J Musculoskelet Neuronal Interact 2008; 8(2):111-120

Mini-review Article

Hylonome

Sympathetic nervous system and bone adaptive response to its mechanical environment

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Abstract

While bone adaptive response to its mechanical environment was considered to be controlled locally by cytokines and systemic hormones, some recent work suggests that it could also be neuronally regulated. Bone is indeed very densely innervated and many experimental and clinical studies have previously shown the involvement of the nervous system in the control of bone metabolism. The demonstration that the central nervous system regulates bone mass via the sympathetic nervous system (SNS) has prompted recent studies aimed to investigate the role of the SNS in the bone mechano-adaptive response. This review will focus on this work and summarize the evidence for a contribution of the β-adrenergic signalling in the response of bone cells to mechanical loading. The apparent conflicting results obtained in diverse experimental models of loading and unloading, at different skeletal sites, and in relation to various hormonal levels, will be discussed. While those studies do not support a major influence of the SNS on the bone mechano-adaptive response, there is nevertheless strong evidence that the SNS is part of a complex system which contributes to the metabolic regulation of bone.

Keywords: SNS, β-adrenergic Signalling, Propranolol, Bone, Mechanical Loading

Introduction

Bone is a dynamic tissue that can adapt its mass and architecture to be constantly structurally suitable to the external mechanical demand. To achieve this, bone cells can enhance bone formation with increasing mechanical demands or dispose of bone in excess in situations of disuse. This bone adaptive response to the strain magnitude, originating from the external environment, defines the mechanostat, a concept postulated by Frost[1]. According to this theory, there is a threshold called "the minimum effective strain" which is the lower strain within the remodelling window of strains under which bone resorption prevails over formation. On the reverse, strains above the upper threshold of the remodelling window will increase formation over resorption. The physiological mechanisms by which a mechanical stimulus is sensed by bone and by which the sensed signal is transduced into biochemical signals by bone cells are not completely understood. Bone cells respond to intermittent, but not to static loading, implying a sophisticated perception system[2]. They detect changes in their strain environment associated with fluid flow and physical deformation. Osteocytes, with their unique location and morphologies, and osteoblasts on the bone surface are believed to be the mechanosensors of bone. They communicate via gap junctions providing a network important for both mechanosensation and mechanotransduction. Within minutes of a mechanical stimulus, those cells release prostaglandins and nitric oxide which respectively enhance bone formation and inhibits bone resorption[3,4]. While this bone adaptive response to its mechanical environment was considered to be controlled locally by growth factors, cytokines and systemic hormones[4], several studies suggest that it could also be neuronally regulated.

Both bone and periosteum are richly innervated[5] and the areas of mineralized bone which receive the greatest mechanical load display the highest density of nerve fibres[6]. An ancient hypothesis was that nerve endings could be the receptors of mechanical stress in bone[7,8]. This is supported by a recent study indicating that load by occlusal force causes an increase in the number of nerve fibers around oral implants[9]. Hert et al. in 1971[8], have however demonstrated

The authors have no conflict of interest.

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Accepted 10 March 2008

that innervated and denervated limbs react to loading in the same way, and several groups have shown that bone explants and isolated bone cells are sensitive to mechanical stimuli[10,11], opposing this assumption. Nevertheless, the idea that the nervous system, and particularly the sympathetic nervous system (SNS), might contribute to the bone adaptive response mechanical loading has emerged after the demonstration that the central nervous system regulates bone formation and bone mass via the SNS acting on β2-adrenergic receptors expressed by osteoblasts[12]. This discovery that neuronal signals make a major contribution to bone mass regulation has been one of the most exciting developments in the field of bone metabolism research in recent years. Although it had a major impact, the idea that the nervous system influences bone remodelling is not new and many experimental and clinical studies have previously shown the involvement of the nervous system in the control of bone metabolism[(for reviews, see 13-18)]. Both sensory and sympathetic nerve fibers are present in bone and bone cells express a variety of receptors for neuromediators, including β-noradrenergic receptors[5,12]. It is still unclear whether the major influence of the nervous system on bone physiology is local via peripheral skeleton innervation or central. It is likely that both central and peripheral controls occur. Direct neural regulation of bone cells via paracrine release of neuromediators by nerve terminals is expected, but there is also increasing evidence that the hypothalamus senses the physical and metabolic needs of the skeleton, and integrates those needs with other homeostatic functions, to control bone metabolism[12,19]. Whether the central nervous system regulates bone cells' activities only via peripheral innervation or also via a soluble factor is unknown. Moreover, neural influences may be exerted on bone cells indirectly via the control of blood supply and immune cell functions[20-22]. The signal transmission from nerve to bone cells is also a subject of a debate as no synapse was identified in bone[5]. It is expected that non-synaptic secretion of neuromediators and neuropeptides occurs, and that GAP junctions between osteoblasts and osteocytes could be involved in the transmission of neuronal signals[5,17,18]. The link between osteocytes, the main sensors of mechanical loading, and bone innervation is unclear. The opportunity for osteocytes to come in direct contact with innervation is limited, but neuromediators may diffuse through the lacunocanalicular system. There is evidence that osteocytes express receptors for neuromediators and that the expression of some of these receptors is modulated by mechanical loading[23-26].

