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Perspective Article

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The role of glutamate transporters in bone cell signalling

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Abstract

The amino acid L-glutamate mediates signals at excitatory synapses in the CNS where its effects are controlled by co-ordinated activities of various types of glutamate receptor and transporter. This signalling mechanism has proved to be far more ubiquitous with many different cell types responding to glutamate. The glutamate transporter GLAST-1 was the first component of this pathway identified in bone where its expression was found to be mechanoresponsive in osteocytes. There is now a wealth of evidence supporting a role for this signalling mechanism in bone. Osteoblasts can release glutamate in a regulated manner and express functional glutamate receptors that influence their differentiation and osteogenic activity. Likewise, osteoclasts express functional glutamate receptors that influence their bone resorbing capacity. This article considers the various functions of glutamate transporters in this signalling pathway, and the evidence supporting an important role of glutamate signalling in regulating bone cell activities.

Keywords: Glutamate, Bone, GLAST, Osteocyte, Mechanical Loading

Introduction

The amino acid L-glutamate is the major mediator of excitatory signals in the mammalian central nervous system (CNS) where the glutamate-mediated signal transduction pathway has been studied extensively. Recently, the expression of glutamate receptors and transporters has been reported in a wide variety of tissues and it is clear that they are functional in many non-neuronal cell types. This has led to speculation that glutamate may represent a more ubiquitous signalling molecule that is also important in non-CNS tissues.

The well-characterised glutamate signalling pathway that operates in excitatory synapses has been used as a model to investigate glutamate signalling in non-neuronal tissues. Activation of the pre-synaptic neuron leads to calcium-dependent glutamate release into the synapse. The released glutamate activates various classes of glutamate receptor on the post-synaptic membrane to perpetuate the excitatory signal. A 'memory' of previous signalling episodes can cause modification of glutamate receptor properties and thus alter the downstream responses. The glutamate signal is terminated by the activities of high affinity glutamate transporters expressed within the plasma membrane of glial cells and neurons. These transporters quickly bind the glutamate within the synapse and transport it into the cells thus preventing neurotoxic activation of the glutamate receptors. The association of the glutamate transporter GLAST-1 with mechanical signal transduction in bone[1] together with the observation that bone cells expressed glutamate receptors that influence osteoclast activity[2] led to investigation of this signalling cascade in bone cells.

Glutamate signalling in bone

The first evidence of a role for glutamate signalling in bone came from a gene screening exercise designed to identify osteocyte-specific genes involved in mechanically-induced osteogenesis in bone[1]. Gene expression in osteocytes underlying a bone surface that had been mechanically loaded to induce bone formation was compared to that in equivalent cells from the control where mechanical loading had not been applied. The glutamate transporter GLAST-1 was down-regulated in these cells after osteogenic mechanical loading. GLAST-1 is a high affinity glutamate transporter and its role in the regulation of glutamate transmission in the CNS led to the intriguing notion that glutamate signalling may also be

Author has patents pending for material and methods relating to a novel splice varient of a Na^+ dependent glutamate transporter.

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important in mechanotransduction in bone.

There is now much evidence to support an important role for glutamate signalling in bone. All aspects of the glutamate signalling pathway have been investigated in bone cells[3]. Thus, osteoblasts release glutamate in a regulated, calcium-dependent manner[4,5] and express functional glutamate receptors[6,7] that regulate their ability to form bone[8-10]. Likewise, osteoclasts express functional glutamate receptors[11,12] that influence their bone resorbing capacity[12-15]. Osteoblasts and osteocytes also express glutamate receptors[2] and transporters[1] *in vivo*. These glutamate transporters are responsive to extracellular glutamate concentrations and localize to the plasma membrane[16,17]. We have been investigating the role of glutamate transporters in this signalling pathway in bone.

