FEBS Letters 559 (2004) 6–12 FEBS 28070

Minireview

Emerging neuroskeletal signalling pathways: a review

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Received 2 December 2003; revised 6 January 2004; accepted 7 January 2004

First published online 23 January 2004

Edited by Jesus Avila

Abstract Recent work has demonstrated that neurotransmitters, signalling molecules primarily associated with the nervous system, can have profound effects on the skeleton. Bone cells express a broad range of neurotransmitter receptors and transporters, and respond to receptor activation by initiating diverse intracellular signalling pathways, which modulate cellular function. Evidence of neuronal innervation in skeletal tissues, neurotransmitter release directly from bone cells and functional effects of pharmacological manipulation support the existence of a complex and functionally significant neurotransmitter-mediated signalling network in bone. This review aims to concisely summarise our current understanding of how neurotransmitters affect the skeletal system, focusing on their origin, cellular targets and functional effects in bone.

Key words: Bone; Marrow; Nerve; Neurotransmitter; Osteoblast; Osteoclast

1. Introduction

Throughout life, old or damaged bone is periodically removed and new bone is formed at discrete sites throughout the skeleton. This regenerative process of bone remodelling is dependent on the coupled formative and resorptive activities of specialised bone cells, osteoblasts and osteoclasts, and serves to prevent inappropriate changes in bone mass whilst allowing precise structural adaptation necessary to meet the varying physical and metabolic demands placed on the skeleton.

Intercellular communication plays a fundamental role in regulating remodelling events, by controlling cell lineage commitment, proliferation, differentiation, function and apoptosis of bone cells. A wide variety of cellular signals are implicated in these processes including mechanical strain, systemic hormones and locally released cytokines and growth factors. In this review we focus on the emerging role of neurotransmitters in bone and describe how these diverse signalling molecules regulate bone formation and bone resorption through effects on osteoblasts and osteoclasts, and the activation of specific intracellular signalling pathways. Bone cells express a range of functional neurotransmitter receptors and transporters including those for glutamate, γ-aminobutyric acid (GABA), purines and pyrimidines, 5-hydroxytryptamine (5-HT), catecholamines (dopamine, adrenaline and noradrenaline), and neuropeptides (vasointestinal peptide (VIP), substance P (SP), and calcitonin gene-related peptide (CGRP)). Their expression and activity provides direct evidence for a complex neurotransmitter-mediated signalling network in bone (Fig. 1). We also discuss neuronal innervation of the skeleton and describe how bone cells themselves release neurotransmitters into the bone microenvironment, which act as local autocrine and/or paracrine signalling molecules, regulating the activity of neighbouring cells. Finally we discuss how combining current knowledge and resources of pharmaceutical companies could provide fast-track treatment for a wide range of debilitating bone disorders including osteoporosis.

2. Neuronal innervation of bone

The existence of nerve fibres in bone is well established, although the heterogeneity and extent of innervation has only recently become apparent. The presence of sensory and sympathetic fibres has been demonstrated in the bone marrow, mineralised bone and periosteum [1–4], with bone marrow containing the greatest number of fibres per unit volume [5]. The effects of chemical and surgical denervation on skeletal function have been widely studied, but their contribution to our understanding of neurotransmitters in bone is less clear. Mechanical loading is a potent stimulator of osteogenesis, which is impaired by lack of movement caused by some types of denervation. As such, it is difficult to determine whether changes in bone remodelling result from direct inhibition of neurotransmitter signalling or from indirect effects on mechanical forces acting on the skeleton. However, skeletal phenotypes observed in some denervated bones suggest that changes in pathology cannot be explained solely by disuse. For instance surgical and pharmacological sympathectomy has been demonstrated to increase the number of osteoclasts and bone resorption in inner ear bones, which are not exposed to mechanical stimulation [107]. Sympathetic nerves innervating bone have recently been directly linked to the control of osteoblast activity. These are stimulated via a hypothalamic relay involving leptin and release noradrenaline into the bone microenvironment, activating β2-adrenergic receptors expressed by osteoblasts, inhibiting their osteogenic activity [7]. Acid-sensing ion channels have also been identified on sensory fibres innervating mineralised bone which may be involved in sensing low pH brought about by resorbing osteoclasts [8,9].