A potential role of the SNS in the adaptation of bone to mechanical signals was suggested by several studies. The first indication came from reports in bedridden patients and astronauts under gravity conditions. Those people lose bone due to the deficit of mechanical stress but the mechanisms underlying such disuse osteoporosis are mostly unknown. The rapid bone loss in unloading conditions could evoke the involvement of the nervous system which can elicit very rapid signals. The well-known effect of exercise on the SNS activation[27], together with the fact that the sympathetic nervous tone was enhanced in the astronauts' muscles after returning from space[28], suggested a possible role of the SNS. β-adrenergic agonists have been widely used as anabolising agents on skeletal muscles[29,30], and studies on hormonal responses to exercise have shown that exercise and growth hormone release are coupled to adrenergic activation[31]. More recent studies in rats and mice have supported the involvement of sympathetic tone in the induction of bone resorption after unloading. They indicate for the first time that the β-adrenergic part of the SNS is a mediator of the physiologic response to skeletal unloading, as treatment with β-blocker propranolol suppressed the unloading-induced reduction in bone mass[32,33]. Those results obtained in the hind limb unloading model of tail suspension have however not been confirmed in the most widely used model for disuse, the sciatic neurectomy model[34]. Other studies strongly suggest that the adrenergic component of the SNS is not involved in the bone osteogenic response to mechanical loading[35], although the observation that mechanical loading of one region of a long bone induces an osteogenic response in a distant skeletal site at which bone strain is not affected by loading[36] may nonetheless evoke the involvement of the nervous system. The evidence for a potential role of the SNS in the bone mechano-adaptive response therefore remains weak. Clinical studies investigating the effects of β-blockers on bone mass have not simplified the interpretation of all these experimental data as they have shown conflicting outcomes. Furthermore, there are no obvious common signalling pathways between adrenergic receptor signalling and the mechanical pathway, although both pathways lead to an increase in the production of prostaglandins E2 in osteoblasts[4,37]. This review will focus on the complexity of this neuronal system and its role on the bone mechano-adaptive response, and will try to clarify those apparent differing results.

The SNS does not influence bone functional adaptation to mechanical loading

Traditionally, bone adaptation to mechanical loading has been considered highly site-specific[38] and not centrally controlled[8-10]. Consistently, using a model for non-invasive *in vivo* axial loading of murine tibia previously developed in our group[39], we showed that the cortical bone gain induced by cyclic loading of tibiae was not modulated by the SNS[34]. In this set of loading experiments, the SNS was inhibited by either a high dose of the non-selective β-adrenergic receptor antagonist propranolol (PRO, 0.5 g/liter in the drinking water) or guanethidine sulphate (GS, 40 mg/kg/day), a treatment that reduces norepinephrine concentration in the peripheral SNS. New cortical bone formation was enhanced by loading in all tibial sites examined and the increases in new bone formation induced in response to mechanical loading were similar in mice treated with either GS or PRO compared to controls, indicating that inactivation of the SNS