The glutamate transporter GLAST-1

The glutamate/aspartate transporter, GLAST-1, is a member of the high affinity sodium and potassium coupled glutamate transporter family that consists of 5 structurally related proteins. These transporters, also referred to as excitatory amino acid transporters (EAATs), are distinct from other glutamate transporters within the plasma membrane and intracellular glutamate transporters in mitochondria and synaptic vesicles. GLAST-1 uses electrochemical gradients of Na^+ and K^+ to drive glutamate uptake[18]. During glutamate transport, 3 Na^+ ions and 1 H^+ are co-transported with glutamate and a potassium ion is counter-transported leading to a net positive charge moving into the cell. If the electrochemical gradients of Na^+ and K^+ are reversed, GLAST-1 transports glutamate out of the cell. In addition to its glutamate transport activities, GLAST-1 also operates as a glutamate gated ion channel[19] and has been implicated in the activation of MAPK pathways[20].

GLAST-1 and GLAST-1a isoforms in bone

GLAST-1 was originally cloned from rat brain where the 4.5Kb cDNA was demonstrated to encode a 543 amino acid protein[21]. To investigate the function of GLAST-1 in bone, we determined whether the bone derived mRNA encoded the same GLAST-1 protein as that transcribed in brain. We cloned the full length cDNA from bone and demonstrated that the open reading frame (ORF) of the bone-derived mRNA is identical to that expressed in brain[22]. In addition, a novel splice variant of this gene, which we have called GLAST-1a, was identified in which exon 3 is absent[22]. Western blot analysis revealed a 69Kd protein in bone that was identical to that in brain[22]. A 55KDa immunoreactive protein correlating to the molecular weight of GLAST-1a was also expressed in brain[22].

The high affinity glutamate transporters all have a common structure within the plasma membrane characterised by an intracellular N terminal, 8 transmembrane domains (TMD), a region containing the transport pore and an intracellular C-terminal. C-terminal interactions are involved in MAPK signalling whereas direct oxidation and phosphorylation of the transporter influences its transport activity and trafficking within the cell. Hydrophobicity analysis predicts that GLAST-1a is likely to assemble in reverse in the plasma membrane downstream of TMD 2 resulting in an extracellular C-terminal[22]. All of the amino acid residues that are required for glutamate binding and transport are present in GLAST-1a, however sequences that are sensitive to oxidation and phosphorylation may be reoriented. Electrophysiological experiments using whole cell clamping to detect glutamate transport, and radiolabelled glutamate uptake assays, reveal that GLAST-1a is a functional glutamate transporter when expressed in Xenopus oocytes (Huggett, Daniels and Mason, unpublished data). However, we believe that the reorientation of GLAST-1a may cause it to have quite different properties to GLAST-1. Strikingly, 4 of the 5 members of this transporter family retain the ability to splice out equivalent domains, despite divergent gene sequences, indicating a functional significance for the GLAST-1a variant (Huggett, Taylor and Mason, unpublished data).

Localization of glutamate transporters in bone cells

To investigate whether GLAST proteins localise to the plasma membrane of bone cells, consistent with their glutamate uptake function, we cloned GLAST-1 and GLAST-1a into expression vectors incorporating Green Fluorescent Protein (GFP) tags. Scanning laser confocal microscopy of MLO-Y4 osteocytes transfected with these constructs revealed GLAST-1 to localise to the plasma membrane and discrete vesicles close to the nucleus[16,17]. GLAST-1a showed a different expression pattern, mostly localising to intracellular vesicles. In the CNS, GLAST-1 is stored in intracellular pools and is quickly trafficked to the plasma membrane in response to high extracellular glutamate concentrations. Intriguingly, the distribution of GLAST-1 in osteocytes was also responsive to extracellular glutamate concentrations, with the transporter localising to the cytosol at low glutamate concentrations and the plasma membrane at high concentrations[16]. The distribution of GLAST-1a appeared unaffected by extracellular glutamate concentration.

