J Musculoskel Neuron Interact 2002; 2(5):415-422

Perspective Article

Hylonome

Functional osteoblastic ionotropic glutamate receptors are a prerequisite for bone formation

A.F. Taylor

Musculoskeletal Research Laboratory, Department of Orthopaedics and Rehabilitation, Penn State College of Medicine, Hershey, PA, USA

Abstract

Many studies have now demonstrated that osteoblasts express the protein components necessary for functional neuronal-like glutamatergic signalling to occur, and as a result a physiological role for receptor mediated osteoblastic glutamate signalling has been proposed. Osteoblastic ionotropic type glutamate receptors (iGluRs) have been shown to be functional; they possess electrophysiological characteristics similar to neuronal iGluR[1,2]) and agonist application modulates the activity of intracellular signalling molecules[3] and osteoblastic transcription factors[4]. The physiological importance of osteoblastic iGluRs is illustrated by the fact that osteoblasts treated *in vitro* with non-competitive iGluR antagonists fail to form mineralized bone. Interestingly compounds known to antagonize specific sub-types of iGluR induce different effects when applied to osteoblasts derived from long and flat bones. These data imply that not only are functional osteoblastic iGluRs a prerequisite for osteoblast differentiation and bone formation, but also that the components of osteoblastic glutamatergic signalling may be adapted to reflect the differential function of osteoblasts from different skeletal sites. This paper reviews the evidence that suggests that iGluR-mediated glutamate signalling plays a fundamental role in the regulation of osteoblast function and bone formation, and discusses the therapeutic potential of manipulation of osteoblastic iGluR to modulate bone homeostasis.

Keywords: Adipogenesis, Bone Formation, Glutamate Receptor, Osteoblast, Osteoclastogenesis

Introduction

The idea that a ubiquitously expressed amino acid could act as a signalling molecule in the brain was in the early 1960s considered a laughable suggestion. Yet it is now nearly impossible to pick up a biology textbook that doesn't discuss the role of the excitatory amino acid glutamate as the major excitatory synaptic neurotransmitter. The proposed therapeutic possibilities and obvious financial rewards to be gained from the manipulation of the glutamatergic synapse has helped to drive modern neurological research and achieve a better understanding of glutamate signalling. As a result of these efforts numerous proteins deemed to be "key" or essential for the regulation of both pre- and post-synaptic glutamate signalling have been identified and characterized.

Corresponding author: Amanda F. Taylor, Musculoskeletal Research Laboratory, Department of Orthopaedics and Rehabilitation, Penn State College of Medicine, 500 University Drive, PO Box 850, Hershey, PA, 17033, USA
E-Mail: ataylor@mrl.hmc.psu.edu

Accepted 6 July 2002

Interestingly, over the past 10 years, researchers working in fields outside the CNS have cloned a selection of these "key" glutamatergic signalling proteins from non-neuronal tissues and as a result a strong case for receptor-mediated glutamatergic signalling in tissues such as the pancreas[5-8] and skin[9-11] has been proposed (reviewed by Genever and Skerry[12]). However, some of the most comprehensive and compelling evidence for receptor mediated-glutamate signalling existing outside the CNS is provided by work from members of the bone field. Although the main focus of this review is evidence pertaining to a physiological function of glutamate signalling through osteoblastic glutamate receptors, it is noteworthy that there is functional evidence for osteoclastic glutamate signalling. Although outside the scope of this review, the well-known and crucial relationship between osteoblasts and osteoclasts in the regulation of bone homeostasis is such that osteoblastic glutamate signalling should not be considered in isolation in view of the presence of functional osteoclastic glutamate receptors (see paper in this issue by Dr. C. Chenu).

Numerous groups have identified protein components required for functional neuronal glutamate signalling in the cellular components of bone, and these studies were the sub-