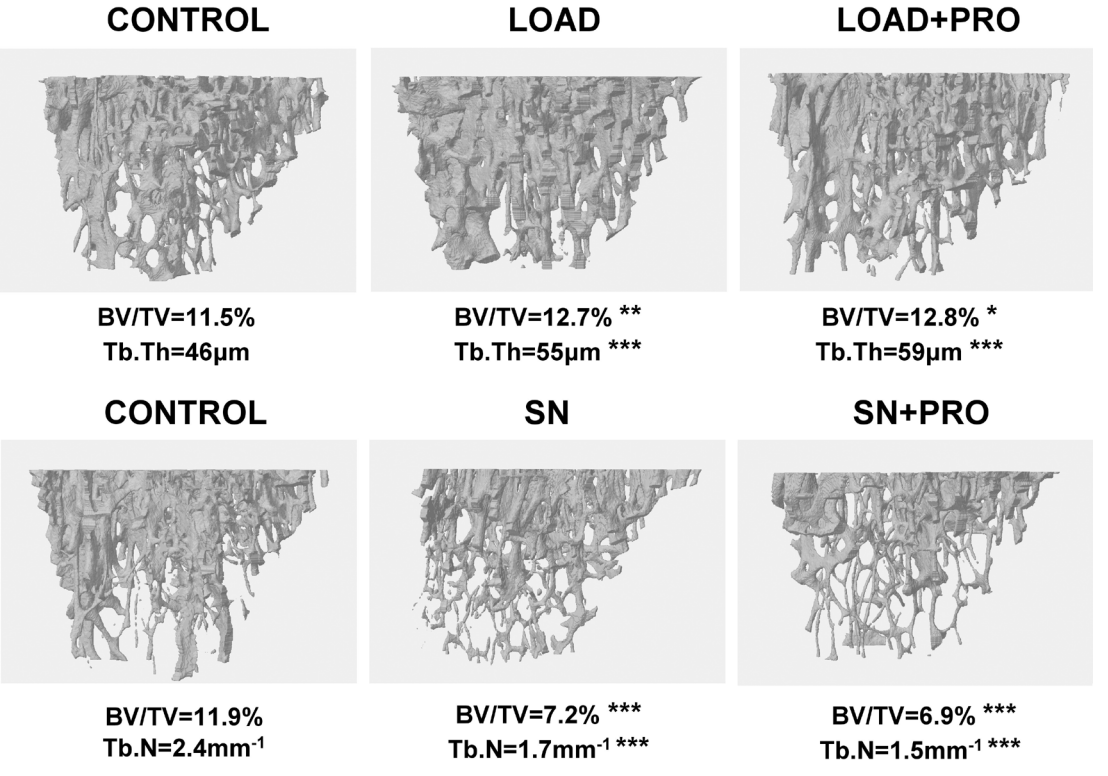

Figure 1. Representative three-dimensional reconstructions of the trabecular bone within the tibia metaphysis. External loading (LOAD) of the tibia increased microarchitectural parameters. Unilateral Sciatic Neurectomy (SN) decreased bone mass and structure. Treatment with propranolol (PRO) did not modulate either the anabolic or the catabolic bone response to the mechanical environment. Data reproduced from Marenzana et al.[34] Statistics: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ versus control tibia determined by 2-way ANOVA and Bonferroni's post hoc analysis.

had no effect on load-induced cortical new bone formation.

However, parallel studies have shown a contribution of the SNS in the bone loss induced by hind limb unloading. In this model, load bearing on the hind limbs is prevented, greatly reducing the strain normally generated during cage activity (sub-physiological strain level). Thus, this model represents the other end of the mechanical spectrum applied to bone in comparison to the tibia axial loading model mentioned above which induces supra-physiological strain levels. Treatment of rats and mice with similar high concentrations of β-blocker PRO and/or GS, prevented the trabecular bone loss induced by hind limb unloading in the tail suspension model[32,33]. Analyses on the cellular bases showed that the SNS mediates the unloading-induced bone loss through suppression of bone formation by osteoblasts and enhancement of resorption by osteoclasts. In those two studies however, the first one did not examine the cortical bone response to unloading[33], while the second one[32] did not demonstrate a significant cortical bone mass recovery induced by PRO treatment in the tail-suspended group. This suggested that the SNS modulation of unloading-induced bone loss is more prominent in the cancellous bone compartment. One hypothesis made following those observations was that the cortical bone response to loading could be mediated primarily by hormonal factors responsible for the anabolism of cortical bone such as estrogen status and insulin levels, whereas neuronal influences may control predominantly trabecular bone mass. This skeletal site-specific bone mass regulation is supported by a study showing that the phenotypic effects of leptin deficiency differ between the long bones and the vertebrae[40]. This hypothesis was nevertheless disproved in a subsequent study performed by our group[34] which demonstrated that the mechano-adaptive response of trabecular bone in the tibia metaphysis was also not affected by blockade of the β-adrenergic receptors using PRO. This held true in conditions of bone loss caused by sub-physiological – near zero – loading in the sciatic neurectomy model as well as in conditions of bone gain induced by supra-physiological external mechanical loading (Figure 1). Thus, these findings strongly suggest that the sympathetic tone is not involved in the modulation of the local bone response to its mechanical environment. As a consequence, the abundant nerve fibers distributed over the periosteum as well as on the trabecular surfaces[5] do not seem to act as mechano-receptors or mechano-transmitters for the mechanical loading in bone[11]. On the contrary, trabecular and cortical bone compartments

