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Perspective Article

Hylonome

Glutamatergic regulation of bone remodeling

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Abstract

L-glutamate (Glu) is the predominant neuromediator in the mammalian central nervous system (CNS). Bone is highly innervated and there is growing evidence of a neural control of bone cell metabolism. The recent discovery of Glu-containing nerve fibers in bone and Glu receptors (GluR) and transporters in bone cells suggest that this neuromediator may also act as a signaling molecule in bone and regulate bone cell function. Our previous studies have demonstrated that ionotropic N-Methyl-D-Aspartate (NMDA) GluR are highly expressed by mammalian osteoclasts. NMDA receptors (NMDAR) are heteromers associating the NR1 subunit and one of the four types of NR2 subunits (NR2A to D). We showed that osteoclasts express NR1, NR2B and NR2D subunits, suggesting a molecular diversity of NMDAR in these cells. Electrophysiological studies have confirmed that NMDAR are functional in mature osteoclasts, and features of Glu-induced current recorded in these cells indicate a major NR2D subunit composition. Using an *in vitro* assay of bone resorption, we showed that several antagonists of NMDAR binding to different sites of the receptor inhibit bone resorption. In particular, the specific NMDAR channel blocker MK801 had no effect on osteoclast attachment to bone and survival while it rapidly decreased the percentage of osteoclasts with actin ring structures that are associated with actively resorbing osteoclasts. NMDAR may thus be involved in adhesion-induced formation of the sealing zone required for bone resorption. NMDAR are also expressed by osteoclast precursors isolated from mouse bone marrow. We recently confirmed the presence of NR1, NR2B and NR2D in these cells and demonstrated their expression at all differentiation stages from osteoclast precursors to mature resorbing osteoclasts. No regulation of these subunits mRNA expression levels was observed throughout the osteoclastic differentiation sequence. Activation of NMDAR may therefore represent a new mechanism for regulating osteoclast formation and activity. While the origin of Glu in bone is still unknown, the possibility of a glutamatergic neurotransmission in this tissue is suggested by the detection of Glu in nerve fibers in close contact to bone cells. Furthermore, we recently demonstrated that sciatic neurectomy in growing rats induces a bone loss associated with a reduction of nerve profiles immunostained for Glu. These results suggest that Glu may be released from glutamatergic nerve profiles present in bone and therefore contribute to the local regulation of bone cell function.

Keywords: Innervation, Glutamate, NMDA Receptors, Bone Resorption

Introduction

Bone is highly innervated and evidence for a regulation of bone metabolism by nerve fibers has been suggested by many clinical and experimental studies[1,2,3]. Innervation has been shown to be important for bone growth, ossification, repair and remodeling. However, the nature of neuromediators involved in these processes has not been well documented. The recent identification in bone of nerve fibers containing glutamate (Glu), the most largely distributed neurotransmitter in the central nervous system (CNS), and the demonstration in bone cells of all the machinery required for glutamate signaling, suggest that glutamate may act as a neuromediator in bone and regulate bone cell activity.

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Identification of glutamate signaling in bone

All the machinery required for Glu signaling in the CNS has been identified in bone in recent years. In the CNS, Glu can stimulate post-synaptic intracellular signaling by acting on two types of membrane receptors, ionotropic (iGluR) and metabotropic Glu receptors (mGluR). Three subtypes of iGluR have been classified according to their activation by specific agonists: NMDA, AMPA and Kainate GluR. Many studies have shown that bone cells express several classes of

GluR, essentially NMDA receptors (NMDAR) and mGluR[4-8]. NMDAR were the most studied and are expressed by mature osteoblasts and osteoclasts on bone surfaces. Several NMDAR subunits were detected in bone cells, supporting a molecular diversity of these channels in bone similar to what was shown for brain[8]. In the CNS, Glu transporters located on surrounding glial cells and neurons rapidly lower the concentration of Glu in the synaptic cleft to avoid the excessive activation of GluR contributing to excitotoxicity. Two Glu transporters were identified in bone by Mason et al.[9] GLAST-1 mRNA was localized in active cuboidal osteoblasts as well as in osteocytes, while the Glu transporter GLT-1 was expressed in bone marrow cells. Recently, a splice variant of GLAST-1 (GLAST-1a), lacking exon 3, was identified in bone[10]. Proteins containing PDZ domains, which interact with the cytoplasmic tails of GluR and connect them to the cytoskeleton and downstream signaling pathways, have also been detected in bone[5,8].

Existence of functional NMDAR on mature osteoclasts

Electrophysiology studies performed on mature osteoclasts have shown that NMDAR are functional in these cells and that their electrophysiological and pharmacological properties are similar to those documented for neuronal cells[11]. The specific agonists of NMDAR, Glu and NMDA, activate whole cell currents recorded in osteoclasts. Furthermore, the induced-currents are sensitive to specific blockers of NMDAR (MK801, magnesium ions, 1-(1,2-diphenylethyl) piperidine). Features of Glu-induced current recorded in these cells indicate a major NR1/NR2D subunits composition[12].

Involvement of NMDAR in the bone resorption process

We have shown that several specific antagonists of NMDAR binding to the ligand, glycine and channel sites of the receptor inhibit bone resorption *in vitro*, suggesting the involvement of these receptors in the regulation of osteoclast activity[4,8]. The effect of the specific NMDAR antagonist MK801 was studied in the different steps leading to osteoclast activation. It has no effect on osteoclast attachment to bone and survival while it rapidly decreases the percentage of osteoclasts with actin ring structures that are associated with actively resorbing osteoclasts[8]. NMDAR expressed by osteoclasts may therefore be involved in adhesion-induced formation of the sealing zone required for bone resorption. A recent report by Gray et al.[13] has shown that, although MK801 reduces the number of pits, this effect is not reproduced with another antagonist of NMDAR, D-AP-5, suggesting that Glu is not a major controller of bone resorption. This study was however compromised by many technical pitfalls[14]. NMDAR are also expressed by osteoclast precursors isolated from mouse bone marrow and by Raw 264,7 cells, a murine myelomonocytic cell line that differentiates into osteoclasts under the influence of receptor activator of nuclear factor-kB ligand (RANK ligand). The subunits NR1, NR2A, NR2B and NR2D are present in these cells and we have demonstrated their expression at all differentiation stages from osteoclast precursors to mature resorbing osteoclasts. No regulation of these subunits mRNA expression levels was observed throughout the osteoclastic differentiation sequence (Chenu et al., 2001, in preparation). Specific antagonists of NMDAR also inhibit osteoclast differentiation[15], indicating that activation of NMDAR is important for the regulation of both osteoclast formation and activity *in vitro*.

Control of bone remodeling through glutamatergic innervation

While the origin of Glu in bone is still unknown, the possibility of a glutamatergic neurotransmission in this tissue is suggested by the detection of Glu in nerve fibers running in bone marrow in the vicinity of hematopoïetic cells and bone cells[3]. Electron microscopy studies have clearly demonstrated some contacts between Glu-immunoreactive nerve terminals and bone cells, but no synaptic vesicles were observed in these structures. Using a model of sciatic neurectomy in growing rats, we have shown that the bone loss induced in this model is associated with a reduction of nerve profiles immunostained for Glu[16]. The nerve fibers, by releasing neuromediators such as Glu, may therefore contribute to the local regulation of bone cell function. Preliminary results by our team indicate that ovariectomy-induced bone loss is also associated with a marked reduction of nerve profile density in rat tibiae[17]. The decrease of innervation associated with bone loss in neurectomy and ovariectomy models suggests that neural regulation may play a role in bone loss during immobilization and in osteoporosis.

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