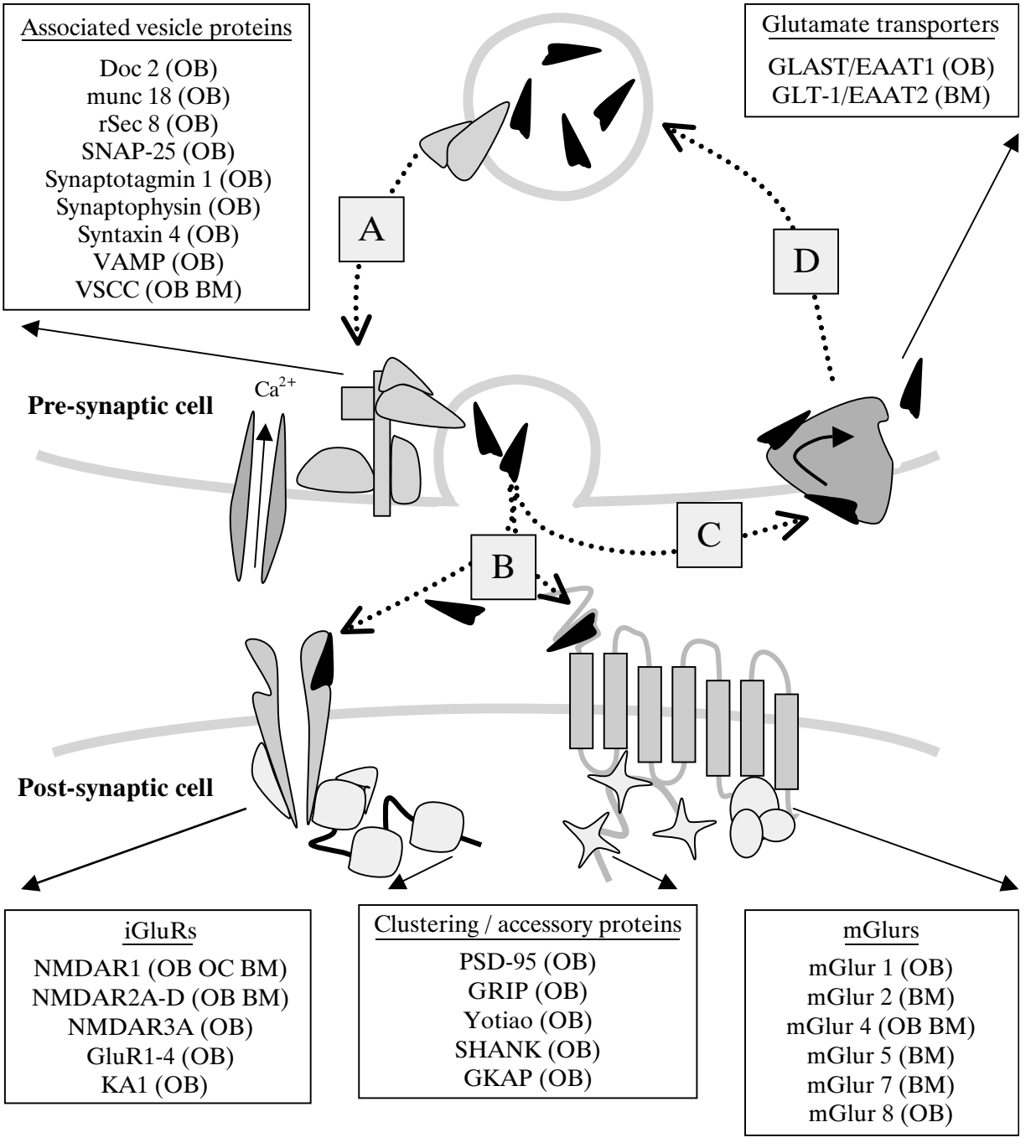

Figure 1. Schematic of the protein components found in a glutamatergic synapse and those identified within bone cells. Within pre-synaptic neurones glutamate is packaged into intracellular vesicles which are then subsequently directed to and tethered at the pre-synaptic membrane (A). In response to specific stimuli glutamate is released from the pre-synaptic cell into the synaptic cleft wherein it binds to and activates both iGluR and mGlur (B). Glutamate is removed from within the synaptic cleft by glutamate transporters terminating this episode of glutamatergic signalling (C) and permitting the recycling of glutamate molecules and the regeneration of the glutamatergic signal (D). Glutamatergic proteins identified in osteoblasts (OB), osteoclasts (OC) and bone marrow (BM) are listed in accompanying boxes[2,13,24, 35,37-39].

ject of a recent review by Genever and Skerry[13] and key findings summarized in Figure 1. The range of glutamatergic proteins expressed by osteoblasts implies that osteoblastic glutamate signalling follows similar mechanisms to those seen in the CNS. Thus, it is prudent before discussing the proposed function of glutamate signalling between osteoblasts to understand the process by which neuronal glutamate acts as a signalling molecule.

Glutamate signalling

The efficacy of an episode of glutamate signalling is dependent on the co-ordinated release, action and recovery of glutamate from within the synaptic cleft, and as such these events are tightly regulated (Figure 1). Briefly, in response to a presynaptic depolarization event, glutamate is released into the synaptic cleft from primed intracellular vesicles located within the pre-synaptic cell. This extracellular glutamate remains contained within the synaptic cleft and acts on a variety of glutamate sensitive receptors present on the post-synaptic cell. This release of glutamate simultaneously triggers transporters located on the pre-synaptic cell, which actively removes the glutamate from within the cleft, enabling it to be recycled and bringing about the cessation of the signalling episode. The detrimental consequences of uncoupling these events often results in permanent neurological due to the continued presence of excitotoxic concentrations of glutamate within the cleft[14,15].

Glutamate receptors and associated signalling molecules

Neuronal glutamate receptors fall into one of two classes. The metabotropic type (mGlur) are large 7 transmembrane G-protein linked receptors whereas the ionotropic type (iGluR) are multimeric and act as glutamate-gated ion channels[16]. Members of these families of glutamate receptors are further classified according to consequences of activation and both kinetic and pharmacological properties (Figure 2)[16,17]. As the majority of published data regarding glutamatergic signalling in bone is based on observations centred on the function of osteoblastic and osteoclastic iGluRs, they will be the mainstay of this review.