appear to have consistent responses to loading which are not modulated by the SNS, thus excluding that differences in the density of sympathetic innervation or changes in the expression of adrenergic receptors between trabecular and cortical bone could play a role in the contrasting responses to mechanical loading observed previously in trabecular and cortical bone. Consistently, the catabolic action of β-agonists on bone[41,42] was not alleviated by treadmill exercise which supplies a potent local remodelling stimulus to the long bones. It combines direct bone straining during the exercise with enduring mechanical stimulation from enhanced muscle mass which is also further increased by the action of the β-agonists[43]. It is worth taking notice of the differences between the treadmill-based model of exercise and the tibia axial compression model. While in the latter the cyclic compression is administrated under complete muscle relaxation (general anaesthesia), the exercise model implies up to 70% maximal O2 consumption[44] involving changes in muscle mass, heart beat rate, blood pressure and even hormone levels, such as leptin[45], besides the changes in bone. Therefore, the model for applying axial loads is more suitable to identify the modulation of bone adaptation to loading independently of other systemic factors, while running exercise takes into account all the physiological changes in a more complex system in which the direct modulation of the bone mechano-adaptive response is less clear to identify.

Contrasting effects of the SNS on the bone loss induced by hind-limb unloading and sciatic neurectomy

The divergent influence of the SNS on the bone loss induced by two different models of disuse, the hind limb unloading and sciatic neurectomy, remains unclear. In both models, the same β-blockade (high dose propranolol, 20 mg/ml) and a similar timeframe of two weeks of unloading were used. Several factors may contribute to those differences. First, there is differential impairment of the bone remodelling homeostasis in these two models, with bone resorption being more markedly increased by neurectomy[46,47] and to a lesser extent by tail suspension[48], while in the latter model bone formation is highly suppressed[49], which might have been targeted preferentially by the SNS blockade. Second, different hind limb muscles are affected by unloading in these two models, although it is unclear how this is related to the greatest effects of the SNS on bone mechanical properties induced by tail suspension compared to neurectomy[50]. Third, the tail suspension model is known to involve several physiological shifts, such as alterations in the SNS activity and in neuromuscular function similar to that observed after spaceflights[28], changes in blood distribution with reductions in plasma volume, perturbations in the arterial vascular tone[51,52], and decrease in femoral intramedullary pressure[53]. In addition, stress-related factors may also play a role in the tail suspension-induced bone loss besides mechanical unloading, which fits with the recent finding demonstrating that stress-induced depression induces bone loss in mice through stimulation of SNS[54]. It is also possible that single housing of rodents, as it is observed in practice in the tail suspension model, might have an impact on bone, analogous to what has been shown in spaceflight[55] in which the housing conditions, isolation versus social, dramatically influenced bone response. Finally, the sciatic neurectomy model involves significant reduction, although not complete deletion, of the innervation in the neurectomized limbs[56]. As the sciatic nerve is a mixed nerve, which contains both sympathetic and sensory nerve fibers[5], it is possible that there is a decreased normal nerve sympathetic transmission in the rat tibia after sciatic neurectomy and/or that other components of the nervous system are activated by or counterbalanced by the β-blockade of the adrenergic signalling pathway. Thus, alternative experimental models of immobilization in rodents, which do not induce significant physiological changes such as the tail suspension, or do not affect the normal neural transmission to/from the limbs such as neurectomy, are needed in order to dissect the true effect of SNS blockade on the bone loss induced by unloading. The possible alternative models, known to induce osteopenia, include casting[57] or muscular disconnection through tenotomy[58], although tenotomy was found difficult to apply in mice (C. Chenu, unpublished observations). Another animal model of disuse in which innervation remains intact is the MyoD-Myf5-deficient mice which lack skeletal muscle, but the use of those mice is limited by the fact that they die soon after birth[59]. A possible way to clarify this contrasting influence of the SNS in these two models of disuse would be to test the protection against unloading-induced bone loss in these two models in mice deficient for β2-adrenergic receptor.