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0014-5793/04/$30.00 © 2004 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(04)00053-5

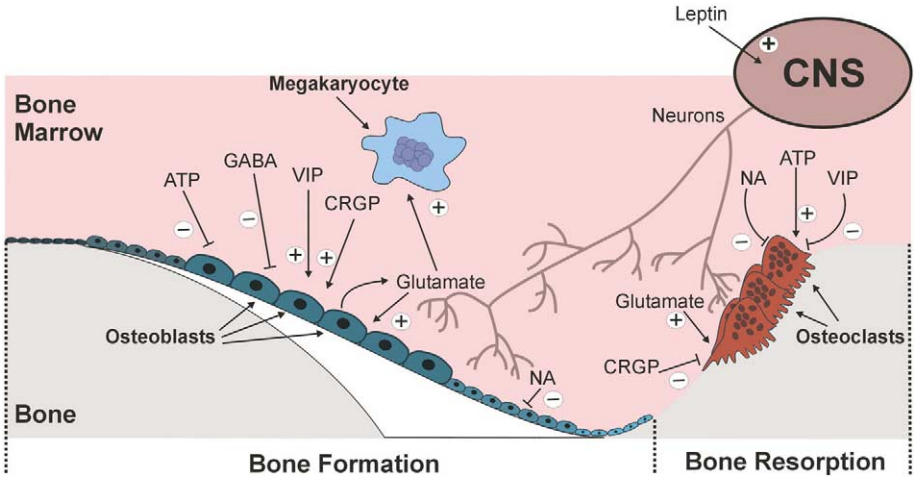

Fig. 1. Effects of neurotransmitters in the bone microenvironment. Schematic representation detailing the putative functions of neurotransmitters involved in the regulation of bone remodelling and megakaryocytopoiesis. Although the origin of several transmitters is still unclear, bone and marrow are highly innervated with peripheral nerves, which likely contribute to local neurotransmitter release. Abbreviations: ATP, adenosine triphosphate; CGRP, calcitonin gene-related peptide; CNS, central nervous system; GABA, γ-aminobutyric acid; NA, noradrenaline; VIP, vasointestinal peptide.

3. Glutamate

Mechanisms of glutamate transport and release, receptor expression and downstream signalling events have been identified in bone (reviewed in [10]). Interest in the field was first stimulated by the finding that the gene encoding the glutamate/aspartate transporter (GLAST/EAAT-1), previously believed to be expressed only in the central nervous system (CNS) [11], was expressed by osteoblasts and newly embedded osteocytes [12]. Other glutamate transporter proteins have also been identified in bone, including GLT-1/EAAT-2, which appears to be expressed by mononuclear bone marrow cells [12].

Subsequent studies, which focused on determining expression of glutamate receptors by bone cells, identified members of the ionotropic family of ligand gated ion channels (α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate receptor subtypes) and metabotropic G protein-coupled receptors. NMDA receptors are the best-characterised glutamate signalling receptors in skeletal tissues and their expression has been demonstrated on osteoblasts, osteoclasts and megakaryocytes in vitro and in vivo [13–17]. In the CNS, NMDA receptors exist as heterotetramers assembled from the NMDA receptor (NR) subunits NR1, NR2A–D, NR3A and B [18]. Although evidence of NR1 expression on osteoblasts, osteocytes and osteoclasts is convincing, disparity exists with regard to expression of the regulatory NR2 subunits. Hinoi and co-workers describe expression of NR2D, but not NR2A, B or C in rat calvarial osteoblasts [19], whilst others report expression of NR2C but not NR2A, B or D [20] or NR2A, B and D but not C [21]. Potential explanations for these differences exist, based on differing detection methods and sample sources, although a definitive answer remains elusive. As receptor composition significantly alters conductivity and sensitivity, it would seem likely that different receptor stoichiometry would exist on different bone cells, allowing them to respond differentially to common signals.