The iGluR family of glutamate receptors are further subdivided into three groups according to their kinetic responsiveness to the artificial ligands: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate. As a result iGluR are referred to as being either NMDA, AMPA or kainate type iGluRs. Although NMDA, AMPA and kainate type iGluR exist and function as independent receptors within the CNS, the AMPA and kainate type iGluRs display very similar properties of activation. This is further complicated by the relative paucity of pharmacological compounds that can differentiate reliably between AMPA and kainate type iGluRs. Hence in some studies iGluR antagonists used are not capable of discriminating between AMPA and kainate type iGluRs and as a result these receptors are routinely grouped together as "non-NMDA" type iGluRs.

A vast number of signalling proteins involved in the initiation of intracellular signalling cascades are found attached/adjacent to the post-synaptic membranes of glutamatergic synapse[18-20]. A number of these proteins have been found to associate with iGluRs (calmodulin CAMKII, PKA, PKC and Yotio)[21] and some cluster to specific iGluR family members (PSD-95 and GRIP to NMDA and AMPA type iGluR respectively)[22,23]. This clustering acts to co-localize iGluR with specific intracellular signalling molecules, thus increasing the efficiency of transducing the signal and also explaining how a single episode of glutamate signalling can trigger numerous different intracellular signalling cascades[18].

Glutamate signalling in osteoblasts

The working model used to describe osteoblastic glutamate signalling is understandably based on the neuronal glutamatergic synapse; hence the events involved in an episode of osteoblastic glutamate signalling are still classified as either "pre-synaptic" or "post-synaptic". The "pre-synaptic" events of regulated osteoblastic glutamate release and recovery are the subject of a forthcoming review to be published in JMNI (P.S. Bhangu), but the key components identified in osteoblasts are illustrated in Figure 1. This paper aims to review the evidence that "post-synaptic" like glutamate signalling plays a role in osteoblast function.

Post-synaptic glutamatergic proteins identified in osteoblasts

To date the majority of data published on osteoblastic post-synaptic glutamatergic proteins has concentrated on osteoblastic iGluRs although a number of different mGlurs have been recently cloned and sequenced from osteoblasts[2,24]. As expected, osteoblastic mGlurs appear to function independently of iGluRs, but there is preliminary evidence that the close association between mGlurs and NMDA type iGluRs seen in the CNS exists within osteoblasts[2]. Despite these recent studies there is to date no data regarding the physiological role of osteoblastic mGlurs, and hence the remainder of this paper concentrates on outlining the possible physiological role of osteoblastic iGluRs.

As mentioned earlier, numerous post-synaptic glutamatergic proteins have been identified in both primary osteoblasts and osteosarcoma cell lines (Figure 1). These include a number of NMDA, AMPA and kainate type iGluR sub-units necessary for the formation of NMDA, AMPA and kainate type iGluRs[25]. It has also been possible to identify and clone from osteoblasts a number of proteins essential for the formation and functionality of neuronal iGluR receptor signalling complexes. The identification of osteoblastic

