the current use of β -blockers was demonstrated to be associated with a reduced risk of fractures, taken alone as well as in combination with thiazide diuretics. Thus, β -blockers generally do cause a reduction in bone fracture risk and

higher bone mineral density. Another prospective study, however, showed no association between β -blocker use and fracture risk in perimenopausal and older women (28–30). Therefore, there is currently no convincing evidence supporting the hypothesis that pharmacological blockade of the β -adrenergic system is beneficial to the human skeleton after menopause. Although β -adrenergic stimulation can be proposed as one of the causes of osteoporosis in experimental studies, the clinical usefulness of β -blockers on fracture risk must be analyzed in several patients with increased sympathetic nervous activity. Specifically, it is important to find a difference between users and nonusers with increased sympathetic tone. Further experimental and clinical studies are desirable to demonstrate the usefulness of β -blockers for the treatment of osteoporosis, by measuring markers of sympathetic nervous activity and bone metabolic activity, and are also needed to find β -blockers with high affinity for bone tissues.

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