Glutamate receptors expressed by bone cells have similar functional properties to those found in the CNS. On agonist-mediated receptor activation, NMDA receptor channel currents and increases in intracellular calcium have been detected in bone cells with primary osteoblasts and osteoclasts exhibiting electrophysiological characteristics very similar to neuronal cells [20,22]. Indeed in primary rat osteoblasts, NMDA receptor activity is regulated by co-expressed metabotropic receptors, which is directly comparable to central glutamatergic synapses [20].

Antagonism of NMDA receptors has been demonstrated to inhibit both bone formation and resorption in vitro [15,23]. These effects appear to be mediated through impaired cell differentiation rather than proliferation or activity of mature cells [15,24,25]. NMDA receptor antagonism has also been shown to attenuate the DNA binding activity of the key transcription factor core binding factor-1 (Cbfa-1), which is required for osteoblast differentiation, and inhibit osteoclast formation in coculture assays of bone resorption [24]. In contrast, AMPA receptors appear to play a role in osteoblast lineage determination, as AMPA receptor blockade promotes adipogenesis in bone marrow stromal cell cultures at the expense of osteoblastic differentiation [26].

Studies on the expression and function of glutamate receptors in bone raised important questions regarding the source of agonist required for receptor activation. Neuronal innervation of bone by glutamate-containing peripheral nerves has recently been demonstrated by immunohistochemistry [27]. Significantly, osteoblasts have been demonstrated to express components of neuronal presynaptic machinery required for glutamate release in the CNS, including molecules involved in synaptic vesicle packaging, targeting and fusion [28]. These also include the vesicular glutamate transporter VGLUT1, whose expression in the CNS is both necessary and sufficient to confer glutamate release characteristics on non-glutamatergic neurones [29]. More compelling evidence for an osteoblastic source of glutamate is provided by studies demonstrating regulated and spontaneous glutamate exocytosis from osteoblasts, prevention of which inhibits cell survival and differentiation [6,30].

Glutamate signalling in bone does not exclusively involve osteoblasts and osteoclasts, and includes bone marrow megakaryocytes, whose primary function is the production and release of functional platelets. Megakaryocytes express NR1, NR2A and NR2D subunits and antagonism of receptor activity inhibits differentiation of cell lines and human primary megakaryocytes in vitro [16,17]. Cells incubated with antagonist also fail to produce proplatelet structures, and lack the cytoplasmic characteristics and organelles essential for the production of functioning platelets [17].

4. GABA

GABA is the principal inhibitory neurotransmitter in the CNS synthesised from glutamate by glutamic acid decarboxylase. GABA mediates its actions through ionotropic ($GABA_A$ and $GABA_C$) or metabotropic ($GABA_B$) receptors. Activated ionotropic receptors conduct Cl^- ions and the metabotropic receptors transduce signals through the G_1/G_0 family of G proteins. GABA receptors have been identified in a range of peripheral tissues, including testes, lung, liver, gastrointestinal tract and mammary gland [31]. Recent evidence also suggests that GABA may act as a trophic factor, with the ability to modulate cell proliferation, differentiation and survival during neuronal development [32]. Following the identification of functional glutamate receptors in bone cells, Fujimori and co-workers [33] determined the expression and activity of different GABA receptors in rodent primary and clonal osteoblastic cells, identifying two $GABA_B$ receptor splice variants ($GABA_B$R-1a and $GABA_B$R-1b). Exposure to the $GABA_B$R agonist baclofen inhibited forskolin-induced cAMP formation in primary osteoblasts and reduced alkaline phosphatase activity and calcium accumulation in MC3T3-E1 cells, over 28 days in culture. Although more work is required in this area, these initial studies at least suggest that both glutamate and GABA signalling mechanisms may co-exist within the bone microenvironment to mediate autocrine and paracrine signalling in a manner analogous to the excitatory/inhibitory neuronal network in the CNS.