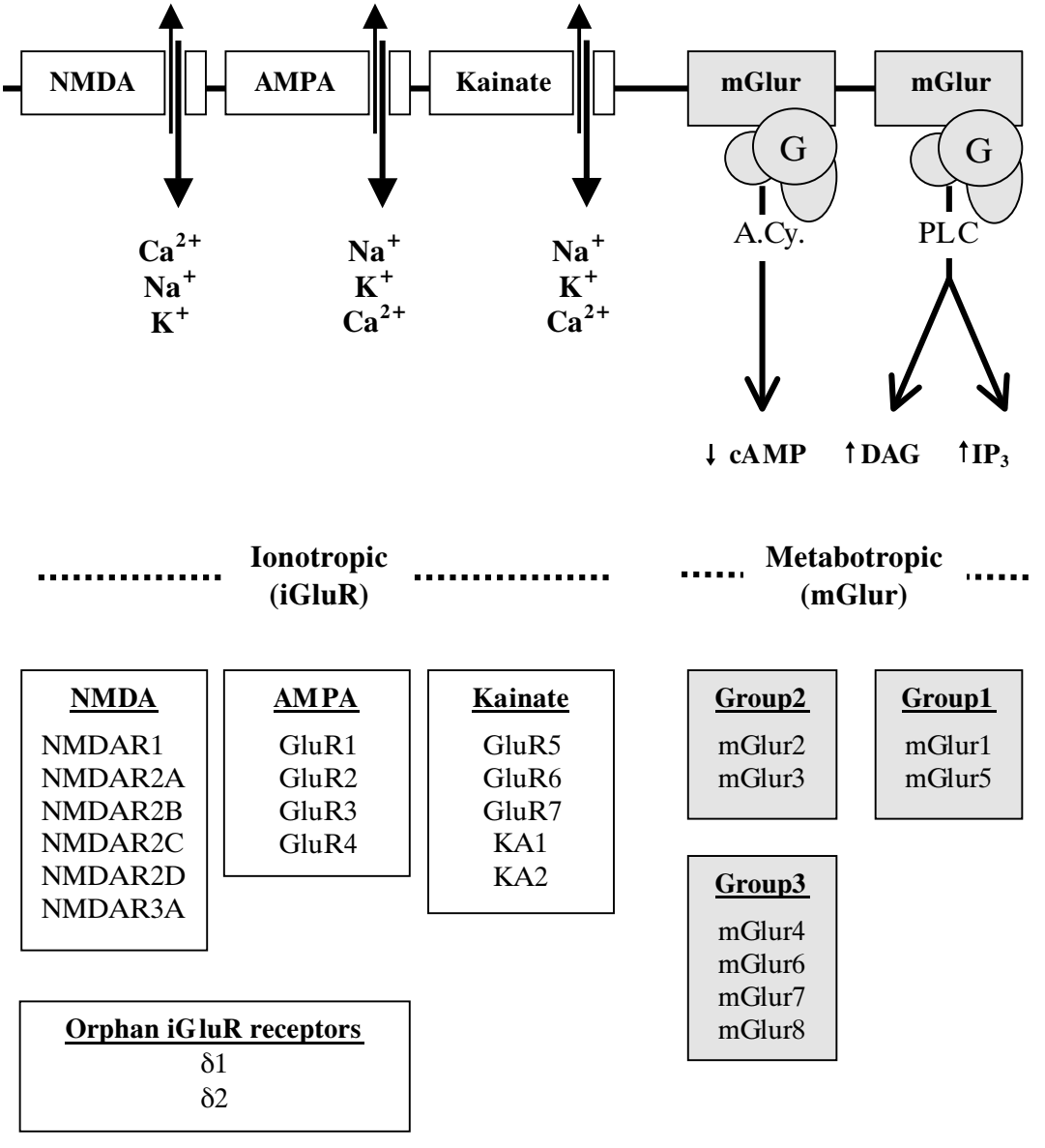

Figure 2. Members of the glutamate receptor family. Glutamate receptors are classified as either ligand gated ion channels or G-protein linked receptors (iGluR or mGlur respectively). The iGluR family is split into 3 sub-groups according to the kinetics of activation and the selectivity of the ion channel (most favoured listed first and least last). Below each sub-group of iGluR are the sub-units that combine to form these receptors. Although classified as iGluR sub-units there is little evidence that orphan receptors form functional iGluR, hence they are only included in this diagram for completeness. The mGlurs are further sub-classified by the intracellular pathways triggered upon activation[17].

PSD-95, GRIP, Yotiao and SHANK gives further weight to the notion that osteoblastic glutamate signalling has a physiological relevance[3].

A number of elegant electrophysiological studies have shown that osteoblastic NMDA type iGluRs display very similar functional characteristics as those seen in neuronal systems[1,2]. Using the patch-clamp technique Laketic-Ljubojevic and colleagues showed in osteoblasts that a rapid increase in membrane potential and intracellular calcium is seen following the application of L-glutamate or NMDA. As in the CNS, the L-glutamate / NMDA-induced membrane depolarization of osteoblasts is blocked by the co-application of a number of antagonists (MK801, R-CPP or magnesium) commonly used against NMDA type iGluR.

Further studies show that the activity of iGluR associated protein kinases (Spencer and Skerry, unpublished data) and the transcription factor AP-1[4] are modulated by the activation of osteoblastic iGluRs, again in a similar manner to that

