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Neurotransmitters in Bone

Introduction

Five of the papers of this issue of the JMNI address different aspects of the same broad subject area, namely the involvement of what have been considered traditionally to be neurotransmitters in bone cell signalling. Before introducing the papers specifically, it is worth considering why there should be such similarities between such apparently disparate tissues as the nervous system and the skeleton.

While the tissues of the body can be divided broadly into those where the cells perform the function ascribed to the tissue, and those where cell products (extracellular matrix) perform that function, it is apparent that even those distinctions are not sufficiently fundamental to provide a link between nervous and bone tissue. In the CNS, it is the cells that interconnect for function, while in the skeleton, even dead bone retains a substantial proportion of the mechanical ability of the vital tissue to support loads that is seen to be the primary function of the skeleton. However, since it is clear that the extracellular matrix of bone is, by definition, formed by the cells within the tissue, and that those cells function in an orchestrated way as a result of the mechanical and biochemical signalling influences upon them, perhaps intercellular communication is the common thread which can be ascribed as a fundamental property of both bone and nervous tissue. If that is true, then other differences between the nervous and skeletal systems appear to be at odds with a common signalling system in each. Intercellular communication in the nervous system is rapid, with the ability to resolve high frequency repetitions, to modulate those signals in response to inhibitory or potentiating stimuli and to retain a persistent record of events as learnt short and long-term memory. Why should the skeleton, which changes its properties over the course of weeks as a result of remodelling, require such a sophisticated system? The answers to this question cannot be stated with complete certainty, but there are several features of the way the skeleton functions where such properties would not only be advantageous, but essential.

The response of bone to mechanical loading is the primary case in point. We have known for many years that different forms of exercise have different "osteogenicities". The variously different skeletons of sprinters, weightlifters, cyclists, and swimmers show that not all muscle-building exercises alter skeletal strength[1]. Since the relevant difference attributed to these different exercise regimens is not the magnitude, but the rate of application of strains, it is sensible to consider how perception of different strain rates could occur. In humans (and animals), strain rates during moderate activity are low – of the order of $0.004sec^{-1}$, while vigorous activity such as landing from a jump on a hard surface engenders strains with rates over $0.1\ sec^{-1}$ (Hillam and Skerry, unpublished data). From this it is simple to calculate that applied high rate loading events reach physiological magnitudes of strain in less than 40msec, while slow rate events could take well over 10 times as long. Where bone responds to high frequency but lower strain magnitude events with high rate components, the window for perception and/or onward signalling is even less. This suggests that bone is capable of resolving differences in the time of application of strains of similar magnitude in a millisecond time scale, a property that brings neuronal levels of speed of response into perspective. I have deliberately refrained from commenting on whether the response of bone cells (or which cells are involved) is to direct tissue strain, fluid flow, or any other consequence of whole bone deformation, because those distinctions are irrelevant in this context, where only the need for a rapid perception/recording mechanism is under consideration.

The second feature of the response of bone to loading that has striking parallels with neuronal signalling arises from the way that only short durations of exercise/loading are necessary to induce a maximal osteogenic effect. In the classic first experiment to demonstrate this property of the skeleton, Rubin and Lanyon showed that in a bone otherwise subjected to disuse for 6 weeks, only 8 seconds of loading (at 0.5Hz, so only 4 cycles) was sufficient to prevent the bone loss that arose in animals where those loads were not applied[2]. Furthermore, increases of loading over 36 cycles per day (a period of 72 seconds) induced a response that was not increased by any further increase in numbers of load cycles. Subsequent to that, we showed that a single period of loading in the same disuse model could, over the course of 5 days, entrain a series of osteogenic effects that began (presumably) with some form of perception/integration /recording of mechanical information, and culminated in periosteal proliferation and new bone formation[3]. This work and later corroborative studies in other animals and humans leads to one inescapable conclusion, that short duration events have some persistent effect on cell behaviour that allows such brief periods of mechanical signalling to provide a long-term influence on cell activity – a memory of recent strain events. While the idea that strain memory could exist has been considered for some years, the data on a possible mechanism for it are sparse. In the 1980s we showed that reorientation of bone proteoglycans occurred in response to loading, and

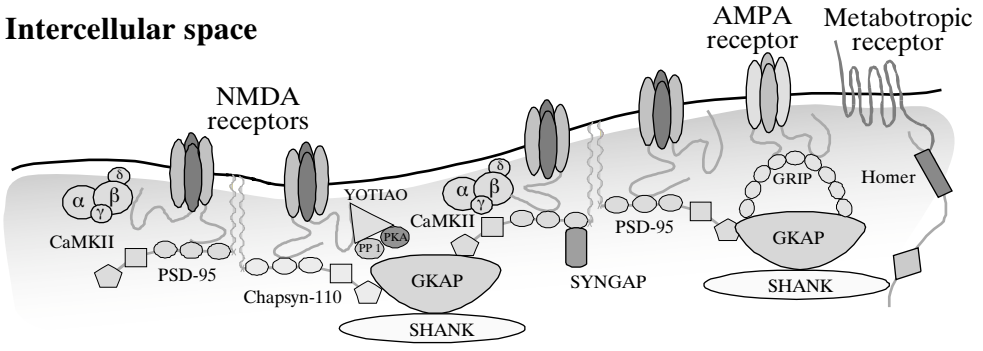

Figure 1. The postsynaptic density.

that this effect persisted for 24 hours, but had returned to control levels after 48 hours[4,5], features with similarities to the way that loading effects persisted for more than one but less than two days. However, a means by which such a change could provide an influence on cell activity remained hypothetical, perhaps altering fluid flow properties of the matrix, or changing charge density around osteocytes.