Finally, the choice of propranolol and the high doses used in those studies are critical. The exact effects of PRO on bone are complex and difficult to dissect since they may vary depending on the dose used. Furthermore, the dose-effect may differ according to the bone site. As demonstrated for other cells[60], propranolol depending on the dosage might produce paradoxical effects on bone cells, simultaneously reducing cAMP accumulation by acting as an inverse agonist while working as an agonist on MAP kinase activation. Most effects on bone of β-adrenergic signalling seem to be mediated by β2-adrenoreceptors expressed by bone cells[12]. However, expression of other β-adrenergic receptors (ADRB) subtypes have been suggested in bone and bone marrow[61] (and personal unpublished results), and we cannot completely exclude the possibility that they may also contribute to the regulation of bone mass by having different or even opposite effects on bone. This is suggested by the phenotype of ADRB1/B2 double KO mice which shows a very different phenotype of ADRB2 KO, illustrated by the reduced trabecular and cortical thickness[62]. The author of this study suggested in a review that stimulation of β2-adrenergic receptors on osteoblasts leads to bone loss via RankL-

mediated osteoclastogenesis, while activation of β1-adrenergic receptors may contribute to maintain cortical bone mass by affecting the GH-IGF-1 anabolic pathway[63]. Therefore, it is possible that propranolol unspecific blockade of all three ADRB subtypes might result in various opposite effects on bone mediated by different adrenoreceptors or subtypes. How these possible effects might be related to the changes in bone mass in these two different unloading models is currently unknown.

Studies on the effects of β-agonists on bone do not help to clarify the discordant effects of ADRB blockade in these two different unloading models. Clenbuterol, a β2-agonist, has been shown to reduce the bone loss induced by both hind limb unloading via tail suspension[64] and denervation[65], which appeared to be correlated to a decrease in muscle wasting. Conversely, the extent of the catabolic effects of β-agonists such as isoproterenol, clenbuterol or salbutamol, established on control rodents[41,42,66,67], appears to be reduced in the presence of pre-existing extensive bone loss, as observed in OVX rats[68] or hind limb unloaded mice[33]. This catabolic action of β-agonists persisted in the OVX rats if they were subjected to exercise, thus abolishing the positive effect on bone of the additional mechanical loading stimulus despite the concomitant anabolic effects of these compounds on the hind limb muscle mass. Although the absence of estrogen limited the influence of β-agonists on bone, the deleterious skeletal effects of β-agonists may also depend on the initial structural bone quantity and quality[68].

Changes in hormonal levels may affect the influence of the SNS on bone mechanoadaptation

The interactions between estrogen, mechanical loading, and the β-adrenergic axis have been recently demonstrated by a series of publications by Bonnet and colleagues[68-70]. They first demonstrated that only low doses of propranolol are beneficial for preserving trabecular bone mass in ovariectomized rats. These results are in agreement with earlier findings showing that rats with bone defects treated with low doses of PRO have increased callus formation and bone union[71]. The protective effect of PRO on the bone loss induced by OVX in mice remains however controversial as PRO was reported to either prevent bone loss[66] or to be ineffective unless combined with PTH treatment[72]. In both of those studies PRO was given at a high dose in the drinking water. It is therefore possible, as previously discussed, that high doses of PRO might have an inverse agonist effect on β-AR which could be detrimental to the SNS modulation of bone loss[70]. This hypothesis is supported by the observation of the bone phenotypes of β-adrenergic transgenic mice. While β2-AR deficient mice have a high bone mass phenotype and are resistant to ovariectomy[12], β1/β2-AR double deficient-mice present a low bone mass phenotype and are not resistant to OVX[62,73]. The interaction among these receptors may also play a role since the deletion of β1-AR solely does not yield any bone phenotype[12], while the triple deletion of all β-ARs generates a high bone mass phenotype and the mice are not protected from OVX-induced bone loss[74]. Interestingly, the fact that high doses of PRO can be effective in rescuing the bone loss induced by OVX, while it has no effect on the bone deficit induced by sciatic neurectomy, suggests that the differences in trabecular microarchitecture and cortical modelling observed in these two osteopenic models[75] might be mediated by different catabolic signalling pathways, with only the estrogen signalling axis being modulated by the SNS.