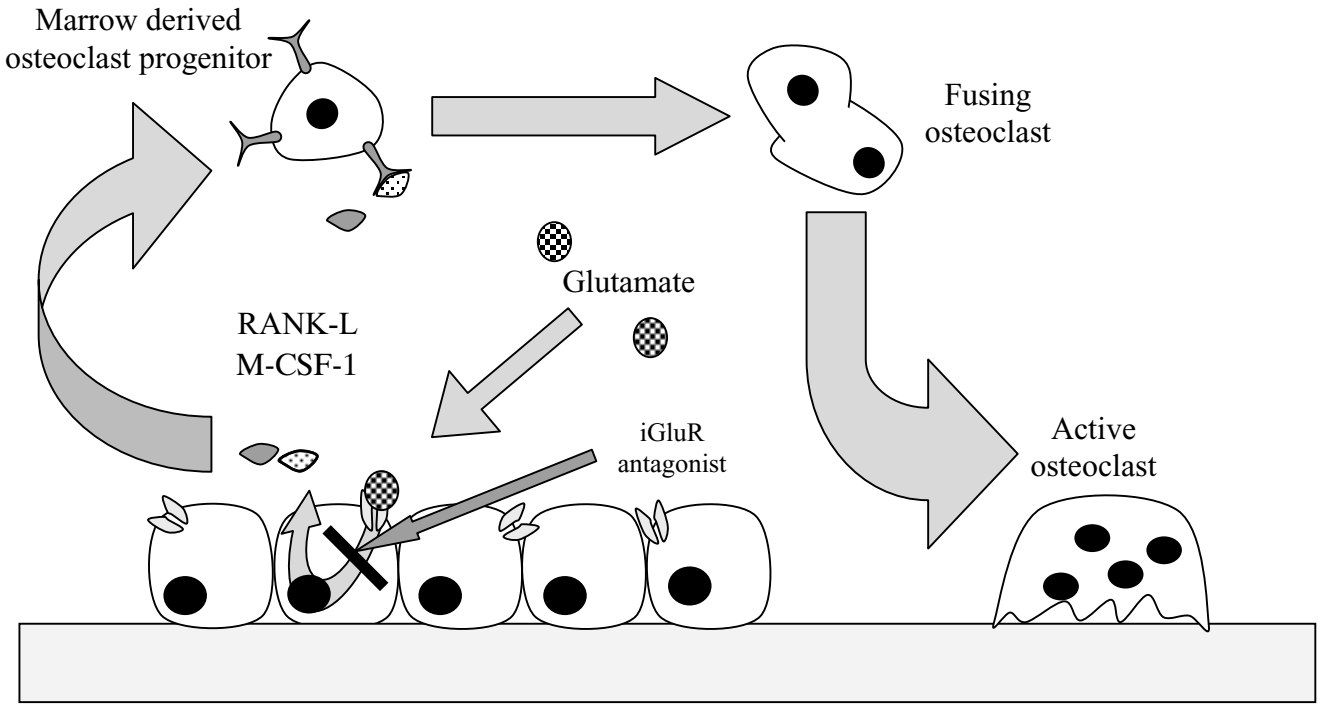

Figure 3. Blocking osteoblastic iGluRs may indirectly modulate osteoclastogenesis. iGluR antagonists modulate the phenotype of osteoblasts present within co-cultures causing a reduction in RANK-L and M-CSF-1 expression; this would have the knock-on effect of reducing the number of osteoclasts generated and hence the resorption observed within the system.

which is seen within neurones. These signalling events are presumably triggered by the elevated intracellular calcium seen to accompany osteoblastic iGluR activation[1]. These data provide compelling evidence for the existence of functional osteoblastic iGluR, which is in turn suggestive that osteoblasts utilize glutamate as a signalling molecule.

The role of osteoblastic iGluR in bone formation

A number of problems are presented when studying non-neuronal glutamate receptor function *in vivo*, the most telling being that the majority of the commercially available glutamate receptor antagonists are specifically designed to readily cross the blood brain barrier and modulate neurological function. Common consequences of these neurological effects are that animals treated with glutamate receptor antagonists often display abnormal behaviour. For example, when administered to rats the classical NMDA type iGluR antagonist PCP (or angel dust) rather unsurprisingly induces a catatonic state (Brabbs and Skerry, unpublished data). Thus, the associated behavioural effects of administering iGluR antagonists to experimental animals often hamper *in vivo* studies of osteoblastic (and osteoclastic) iGluR function and as such, useful data are limited[26]. As a result of these constraints, the majority of the information gleaned about the role of osteoblastic glutamatergic signalling has been obtained from *in vitro* rather than *in vivo* studies[26].

Unfortunately, conducting studies *in vitro* present some additional problems, the first one being that glutamate is a prerequisite normal cell growth and thus it is impossible to culture osteoblasts under osteogenic conditions in the absence of glutamate, so agonist studies are problematic. The large amounts of glutamate / glutamine present within tissue culture media and serum also present a serious experimental problem where competitive antagonists are used. In culture, the high concentrations of endogenous glutamate act to compete out competitive iGluR antagonists at physiologically relevant doses. As a result, bone formation in osteoblastic cultures treated with competitive iGluR antagonists such as R-CPP and A-PV rarely differ from controls (Taylor, unpublished data). Thus, evidence for the necessity of glutamate signalling in bone formation is for the most part based on *in vitro* studies conducted using non-competitive iGluR antagonists. A number of studies performed using both calvarial and long bone derived osteoblasts have shown that non-competitive blockade of either NMDA or non-NMDA type osteoblastic iGluRs results to inhibit bone formation *in vitro*[28,29].

Whilst non-competitively inhibiting glutamatergic signalling through either type of iGluR results to prevent *in vitro* bone formation, the manner and resulting outcome of this inhibition is highly dependent on which type of iGluR is inhibited. Within the marrow cell CFU-F culture system,

inhibition of non-NMDA type iGluR via the application of CFM-2, prevents the adherence of osteoblast precursors, whereas inhibiting NMDA type iGluR results to convert marrow-derived cells from a pre-osteoblastic to a pre-adipocytic phenotype. In contrast, cultures treated with MK801 (a non-competitive antagonist of NMDA type iGluR), had decreased levels of collagen type 1, osteopontin and osteocalcin expression and reciprocal increases in PREF-1 and C/EBP-α expression[28]. In calvarial-derived osteoblasts the inhibition of NMDA type iGluR abolishes mineralization of nodules but there is apparently little effect on osteoblastic maturation and treated cultures express similar levels of collagen type 1, osteopontin and osteocalcin as controls[29]. Within the same culture system, the application of antagonists against non-NMDA type iGluRs generates mature aP2, C/EBP-α, lipoprotein lypase and PPARγ expressing adipocytes[29]. Thus, data from the *in vitro* studies described above appear to indicate that the function of osteoblastic NMDA and non-NMDA type iGluR may be different in osteoblasts derived from different sources and thus glutamate signalling tailored to meet specific skeletal requirements.