5. Purines and pyrimidines

Receptors to purines and pyrimidines have been identified and classified according to their responses to the physiological agonists: adenosine (P1 receptors), ATP, ADP, UTP and UDP (P2 receptors) and adenine dinucleotides (P4 receptors) [34,35]. P2 receptors are further subdivided on the basis of molecular structure, pharmacology and signal transduction mechanisms into P2X (ligand-gated ion channels) and P2Y (G protein-coupled receptors). To date ligand binding studies and molecular cloning have identified four P1 adenosine receptors (A1, $A2_A$, $A2_B$, A3), seven P2X receptors ($P2X_{1-7}$) and five P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$) [34].

Pharmacological evidence exists to support the existence of A2 adenosine receptors on osteoblasts derived from mouse calvaria, which are capable of inducing cAMP formation. Adenosine has also been reported to be mitogenic for MC3T3-E1 cells and has been demonstrated to act synergistically with platelet-derived growth factor to induce DNA synthesis [36,37].

To our knowledge expression of P4 receptors sensitive to adenine dinucleotides has not been identified on bone cells although studies have identified expression of numerous P2 receptors including $P2X_2$, $P2X_5$, $P2X_7$, $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ by osteoblasts, $P2X_1$, $P2X_2$, $P2X_4$ $P2X_5$, $P2X_6$, $P2X_7$, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ by osteoclasts, and $P2X_2$, $P2X_5$, $P2Y_1$ and $P2Y_2$ by chondrocytes using a variety of detection techniques and range of tissue samples [38]. Extracellular nucleotides acting via $P2Y_1$ and $P2Y_2$ receptors have been demonstrated to increase inositol 1,4,5-trisphosphate production and transiently increase intracellular calcium [39–41] and ATP has been demonstrated to dose-dependently inhibit bone formation by osteoblasts in vitro [42,43]. Mitogenic effects of ATP and synergistic actions with growth factors on osteoblast proliferation have also been reported [38]. More recently P2X receptors have been implicated in the control of osteoblast apoptosis ($P2X_7$) and propagation of intercellular calcium signals between osteoblasts ($P2Y_2$), and between osteoblasts and osteoclasts ($P2X_7$) [44–46].

Osteoblastic P2Y receptors ($P2Y_1$, $P2Y_2$ and P2Y ADP) are also implicated in signalling cross-talk with other pathways important in regulating bone cell function. Activation of P2 receptors has been demonstrated to potentiate the actions of parathyroid hormone (PTH), a principal regulator of bone formation and bone resorption, increasing inositol trisphosphate generation and intracellular calcium concentrations in rat osteoblastic cells [47]. P2 receptors also synergise with PTH to upregulate expression of the transcription factor c-*fos*, providing a mechanism of regulating osteoblastic gene expression [48].

Electrophysiological studies are consistent with expression of functional P2X and P2Y receptors by disaggregated rabbit osteoclasts with characteristic properties of $P2X_4$, $P2X_7$ and $P2Y_1$ [49–51]. Extracellular ATP has been shown to stimulate bone resorption by cells derived from human osteoclastoma and although $P2Y_2$ receptors were originally proposed to mediate these effects, subsequent investigations demonstrated little effect of $P2Y_2$ receptor agonists [52]. ADP, acting via the $P2Y_1$ receptor, has been reported to stimulate osteoclast formation and bone resorption in numerous assays of osteoclast activity [53]. A potential role for the acid-sensitive $P2X_2$ receptor in stimulating bone resorption has also been suggested from observations that the pro-resorptive effects of ATP are amplified at low pH [54]. At a molecular level, ATP increases intracellular calcium through two distinct mechanisms in osteoclasts: P2Y-mediated, thapsigargin-sensitive, calcium release from intracellular stores and influx of extracellular calcium through $P2X_7$ receptors [51,55,56]. Roles for $P2X_7$ receptors in the fusion of osteoclast progenitors and apoptosis of mature osteoclasts have also been suggested [38]. It has been proposed that at least some of the pro-resorptive effects of extracellular nucleotides may be mediated indirectly by osteoblasts through increased expression of the osteoclast differentiation factor, receptor activator of NF-κB ligand or release of pro-resorptive cytokines [57].