More recently though, the potential involvement of neurotransmitter mediated signalling in bone cells has raised the possibility that neuronal-like memory mechanisms may be involved in bone. In the CNS, synaptic plasticity, and particularly long-term potentiation (LTP) is the mechanism by which transient or brief signalling events can potentiate subsequent trains of depolarisations. During LTP, trains of repetitive activation lasting only seconds, cause a long-term increase in synaptic strength that can remain for days[6,7]. At a molecular level, the initiation of LTP in neurons is dependent on an increase in intracellular calcium, which is caused by the activation of postsynaptic NMDA type glutamate receptors on depolarised cells[8,9]. Elevated intracellular calcium concentrations activate calcium-calmodulin dependent protein kinase II-a (CaMKII-a) which promotes the autophosphorylation of Thr286 within the autoinhibitory domain of CaMKII, generating a long-lasting calcium-independent form of the enzyme whose activity leads to the redistribution of AMPA receptors, resulting in the persistent change in subsequent electrophysiological response that is LTP.

LTP therefore requires the activities of NMDA and AMPA-type glutamate receptors to be spatially and temporally coupled. This is achieved through specific interactions between the C-termini of glutamate receptor subunits and PDZ (PSD-95/Dlg/ZO-1) domains of multivalent clustering proteins located at the cytoplasmic face of the plasma membrane. PDZ domain-containing proteins also act as intracellular scaffolding molecules contributing to the assembly of downstream signalling complexes, which are essential for LTP function. In the CNS, these interactions result in the generation of the postsynaptic density (PSD), a highly specialised electron-dense structure enriched with receptors, cytoskeletal proteins and downstream signalling molecules (Figure 1). Retrograde messengers released from postsynaptic cells modify presynaptic glutamate release and potential candidates for this role include nitric oxide (NO) and carbon monoxide, which have already been demonstrated to have a role in the adaptive response of bone to loading[10,11].

In studies of glutamate signalling in bone cells, we have preliminary data suggesting that all the components necessary for an LTP-like process are present and functional in bone, and that CaMKII autophosphorylation occurs in cells loaded *in vitro* in a way that has striking parallels with LTP in the CNS[12]. This implies that a neuronal-like memory system may account for at least part of the ability of bone to respond to such brief periods of loading as it does. In retrospect perhaps, these findings are unsurprising. Evolution often results in the development of sophisticated physiological systems that are conserved across species and long periods of time, because they function well and are relatively fail safe. It is not illogical therefore to see that a memory system developed for brain function could be entirely appropriate for a similar function albeit at a less complex and sophisticated level in bone.

Neurotransmitter signalling in bone cells has been researched sporadically for some years. In the 1990s, Rahman et al.[13,14] showed effects of bradykinin and VIP on bone cell activity *in vitro*, but it was not until some years later that other experiments explored the subject more fully. The almost simultaneous discovery that glutamate could act as a signalling molecule in bone by two different groups[15,16] has grown so that there are now compelling data to suggest that both osteoblast and osteoclast function are regulated by glutamate. This work is the subject of 3 articles in this issue of JMNI. Mason and Huggett explore the role of glutamate transporters in bone. Taylor describes the expression and function of glutamate receptors in osteoblasts, and Chenu reviews the data on osteoclastic bone resorption and glutamate signalling. After this, are two articles on different transmitter systems, P2 receptor expression and function in bone by Gallagher and Buckley, and finally a review of other neurotransmitters by Lerner. Together these papers summarise most of the work on neurotransmitter signalling in bone, with the major exception of work on dopamine and

serotonin signalling by Bliziotes' group, that has already featured in a recent issue of JMNI[17].

Before concluding, it is worth considering one further aspect of this subject. Recent studies by Karsenty's group have illustrated possible mechanisms by which leptin influences skeletal mass[18]. These studies point towards a central, not local action of leptin that is effected by a neuronal action involving as yet unidentified specific nerve fibres, although others have hypothesised a more local effect of leptin[19]. While local paracrine release of neurotransmitters by bone cells may be one mechanism of activation of the relevant neurotransmitter receptors in the skeleton, it is possible that neuronal release could also be involved in a central regulatory effect on bone cells. Such a phenomenon is clouded by the differences in findings of leptin receptors in bone cells, and it is likely that at least some central influence is exerted on bone cells, whether directly or as a result of, for example, control of vasomotor tone to regulate blood supply to the tissue. It is unlikely that such a control could be more than another systemic influence however, since it was shown many years ago that denervated bones respond normally to applied mechanical loads[20]. Furthermore, the response of isolated bones and cells to mechanical influences shows clearly that whatever central influences may exist, there are also local ones with which others must be integrated.

When signalling systems that are well characterised in one tissue or system are discovered to function in another site, there is the potential for unusually rapid research, because progress in the first field provides resources and understanding that is relevant to the second. For this reason, the identification of neurotransmitter-mediated processes in bone could lead to novel insights into drug discovery for treatment of bone diseases, and that makes these research areas exciting and progressive. Whether new drugs are developed based on the findings outlined in the papers in this journal would be a decision influenced by many other factors, but the potential for such development is there.

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Tim Skerry

Department of Veterinary Basic Sciences, Royal Veterinary College, London, UK