A somewhat unexpected observation is that calvarial cultures treated with low doses of CFM-2 consistently and significantly form more bone *in vitro* than controls. The ability of an iGluR antagonist to both positively and negatively modulate bone formation concurrent to inducing adipogenesis seems at first a little contradictory; how can one compound have apparently multiple and conflicting effects? However further studies conducted using adipogenic agents, CFM-2 and a variety of different non-NMDA type iGluR antagonists (including GYKI 52466) corroborate these findings[29].

A simple explanation for these observed responses of CFM-2 treatment is that CFM-2 acts to antagonize individual non-NMDA type iGluRs with differing potency. Within the CNS, CFM-2 has been shown to preferentially antagonize AMPA type iGluRs over the kainate type[30]. Thus, it is possible that low doses of CFM-2 act mainly to block signalling through AMPA type iGluRs, but as the concentration of CFM-2 increases more of the kainate type iGluRs are affected. This would suggest that signalling through osteoblastic AMPA and kainate type iGluRs mediate different intracellular effects, a theory consistent with previously observed differences between AMPA and kainate-induced increased AP-1 activity. Possibly glutamatergic signalling through kainate type iGluRs regulates adipogenesis whilst AMPA type iGluRs regulate bone formation.

Of considerable interest to these data are the recent findings of Arai[31] who reported that when applied to pyramidal neurones from the hippocampus, the non-NMDA type iGluR antagonist GYKI 52466, both positively and negatively regulate AMPA type iGluR in a dose-dependent manner. One explanation Arai proposes for the action of GYKI 52466 is that it shares its binding site with a class of compounds known to prevent the sensitization of AMPA type iGluRs to glutamate. If true, this theory suggests that GYKI 52466 and the structurally related CFM-2 interact with osteoblastic AMPA type iGluR in a manner that initially protects them from becoming sensitized to glutamate, thus prolonging the signalling episode. This also implies that iGluR anti-desensitization agents may well provide a method of modulating osteoblastic activity. Further support for this possibility comes from data showing that such antidesensitization agents affect bone mass when given systemically.

Against these data, in one paper, it has been suggested that glutamate has no role in bone. Gray et al.[27] suggest that glutamatergic signalling plays little or no important role in bone formation because animals lacking the GLAST transporter have no overt skeletal phenotype. Additional support for this assertion is drawn from a number of *in vitro* studies conducted using competitive antagonists of NMDA type iGluR, experiments with the drawbacks previously described. So although their results raise some questions as to the role of glutamate in the skeleton, lack of consideration of the level of endogenous glutamate within their cultures with the concentration of competitive antagonist utilized[32,33] leave the case unproven. In their assessment of skeletal abnormalities in GLAST knockout mice, Gray et al. have also failed to consider the number of genes which have redundancy in their function. This phenomenon was discussed in Nature[34], where the pitfalls of knockout mice were attributed to high degrees of protein redundancy and the need for stringent phenotyping.

A role for osteoblastic iGluR in regulating osteoclastogenesis?

Studies from our laboratory using the co-culture system of maturing osteoclastic precursors by simultaneously culturing them with pre-osteoblasts have shown that application of non-competitive NMDA type iGluR antagonists inhibits *in vitro* osteoclastogenesis. In light of the recent studies described above which implicate glutamate signalling as a prerequisite of osteoblast function, it is no longer possible to be certain that the effects seen within the co-culture model are due to iGluR antagonists acting directly on osteoclast precursors. Instead the effects of NMDA type iGluR antagonists observed by Peet et al.[35] may be due to modulating the osteoblastic activity / phenotype of pre-osteoblast present within these cultures which would have the secondary effect of inhibiting *in vitro* osteoclastogenesis (Figure 3).

To support this theory, there are striking similarities between the profile of inhibition seen when osteoclasts generated in co-cultures are treated with iGluR antagonists and the inhibition profile of osteoblasts treated with the same iGluR antagonists. This theory is further supported by the fact that when administered to co-cultures, low doses of non-NMDA type iGluR antagonists increase *in vitro* osteoclastogenesis at doses similar to those shown to increase the osteogenic potential of calvarial osteoblasts[26] (manuscript in preparation). Although there is as yet little direct evidence

to confirm that iGluR antagonists act directly on the pre-osteoblast present within the co-culture system to modulate *in vitro* osteoclastogenesis, further studies investigating expression of osteotropic factors, such as RANK-L may better clarify this situation.