In addition to osteoblasts and osteoclasts, P2 receptors are also implicated in the control of chondrocyte function. Activation of $P2Y_2$ receptors has been demonstrated to elevate intracellular calcium and enhance basic fibroblast growth factor-induced proliferation of sheep chondrocytes [58].

Although peripheral nerves innervating bone provide one potential source of nucleotides in the bone microenvironment,

local release from osteoblasts, chondrocytes and megakaryocytes has all been described [59–61]. Other sources include nucleotides released as a consequence of cell death or agonists generated by interconversion through the actions of ecto-nucleoside diphosphokinase expressed by osteoblasts [59].

6. 5-HT

Expression of 5-HT receptors (5-HT_{1A}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}) and specific 5-HT transporter has been demonstrated in primary rat osteoblasts and clonal osteoblastic cells lines in vitro [62]. These transporters are functional and their activity increases during osteoblast differentiation in vitro. Expression of 5-HT_{2B} receptors has also been shown in chick osteoblasts, osteocytes and periosteal fibroblasts, a cell population containing osteoblast progenitor cells [63]. In functional studies, 5-HT has been reported to potentiate PTH-induced increases in activity of the AP-1 transcription factor complex and increase proliferation of periosteal fibroblasts. 5-HT receptor activation is also implicated in regulating mechanotransduction by osteoblasts, inhibiting the production of nitric oxide in response to mechanical strain [62,63]. These data provide some evidence for a functional serotoninergic signalling system in bone comprised of essential cellular mechanisms required to respond to and recycle 5-HT.

7. Catecholamines

The catecholamines include dopamine, adrenaline (epinephrine) and noradrenaline (norepinephrine), which are formed from tyrosine following a common synthetic pathway. Adrenaline and noradrenaline exert their effects through interaction with different G protein-coupled α- and β-adrenergic receptors (ARs). All ARs are members of the G protein-coupled, seven transmembrane domain containing superfamily of receptors, and use a variety of intracellular signalling mechanisms to modulate cellular function. In early studies in bone it was shown that the β-adrenergic agonist isoproterenol could stimulate adenylate cyclase in clonal rat osteoblast-like cells [64,65], in a manner sensitive to dexamethasone and retinoic acid exposure [66,67]. In the mouse preosteoblastic cell line MC3T3-E1, adrenaline was shown to increase prostaglandin E_2 (PGE_2) production [68], whereas noradrenaline has been reported to enhance cAMP formation in MC3T3-E1 cells, as well as ROS17/2.8, UMR-106-01 and SaOS-2 osteoblastic cells [69,70]. It was later demonstrated by reverse transcription polymerase chain reaction (RT-PCR) analysis and receptor binding studies that the β_2AR subtype, but not β_1AR, was expressed by ROS17/2.8 cells [71]. However, Northern blot analyses have identified β_1AR and β_2AR expression in several osteoblast-like cell lines including SaOS-2, TE-85, MG-63 (β_2AR only) and OHS-4 (β_1AR only) [72]. These authors also demonstrated that specific activation of β_2ARs induced immediate early c-*fos* expression in SaOS-2 cells [72]. Catecholamines have also been shown to enhance DNA synthesis and increase alkaline phosphatase activity in MC3T3-E1 cells, although without affecting collagen synthesis or osteocalcin production, with pharmacological evidence suggesting that these effects were mediated through α_1ARs [73]. Reports from the same group suggest that mitogen-activated protein (MAP) kinases are essential intracellular mediators of the actions of adrenaline on MC3T3-E1 cells, with the extracellular signal-regulated kinase pathway mediating proliferative responses and the p38 MAP kinase pathway inducing increased alkaline phosphatase activity [74]. In addition, adrenaline has been shown to stimulate sodium-dependent inorganic phosphate transport in MC3T3-E1 cells [75].

