Journal of Oral Biosciences 56 (2014) 73–76

Contents lists available at ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

Signaling pathway and physiological role of the alpha-1 adrenergic receptor in human osteoblasts

Daisuke Kodama *, Akifumi Togari

Department of Pharmacology, School of Dentistry, Aichi-Gakuin University, 1-100 Kusumoto-cho, Chikusa-ku, Nagoya 464-8650, Japan

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form
4 March 2014
Accepted 18 March 2014
Available online 24 April 2014

Keywords:
Alpha1-adrenergic receptor
Osteoblast
Sympathetic nervous system
Bone metabolism

ABSTRACT

Background: In recent years, the role of the sympathetic nervous system in bone metabolism has been revealed. Many studies have suggested that bone loss can be induced by continuously high sympathetic tone resulting from up-regulation of osteoclastogenesis and osteoclastic activity via β_2-adrenergic receptors. Although the expression of α-adrenergic receptors in osteoblasts and osteoclasts has been demonstrated, the physiological roles of these receptors in bone metabolism remain unclear. In the present review, we provide an account of the role of α_1-adrenergic receptors in bone metabolism.
Conclusion: Experimental studies in osteoblasts suggest that not only the suppression of β_2-adrenergic receptors but also the activation of α_1-adrenergic receptors could lead to a treatment for osteoporosis. Studying the signaling pathway will help elucidate the mechanism underlying the regulation of bone metabolism via the α_1-adrenergic receptor and also facilitate the development of a novel therapeutic strategy for osteoporosis.

Contents

1. Introduction

In recent years, neural regulation of bone metabolism mediated by osteoblasts and osteoclasts has been demonstrated [1–4]. Previous studies showed that mRNAs of α- and β-adrenergic receptors are expressed in osteoblasts and osteoclasts [5,6]. Additionally, these cells also express neurotrophins and axon guidance molecules for growing nerve fibers [7]. In immunohistochemical studies, bones were shown to be widely innervated by sympathetic nerves [8,9]. The presence of peripheral nerve axons coursing through the marrow adjacent to osteoblasts in bone tissue was shown by electron microscopy [10]. Direct nerve-osteoblastic and nerve-osteoclastic cell communication has been demonstrated using an in vitro co-culture model comprising sympathetic nerve cells derived from the mouse superior cervical ganglion and MC3T3-E1 cells, mouse calvaria-derived osteoblastic cells, or osteoclasts induced from RAW264.7 cells [11,12]. These findings suggest that the sympathetic nervous system plays a direct role in the regulation of bone metabolism.

Abbreviations: ALP, alkaline phosphatase; BrdU, 5-bromo-2′-deoxyuridine; HOS, human osteosarcoma-derived cells; MC3T3-E1, mouse calvaria-derived osteoblastic cells; MG-63, human osteosarcoma-derived cells; OPG, osteoprotegerin; PI-PLC, phosphoinositide-phospholipase C; Pit-1, sodium-dependent inorganic phosphate transporter; PKA, protein kinase A; PLC, phospholipase C; RANK, receptor activator of NFκB; RANKL, receptor activator of NFκB ligand; RAW264.7, mouse macrophage-like cells; SaM-1, human periosteum-derived osteoblastic cells; SaOS, human osteosarcoma-derived cells; WST, water-soluble tetrazolium

* Corresponding author. Tel.: +81 52 751 2561; fax: +81 52 752 5988.
E-mail addresses: kodamads@dpc.aichi-gakuin.ac.jp (D. Kodama), togariaf@dpc.aichi-gakuin.ac.jp (A. Togari).

http://dx.doi.org/10.1016/j.job.2014.03.004
1349-0079/

Endogenous agonists of adrenergic receptors such as noradrenaline and adrenaline stimulate both α- and β-adrenergic receptors. It is well known that α_1- and β_1-adrenergic receptors have a similar sensitivity to these agonists, but the β_2-adrenergic receptor is less sensitive to noradrenaline than to adrenaline. In experimental studies, bone loss was induced by continuously high sympathetic tone, and this induction was reversed by β-adrenergic receptor blockade [13–15]. Many studies have suggested that up-regulation of osteoclastogenesis and osteoclastic activity via β_2-adrenergic receptors enhances bone resorption [3,16,17]. Additionally, suppression of bone formation by β-adrenergic receptor activity has also been reported [15,17]. Furthermore, it has been reported that the use of β-blockers reduces the risk of bone fracture and increases bone mineral density [18–20]. The physiological role of the β-adrenergic receptor in bone metabolism has been demonstrated by many studies, whereas that of the α-adrenergic receptor has been less well studied. However, clinical studies have also reported that blockade of the α_1-adrenergic receptor increased the risk of hip/femur fracture, and that hypertensive patients treated with α_1-blocker showed an increased risk of osteoporosis [20,21]. These studies suggest that the α-adrenergic receptor also plays a role in sympathetic effects on bone metabolism.

In the present review, we summarize the physiological role and signal transduction pathway of the α_1-adrenegic receptor in osteoblasts based on our recent findings.

2. Effects of α_1-adrenergic agonists on the physiology of osteoblasts

In our study, mRNAs of the α_{1B}-, α_{2B}-, and β_2-adrenergic receptors were found to be expressed in SaM-1 cells, which are human periosteum-derived osteoblasts, and in the SaOS, HOS, and MG-63 human osteosarcoma-derived cell lines [5,6]. Huang et al. [22] also demonstrated the expression of α_{1B}- and β_2-adrenergic receptors using RT-PCR and Western blotting. Additionally, on immunofluorescence microscopy, these receptors were shown to localize to the cell surface of human osteoblasts.