Therapeutic implications

Traditional therapies for treating bone disorders have concentrated on controlling the generation and activity of osteoclasts, although a number of recently proposed therapeutic approaches work by regulating the differentiation of mesenchymal precursors and marrow adipogenesis (reviewed by Nuttall and Gimble[36]). *In vitro* studies utilizing non-competitive antagonists show that functional osteoblastic iGluRs are a prerequisite for bone formation; these data also demonstrated an association between inhibiting osteoblastic iGluR mediate glutamate signalling and increase adipogenesis. As a result, the modulation of osteoblastic glutamate signalling may provide a novel therapeutic approach to treating osteopenic disorders where a reciprocal relationship between adipogenesis and osteoblastogenesis is often observed.

Data from *in vitro* bone formation studies also indicate distinct functional differences between osteoblastic NMDA and non-NMDA iGluR-mediated glutamate signalling in marrow and calvarial-derived osteoblasts, a phenomenon often observed in the CNS which is directly attributed to regional variations in the composition of glutamatergic synapse. Thus, it is possible that the differences observed in antagonist studies conducted on marrow and calvarial-derived osteoblasts may be attributed to subtle variations in the expression of iGluR or iGluR-associated signalling proteins within these cells. These data imply that the role of iGluR-mediated glutamate signalling in osteoblasts may be dependent on skeletal location, and may explain the subtle difference in osteoblastic iGluR identified by various groups[2,25,37,38]. Although to date studies conducted have not investigated the possibility of site directed osteoblastic iGluR expression the repercussions on potential glutamate-based therapeutic intervention in bone are vast. If the role of glutamate signalling in bone and osteoblastic iGluR expression were dependent on the skeletal site, therapeutic approaches utilizing iGluR antagonists may potentially enable site directed modulation of osteoblastic activity.

A clear advantage of exploring the therapeutic potential of osteoblastic glutamate signalling is the vast amount of work by members of the neuronal field who have already developed therapeutic agents to target neuronal glutamate signalling which may be utilised in bone. It is also possible that existing compounds originally discounted as being therapeutically useful due to poor passage over the blood brain barrier may prove to be potent manipulators of osteoblastic glutamatergic signalling pathways and thus be financially attractive therapeutic bone agents!

Acknowledgments

I would like to acknowledge the staff at both the Royal Veterinary College and the University of York for their scientific contributions, which aided the writing of this review. In particular I would like to thank Drs. K. Dobson, P. Genever, N. Peet and G. Spencer for discussing unpublished work with me.

References

1. Laketic-Ljubojevic I, Suva LJ, Maathuis FJ, Sanders D, Skerry TM. Functional characterization of N-methyl-D-aspartic acid-gated channels in bone cells. Bone 1999; 25:631-637.
2. Gu Y, Publicover SJ. Expression of functional metabotropic glutamate receptors in primary cultured rat osteoblasts. Cross-talk with N-methyl-D-aspartate receptors. J Biol Chem 2000; 275:34252-34259.
3. Spencer GJ, Genever PG, Grewal TS, Bhangu PS, Skerry TM. P-203: Mechanisms for intracellular localization of NMDA receptors in osteoblasts and their association with downsrteam signalling complexes. Calcif Tissue Int 2000; S1:S103 (abstract).
4. Taylor AF, Birch M, Skerry TM. c-fos dependent and independent AP-1 translocation induced by NMDA, AMPA and kainate glutamate receptors in bone. J Bone Miner Res 1999; 14:14 (abstract).
5. Weaver CD, Yao TL, Powers AC, Verdoorn TA. Differential expression of glutamate receptor subtypes in rat pancreatic islets. J Biol Chem 1996; 271:12977-12984.
6. Inagaki N, Kuromi H, Gonoi T, Okamoto Y, Ishida H, Seino Y, Kaneko T, Iwanaga T, Seino S. Expression and role of ionotropic glutamate receptors in pancreatic islet cells. FASEB J 1995; 9:686-691.
7. Bertrand G, Gross R, Puech R, Loubatieres-Mariani MM, Bockaert J. Evidence for a glutamate receptor of the AMPA subtype which mediates insulin release from rat perfused pancreas. Br J Pharmacol 1992; 106:354-359.
8. Bertrand G, Gross R, Puech R, Loubatieres-Mariani MM, Bockaert J. Glutamate stimulates glucagon secretion via an excitatory amino acid receptor of the AMPA subtype in rat pancreas. Eur J Pharmacol 1993; 237:45-50.
9. Genever PG, Maxfield SJ, Kennovin GD, Maltman J, Bowgen CJ, Raxworthy MJ, Skerry TM. Evidence for a novel glutamate-mediated signaling pathway in keratinocytes. J Invest Dermatol 1999; 112:337-342.
10. Morhenn VB, Waleh NS, Mansbridge JN, Unson D, Zolotorev A, Cline P, Toll L. Evidence for an NMDA receptor subunit in human keratinocytes and rat cardiocytes. Eur J Pharmacol 1994; 268:409-414.
11. Aramori I, Nakanishi S. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. Neuron 1992; 8:757-765.
12. Genever PG, Skerry TM. Non-neuronal glutamate signalling pathways. Emerging Therapeutic Targets 2000; 4(3):333-345.
13. Genever PG, Skerry TM. Glutamate signalling in bone: a therapeutic target for osteoporosis? Emerging Therapeutic Targets 2000; 4:207-218.
14. Obrenovitch TP, Urenjak J. Altered glutamatergic transmission in neurological disorders: from high extracellular glutamate to excessive synaptic efficacy. Prog Neurobiol 1997; 51:39-87.