Bonnet et al.[69] also showed an additive effect of low doses of PRO and exercise on cortical porosity and overall bone mechanical strength in OVX rats compared to the effects of PRO and exercise alone in those rats, although no combined effects were observed on trabecular microarchitecture. In contrast, PRO inhibited the effect of exercise and exercise inhibited the effect of PRO on trabecular bone, suggesting that the SNS is involved in the trabecular response to exercise in the absence of estrogen but not the cortical response. However, the absence of synergistic effect of mechanical loading with propranolol treatment in estrogen-intact animals[34] strongly suggests that the SNS modulation of bone response to its mechanical environment is activated primarily in the presence of a hormonal imbalance. This view is also supported by the finding that hind limb suspended rats, whose bone loss has been shown to be modulated by SNS blockade[33], have significant decreased serum leptin levels, while exogenous leptin administrated peripherally restores their bone mass[76]. Leptin regulation of bone mass and its connection to the SNS is complex as leptin can have both direct anabolic effects on bone formation and multifaceted central effects including the stimulation of the GH-IGF-1 axis, the suppression of neuropeptide Y a potent inhibitor of bone formation, and an increase in trabecular bone remodelling mediated by the SNS[63]. It seems presently unclear how the decrease in peripheral leptin in the hind limb suspension model could be linked to the rescuing effect of the SNS blockade on the unloading-induced bone loss. Nonetheless, those observations further support the suggestion that the SNS influence on bone remodelling may be dependent on hormonal changes such as estrogen deficiency and decreased serum leptin levels.

Unsolved questions remain concerning the high bone mass phenotype achieved by the transgenic mice lacking β2-AR. Indeed, this phenotype is acquired in the presence of estrogen and those mice are protected from OVX-induced bone loss[12]. One may question whether the deletion of β2-AR combined with the exercise and the normal mechanical loading experienced by the mice in their cages, results in higher bone mass compared to wild type littermates. The answer to this question would need not yet available data regarding bone responses to external mechanical loading and unloading of these transgenic mice compared to wild type mice. However, the fact that bone mass was increased equally in both appendicular (heavily subjected to load bearing) and axial (less load bearing) skeleton might be an indi-

cation that deletion of β2-AR induces enhanced bone formation systemically, irrespectively of the local mechanical stimuli. Generally, the interpretation of transgenic models in relation to their adaptive response to mechanical loading is also complicated by the fact that the deletion is present from birth, and therefore the acquisition of the phenotype might involve the modulation of the fast growth phase rather than the subsequent slower modelling/remodelling process. The demonstration that high dose propranolol has a protective effect on bone of young OVX mice (6-weeks-old)[66], but not of adult OVX mice (15-weeks-old)[72] and rats (6-months-old)[70], might suggest that β-ARs signalling could interact preferentially with the fast growing phase in rodents, but this needs to be investigated.

Clinical studies

Several clinical retrospective studies have investigated the use of β-blockers as potential therapeutic options for osteoporosis[77-85]. Those studies have revealed conflicting results, although they generally showed a positive correlation between the use of β-blockers and bone mineral density. The β-blockers used in those studies were mainly β1-selective or nonselective, indicating that the protective effects of those β-blockers on bone mass might be mediated via sympathetic blockade of β1-adrenoreceptors[86]. This argues against the view that all effects of the SNS on bone are mediated by actions on β2-receptors expressed by osteoblasts[66]. Those clinical data mean that the effects of β-blockers on bone in humans are complex and involve different β-adrenergic signalling pathways. The relationship with exercise was not often investigated in those studies. When physical activity was documented, the correlation between β-blocker use and BMD was independent of the correction for physical activity[81,84], further supporting the view that the SNS is not involved in the local bone mechanotransduction. Interestingly, in the last cross-sectional study performed by Bonnet et al.[84] on postmenopausal women, the positive effect of β-blockers on BMD was paralleled by a positive effect on the trabecular architecture in the calcaneus, which is arguably one of the most sensitive bone sites in regards to mechanical loading induced by physical activity. Correction for physical activity in this cohort of patients again did not influence the effect of β-blockers on trabecular bone microarchitecture.