More recently using RT-PCR, it has been suggested that activation of β-adrenergic signalling in MC3T3-E1 cells causes increased expression of interleukin (IL)-6, IL-11, PGE_2, RANKL and OPG, which results in increased osteoclastogenesis [76,77], and application of noradrenaline and isoproterenol has been shown to stimulate bone resorption in organ cultures [71]. The importance of adrenergic signalling in bone remodelling has been demonstrated in vivo. Application of the non-selective antagonist of β_2ARs, propranolol, enhanced endochondral bone formation rates during the repair of excisional defects in surgical rat models as well as increasing torsional strength in non-surgical models [78]. In vivo administration of clenbuterol, a β_2AR agonist, has also been reported to inhibit the reduction in bone mineralisation induced by sciatic neurectomy [79]; however, it was unclear from this study whether the observed effects could have been attributed to anabolic actions of the β_2AR agonist on muscle. Later reports indicated that clenbuterol inhibited longitudinal bone growth [80] and reduced bone mineral density when administered to rats undergoing different exercise regimes [81].

8. Acetylcholine

Acetylcholine (ACh) is synthesised by the action of choline acetyltransferase on acetyl-CoA and transmits nerve impulses at cholinergic synapses and motor end plates of neuromuscular junctions. Following vesicular release, ACh binds to either ionotropic (Na^+ and K^+ gating) nicotinic ACh receptors (AChR) or metabotropic, G protein-coupled muscarinic AChR to initiate downstream signalling events. Signalling is terminated by acetylcholinesterase (AChE), which hydrolyses ACh into choline and acetate.

We have found little evidence to suggest that muscarinic AChRs are expressed by bone cells [82], although one study, using in situ hybridisation to investigate the distribution of nicotinic AChRs in the inner ear of rats, did identify α9 AChR mRNA expression in developing osteoblast and/or chondrocytes in the cochlear capsule [83]. Others have identified expression of α7 and α4 nicotinic AChR mRNAs in periosteal cells and human primary bone [84,85]. Nicotine has been reported to regulate osteoblast proliferation and induce expression of the AP1 transcription factor complex and the bone matrix protein osteopontin. These effects are at least partly sensitive to the nicotinic receptor antagonist D-tubocurarine, consistent with a specific receptor-mediated affect [85]. Nicotine has also been reported to inhibit the formation of osteoclasts and bone resorption in osteoblast/mononuclear cell cocultures although it is unclear whether these effects are directly or indirectly mediated, or indeed whether they involve activation of specific receptors [86].

We and others have recently shown that AChE is expressed by osteoblasts and can regulate cellular activity in bone [82,87]. AChE expression has often been identified in the absence of any clear cholinergic signalling and there is growing evidence indicating that AChE has multiple non-cholinergic functions in a range of tissue types [88]. In bone, AChE ap-

pears to be able to augment osteoblast adhesion and differentiation and several putative binding sites for osteogenic factors have been identified in the human AChE promoter region [82,87]. These include consensus binding motifs for Cbfa-1 as well as vitamin D receptor and oestrogen receptor binding elements. The complexity of intrinsic cholinergic-like signalling mechanisms in bone tissue is still unclear, but these early studies do suggest that the role of AChE in bone remodelling and development warrants further investigation.

9. Neuropeptides

Nerves immunoreactive to VIP, SP, CGRP and neuropeptide Y (NPY) have been identified in bone innervating the periosteum, vasculature, growth plate and marrow, implicating a complex role for neuropeptides in the control of bone cell function [89].