15. Choi DW. Excitotoxic cell death. J Neurobiol 1992; 23:1261-1276.
16. Hollmann M, Heinemann S. Cloned glutamate receptors. Annu Rev Neurosci 1994; 17:31-108.
17. Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. Pharmacol Rev 1999; 51:7-61.
18. Blackstone C, Sheng M. Protein targeting and calcium signaling microdomains in neuronal cells. Cell Calcium 1999; 26:181-192.
19. Sheng M, Pak DT. Glutamate receptor anchoring proteins and the molecular organization of excitatory synapses. Ann N Y Acad Sci 1999; 868:483-493.
20. Sheng M, Pak DT. Ligand-gated ion channel interactions with cytoskeletal and signaling proteins. Annu Rev Physiol 2000; 62:755-778.
21. Smart TG. Regulation of excitatory and inhibitory neurotransmitter-gated ion channels by protein phosphorylation. Curr Opin Neurobiol 1997; 7:358-367.
22. Niethammer M, Kim E, Sheng M. Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci 1996; 16:2157-2163.
23. Dong H, O'Brien RJ, Fung ET, Lanahan AA, Worley PF, Huganir RL. GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. Nature 1997; 386:279-284.
24. Hinoi E, Fujimori S, Nakamura Y, Yoneda Y. Group III metabotropic glutamate receptors in rat cultured calvarial osteoblasts. Biochem Biophys Res Commun 2001; 281:341-346.
25. Patton AJ, Genever PG, Birch MA, Suva LJ, Skerry TM. 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 1998; 22:645-649.
26. Taylor AF, Brabbs AC, Peet NM, Laketic-Ljubojevic I., Skerry TM. Bone/resorption and osteoblast/adipocyte plasticity mediated by AMPA / Kainate glutamate receptors *in vitro* and *in vivo*. J Bone Miner Res 2000; 15(S.1):275 (abstract).
27. Gray C, Marie H, Arora M, Tanaka K, Boyde A, Jones S, Attwell D. Glutamate does not play a major role in controlling bone growth. J Bone Miner Res 2001; 16:742-749.
28. Dobson KR, Skerry TM. NMDA-type glutamate receptor antagonism inhibits differentiation of rat bone marrow osteoprogenitors. Manuscript under review.
29. Hirai H. Modification of AMPA receptor clustering regulates cerebellar synaptic plasticity. Neurosci Res 2001; 39:261-267.
30. Chimirri A, De SG, De SA, Gitto R, Grasso S, Quartarone S, Zappala M, Giusti P, Libri V, Constanti A, Chapman AG. 1-Aryl-3,5-dihydro-4H-2,3-benzodiazepin-4-ones: novel AMPA receptor antagonists. J Med Chem 1997; 40:1258-1269.
31. Arai AC. GYKI 52466 has positive modulatory effects on AMPA receptors. Brain Res 2001; 892:396-400.
32. Chenu C, Itzstein C, Espinosa L. Absence of evidence is not evidence of absence. Redundancy blocks determination of cause and effect. J Bone Miner Res 2001; 16:1728-1729.
33. Skerry TM, Genever PG, Taylor AF, Dobson KR, Mason D, Suva LJ. The shortcomings of the GLAST knockout mouse. J Bone Miner Res 2001; 16:1729-1730.
34. Pearson H. Surviving a knockout blow. Nature 2002; 415:8-9.
35. Peet NM, Grabowski PS, Laketic LI, Skerry TM. The glutamate receptor antagonist MK801 modulates bone resorption by a mechanism predominantly involving osteoclast differentiation. FASEB J 1999; 13:2179-2185.
36. Nuttall ME, Gimble JM. Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis? Bone 2000; 27:177-184.
37. Chenu C, Serre CM, Raynal C, Burt PB, Delmas PD. Glutamate receptors are expressed by bone cells and are involved in bone resorption. Bone 1998; 22:295-299.
38. Itzstein C, Cheynel H, Burt-Pichat B, Merle B, Espinosa L, Delmas PD, Chenu C. Molecular identification of NMDA glutamate receptors expressed in bone cells. J Cell Biochem 2001; 82:134-144.
39. Huggett J, Vaughan-Thomas A, Mason D. The open reading frame of the Na(+)-dependent glutamate transporter GLAST-1 is expressed in bone and a splice variant of this molecule is expressed in bone and brain. FEBS Lett 2000; 485:13-18.