Perspectives

The overview of animal studies and clinical data using β-adrenergic receptors antagonists and agonists points towards the exclusion of a direct modulation of the SNS on bone modelling/remodelling in response to the mechanical environment. There are presently no data supporting a role for the SNS in the regulation of load-induced bone formation, indicating that other mechanotransduction pathways regulate bone formation in loaded bones. Most reports also exclude a contribution of the SNS in the bone loss induced by removal of the mechanical stimuli, although protective effects of β-blockers on this bone loss were reported in a model of hind limb unloading. This model exhibits however several intrinsic physiological changes including alterations in the SNS activity and in neuromuscular function, rendering the interpretation of these results difficult. These findings do not rule out however that other neuronal pathways, that do not involve β-adrenergic signalling, contribute to bone adaptation to its mechanical environment, since bone is rich in sensory innervation that also affects bone metabolism[87].

While some discrepancies exist regarding the effects of β-blockers on bone mass *in vitro* and *in vivo*, there is more consistency about the anabolic effects of β-blockers on bone in animal models subjected to OVX and in cross-sectional studies of postmenopausal women, which suggests that the influence of the SNS on the regulation of bone mass is enhanced in the presence of hormonal changes such as the absence of estrogen. Similarly, the protective effects of β-blockers on the bone loss induced by unloading might be related to changes in serum leptin levels. Those findings underpin the hypothesis that the SNS acts more as a modulator of the hormonal effects on bone rather than being a direct effector. The protective effects of β-blockers on bone mass under estrogen deprivation do not however combine with physical activity, at least at trabecular bone sites, suggesting that extreme care should be given to the treatment of osteoporotic patients undergoing exercise. Further studies are needed to identify whether direct mechanical loading applied to the bone, or exercise which involves several other physiological changes including variations in SNS activity due to alterations in energy expenditure, is synergising with the SNS blockade during estrogen deprivation. For those studies suitable animal models, such as the tibia external loading model which allows measurements of the anabolic stimuli in both trabecular and cortical compartments[39], and use of transgenic mice with deletion of the β2-adrenergic receptor are now available tools. The demonstration of the importance of the systemic interaction of the SNS with osteoregulatory hormones, such as estrogen, leptin and PTH[72], implies that their levels in plasma should be carefully monitored in animal experiments in the future. Studies using different types of β-blockers will be necessary as well to better understand the action of the three β-adrenoreceptors on bone resorption and formation. There is also a need for new prospective clinical studies on postmenopausal women to better monitor the interactions between the type of β-blocker and the other parameters which affect bone metabolism including the diet and physical activity. Studies involving the administration of various β-blockers together with the use of vibrating platforms at regimes known to affect bone mass could be considered. Finally, investigating the physiological role of the SNS in the skeleton requires new experimental and clinical approaches. Although innervation has been shown to affect fracture repair[88-90], very little is known for example on the contribution of the SNS and the possible

beneficial effects of β-blockers on osteoporotic fractures healing.

In conclusion, while the SNS is not the master controller of bone metabolism, there is increasing confirmation that it is part of a complex system which significantly contributes to its regulation. There is however still much to learn about the complicated relationships between the SNS, the hormones that regulate bone mass, and mechanical loading of bone. Despite the evidence for peripheral and central neuronal regulatory components of the bone remodelling process and the multiple clinical associations between bone and nerves, the role of the nervous system in the physiology and pathology of musculoskeletal disorders has been mostly ignored. The discovery that the SNS plays a significant role in the control of bone mass may bring it into the spotlight.

Acknowledgements

The work performed in our lab was funded by the Arthritis Research Campaign.

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