9.1. VIP

Based on pharmacological studies and cAMP assays, expression of VIP (VIP_1 and VIP_2) and CGRP receptors has been demonstrated on osteoblasts [70,90,91] and their expression, along with NPY, has subsequently been confirmed by RT-PCR [92,93]. VIP increases intracellular calcium concentrations in osteoblasts and stimulates PGE_2 production in a range of human bone cells [90,94], providing evidence for functional coupling of receptor activation to intracellular signalling pathways. In functional assays, VIP has been demonstrated to increase alkaline phosphatase activity, regulate expression of a number of osteoblastic markers and stimulate mineralisation in vitro, consistent with an anabolic effect [95]. VIP has also been reported to both inhibit and stimulate bone resorption, through a direct effect on mature osteoclasts and indirect effects on RANKL expression by osteoblasts ([95] and references therein). Thus VIP can be considered to have a pleiotropic effect on bone, regulating both bone formation and bone resorption.

9.2. SP

SP receptors have also been identified by immunohistochemistry on osteoclasts [96] and their activation has been demonstrated to stimulate calcium influx and bone resorption in vitro [97]. Expression of SP receptors by osteoblasts is less clear and requires further clarification in light of conflicting reports confirming and refuting their presence [92,96].

9.3. CGRP

Like VIP, CGRP also has pleiotropic effects on bone, stimulating bone formation and inhibiting bone resorption. Activation of osteoblastic CGRP receptors has been demonstrated to increase cAMP formation [98] and stimulate the proliferation of osteoblasts in vitro [99]. Transgenic mice overexpressing osteoblast-derived CGRP under the control of the osteocalcin promoter have a skeletal phenotype consistent with anabolic effects on bone [100], furthermore, systemic administration of CGRP partially reverses the osteoporotic phenotype of ovariectomy [101]. More recently calcitonin/CGRP knockout mice have been generated by heterologous recombination, which, like CGRP-overexpressing mice, exhibit an anabolic phenotype. These mice are also protected against ovariectomy and are more sensitive to PTH-stimulated bone resorption [102]. CGRP has also been demonstrated to inhibit bone resorption in vitro and in vivo, raising the possibility that at least some of the stimulatory effects of CGRP on bone mass may result from decreased bone resorption [103,104].

10. Conclusions

It is clear that neurotransmitters have profound effects on bone, influencing the differentiation, proliferation, activity and apoptosis of osteoblasts and osteoclasts. As such, manipulation of these signalling pathways offers real therapeutic potential for the treatment of a wide range of bone disorders characterised by inappropriate and excessive changes in bone formation and/or bone resorption. For example, it has been suggested that osteoporosis aetiology results from an age-related failure in the adaptive osteogenic responses of bone cells to mechanical stimulation which plays an important role in maintaining adequate bone mass [105]. There is evidence to suggest osteoblasts use glutamate as a signalling molecule to perceive, record and respond to these changes through a mechanism similar or identical to neuronal long-term potentiation, a process of synaptic plasticity implicated in long-term memory formation in the CNS [106]. If this hypothesis and that of memory formation in bone are true it may be possible to pharmacologically prime the skeleton to mimic, retrieve or enhance the osteogenic response of exercise for the treatment of osteoporosis. To this end it is noteworthy that thousands of compounds line the shelves of pharmaceutical companies, developed for the treatment of central disorders and rejected on the basis of their inability to cross the blood–brain barrier. These compounds, used to treat 'peripheral' bone disorders, would lack any unwanted central side effects, and may therefore provide a 'fast-track' pharmaceutical approach for the treatment of bone disorders.

Acknowledgements: We acknowledge financial support from Nuffield Foundation, British Heart Foundation and Arthritis Research Campaign and apologise to any colleagues whose citations were omitted due to space constraints.

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