Suzuki et al. [23] demonstrated that adrenaline and phenylephrine, an α_1-adrenergic receptor agonist, increased DNA synthesis in a concentration-dependent manner in MC3T3-E1 cells as determined by ^{3}H-thymidine incorporation. The effect of adrenaline was suppressed in the presence of α_1-adrenergic receptor antagonists. Similarly, Huang et al. [22] demonstrated that DNA synthesis was enhanced by cirazoline, an α_1-adrenergic receptor agonist, but inhibited by fenoterol, a β_2-adrenergic receptor agonist, in human osteoblasts. We evaluated the effect of noradrenaline on cell proliferation activity according to DNA synthesis as determined by 5-bromo-2′-deoxyuridine (BrdU) incorporation, and we also evaluated the number of live cells according to dehydrogenase activity as determined by the water-soluble tetrazolium (WST) assay. In SaM-1 cells, noradrenaline increased BrdU incorporation at submicromolar concentrations and suppressed it at higher-than-micromolar concentrations. In contrast, noradrenaline only increased formazan formation in the WST assay, over the whole concentration range that we tested. In the presence of prazosin, an α_1-adrenergic receptor antagonist, noradrenaline showed only suppressive effect on cell proliferation in both the BrdU assay and the WST assay. In contrast, in the presence of propranolol, a β-adrenergic receptor antagonist, the effect of noradrenaline was facilitatory in both assays [24]. These results suggest that cell proliferation is enhanced by α_1-adrenergic receptors and inhibited by β-adrenergic receptors in human osteoblasts.

Treatment with adrenaline also increased alkaline phosphatase (ALP) activity and sodium-dependent inorganic phosphate transporter (Pit-1) expression through the α_1-adrenergic receptor in MC3T3-E1 cells [25]. ALP reduces extracellular pyrophosphate, a potent inhibitor of calcification, and Pi is taken up by Pit-1. These molecules play important roles in the initial events of bone matrix calcification. These findings suggest that α_1-adrenergic receptor activation enhances osteoblast-mediated bone formation.

It is well known that the binding of receptor activator of nuclear factor kappa-B ligand (RANKL) to its receptor RANK is an essential signal for osteoclastogenesis. In MC3T3-E1 cells, the expression of RANKL and its decoy receptor, osteoprotegerin (OPG), was increased by adrenaline via the β-adrenergic receptor and α_1-adrenergic receptor, respectively [26]. It has also been reported that the effects of α_1-adrenergic receptor agonists on OPG expression were eliminated by the knockdown of the α_{1B}-adrenergic receptor in human osteoblasts [22]. These results suggest that osteoclastogenesis is suppressed by α_1-adrenergic receptor agonists.

In summary, these studies suggest that bone metabolism is positively regulated by the α_1-adrenergic receptor expressed in osteoblasts.

3. Signaling pathways involved in the effects of α_1-adrenergic receptors

α_1-adrenergic receptors belong to the G-protein-coupled receptor family. In general, activation of α_1-adrenergic receptors increases the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by inducing Ca^{2+} release from the endoplasmic reticulum via the Gq/phosphoinositide-phospholipase C (Gq/PI-PLC) pathway. However, recent studies have demonstrated that Ca^{2+} influx through Ca^{2+}-permeable channels and the Na^+/Ca^{2+} exchanger is involved in α_1-adrenergic receptor-mediated Ca^{2+} elevation. The molecular components underlying Ca^{2+} influx and their importance in Ca^{2+} signaling differ among tissues. In our study, noradrenaline induced $[Ca^{2+}]_i$ elevation via the α_1-adrenergic receptor in MC3T3-E1 cells and SaM-1 cells. The effect of noradrenaline was suppressed not only by pretreatment with a PLC inhibitor, U73122, but also by removing extracellular Ca^{2+}. The effect of noradrenaline was completely abolished in Ca^{2+}-free extracellular solution. Therefore, Ca^{2+} influx plays a predominant role in α_1-adrenergic receptor-mediated Ca^{2+} signaling in SaM-1 cells. Additionally, noradrenaline-induced $[Ca^{2+}]_i$ elevation was inhibited by pretreatment with either thapsigargin or store-operated Ca^{2+} channel inhibitors. These results suggest that activation of Gq-protein-coupled α_1-adrenergic receptors induces $[Ca^{2+}]_i$ elevation mainly via store-operated Ca^{2+} channels in human osteoblasts [27].

We have also demonstrated that noradrenaline reduces the whole-cell current in patch-clamp recordings from SaM-1 cells. The inhibitory effect of noradrenaline on the whole-cell current was eliminated by chloroethylclonidine, an α_{1B}-adrenergic receptor-selective inhibitor, and CsCl, a potassium channel blocker. These results suggest that activation of the α_{1B}-adrenergic receptor also suppresses potassium channels [28]. However, the inhibitory effect of noradrenaline on the whole-cell current was not affected by pretreatment with U73122 or by chelation of intracellular Ca^{2+}. These results suggest that noradrenaline-induced activation of store-operated Ca^{2+} channels and inhibition of potassium channels are individual effects. Then, we examined whether the Gi/o-protein is involved in the inhibitory effect of noradrenaline on the whole-cell current. Upon pretreatment with pertussis toxin, an inhibitor of the Gi/o-protein-coupled receptor, noradrenaline-induced inhibition of potassium channels was significantly suppressed. Pertussis toxin inhibits effects via the Giα-protein and also the Gβγ-protein. We next examined which pathways are involved in the inhibitory effect of noradrenaline on the whole-cell current. Pretreatment with H89, a PKA inhibitor, attenuated