Glutamate transporters and bone cell signalling

Since GLAST-1 is critically important in regulating extracellular glutamate concentrations, and thus receptor activation in the CNS, we have proposed that this transporter plays an active role in controlling glutamate signals in bone [3,17]. Activation of glutamate receptors on osteoblasts has been demonstrated to increase their differentiation, as well as increase expression of the transcription factor *Cbfa* I and bone matrix proteins[9,10]. If GLAST-1 activity controls extracellular glutamate levels in the bone microenvironment it may represent an important modulator of osteoblast phenotype. Since osteoclasts are also responsive to glutamate[12,13,15,23], regulation of extracellular glutamate may influence the balanced activities of these cell types.

Whilst functional glutamate signalling has been clearly demonstrated in osteoblasts and osteoclasts *in vitro*, clear effects of modulation of glutamate signalling *in vivo* have proved elusive. Various glutamate receptor and transporter knockout mice strains have been generated but no major skeletal abnormalities have been reported. However, gene ablation in these mice does not always affect the gross morphology of the brain either and differences in neuronal or glial responses to defined stimuli are necessary to reveal the functional significance of each gene. Such detailed analyses of bone cell responses in these knockout animals are yet to be performed. Whilst GLAST-1/1a knockout mice reveal no changes in bone length[14], it is yet to be discovered whether these animals have a more subtle phenotype reflecting altered responses of bone cells.

Data from humans is also limited. Although some human disease mutations that link to the GLAST-1 locus include bone abnormalities, none of these has been directly mapped to the GLAST gene. Patients with nerve damage, and animals that have undergone surgical or chemical denervation of bones, reveal bone loss that has been attributed to lack of innervation of the bones[24]. Since nerve fibres containing glutamate, and other neurotransmitters, infiltrate cortical and trabecular bone, osteopenia associated with nerve damage may result from local bone cell responses to a lack of glutamate release from nerve terminals.

Glutamate signalling and mechanotransduction in bone

Although there is good evidence that individual components of glutamate signalling are expressed and functional in bone cells, the stimulus that commences an episode of glutamate signalling in bone *in vivo* is not known. Since GLAST-1 was originally identified in bone in response to mechanical stimuli, a role for glutamate signalling in the adaptive response of the skeleton to its mechanical environment seems likely. It has been noted by others that the specific responses of bone cells to mechanical stimuli are well matched to the properties of glutamate signal transduction[25-27]. Glutamate signalling can respond very quickly (and thus has the potential to discriminate differences in strain rates) and can self modify to have a 'memory' of previous signalling episodes allowing maximal responses to be achieved by relatively few signalling events. Established mechanoresponsive signalling pathways in osteocytes can be sensibly linked to glutamate signalling mechanisms[3,25-27] and testing these relationships will reveal the importance of glutamate as a mediator of mechanically-induced osteogenesis.

Conclusion

It is clear that glutamate-mediated signalling can influence the phenotype of bone cells. The associations of this signalling pathway with mechanically-induced osteogenesis and increased bone forming activity of osteoblasts make it a good anabolic target in bone. The diversity of receptors and transporters that influence glutamate signalling make elucidation of this pathway difficult, but also provide the potential for subtle and targeted manipulation of bone cell responses. In addition to glutamate, a wide range of signalling molecules associated with neurotransmission, have now been identified in bone. These transmitters include VIP, substance P, NPY, CGRP, ATP, seratonin and dopamine. Receptors for these signalling molecules are expressed by bone cells and often activate convergent signalling cascades to influence the phenotype of both osteoblasts and osteoclasts. Since at least some of these neurotransmitters are known to be released by bone cells, it is unclear how transmitters released from nerve fibres contribute to, and interact with, local control of bone cell phenotype. However, the profound effects of these molecules on bone cell phenotype means that further investigation is essential to elucidate their role in maintenance of healthy bone and exploit their effects in the treatment of bone pathology.

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