Hylonome

Review Article

Adrenergic control of bone remodeling and its implications for the treatment of osteoporosis

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Abstract

Evidence that leptin regulates bone turnover in part through a central nervous system (CNS)/β-adrenergic system relay has driven attention towards the potential therapeutic benefits of β-adrenergic blockade to improve bone mass and strength. β2-adrenergic receptor-mediated signaling in osteoblasts inhibits bone formation and triggers RANKL-mediated osteoclastogenesis and bone resorption. Mouse models of adrenergic-deficiency, particularly the mouse lacking the β2-adrenergic receptor, have increased bone mass, more specifically increased trabecular bone volume. In turn, β-blockers, such as propranolol, were reported to inhibit ovariectomy-induced bone loss. In contrast, a number of experiments in mice and rats suggest that inhibition of β-adrenergic receptor-mediated signaling does not improve, and could actually be detrimental, for bone mass and microstructure. In humans, epidemiological observations suggested that users of β-blockers have higher bone mineral density (BMD) and/or a reduced risk of fractures, yet not all studies were concordant. Here we review the evidence for a role of the adrenergic system in the regulation of bone metabolism *in vitro* and *in vivo* and provide some new evidence for a dual role of β-adrenergic receptors 1 and 2 on bone turnover. Furthermore, we will examine the similarities and disparities that may exist in the effects of β-adrenergic and PTH stimulation on bone metabolism.

Keywords: Adrenergic Signaling, Bone Remodeling, Animal and Human Studies, Osteoblast, Osteoclast

Introduction

The process of bone modeling and remodeling ensures adaptation of the size, shape, microarchitecture and mineral content of the skeleton, as well as the repair of bone damage, in response to growth, aging and mechanical constraints[1]. At the tissue level, alterations of the bone modeling activity, particularly during growth, and of the bone remodeling rate, as occurs with the menopause and aging, in turn will lead to decreased peak bone mass and osteoporosis, respectively[2]. At the cellular level, physiological bone remodeling depends on the balance between bone resorption and bone formation, i.e., on the finely regulated and coordinated activity of osteo-clasts and osteoblasts within the BMU. The development and activation of osteoblasts and osteoclasts is controlled by growth factors and cytokines produced by bone cells themselves as well as by surrounding bone marrow cells. More recently, the neuroendocrine system has been implicated in the regulation of bone remodeling.

Histological studies have shown that both bone and the periosteum receive a rich supply of sensory and sympathetic nerve fibers, whose density is the greatest around the growth plates and in the metaphysis of long bones[3]. Nerve endings have been found to be in direct contact with bone cells, and catecholamine-containing axons have been identified near osteoblasts *in vivo*[3], suggesting a neuroendocrine regulation of bone remodeling. Recently, the role of bone innervation has regained attention with the description of a leptin-dependent central regulation of bone turnover through the β-adrenergic system[4,5]. Leptin may act on a population of neurons located in the ventromedial hypothalamus, which in turn stimulate the activity of intraosseous sympathetic nerve fibers[4]. These fibers release norepinephrine (NE), which binds to adrenergic receptors expressed on osteoblasts, thereby inhibiting their bone forming activity. Furthermore,

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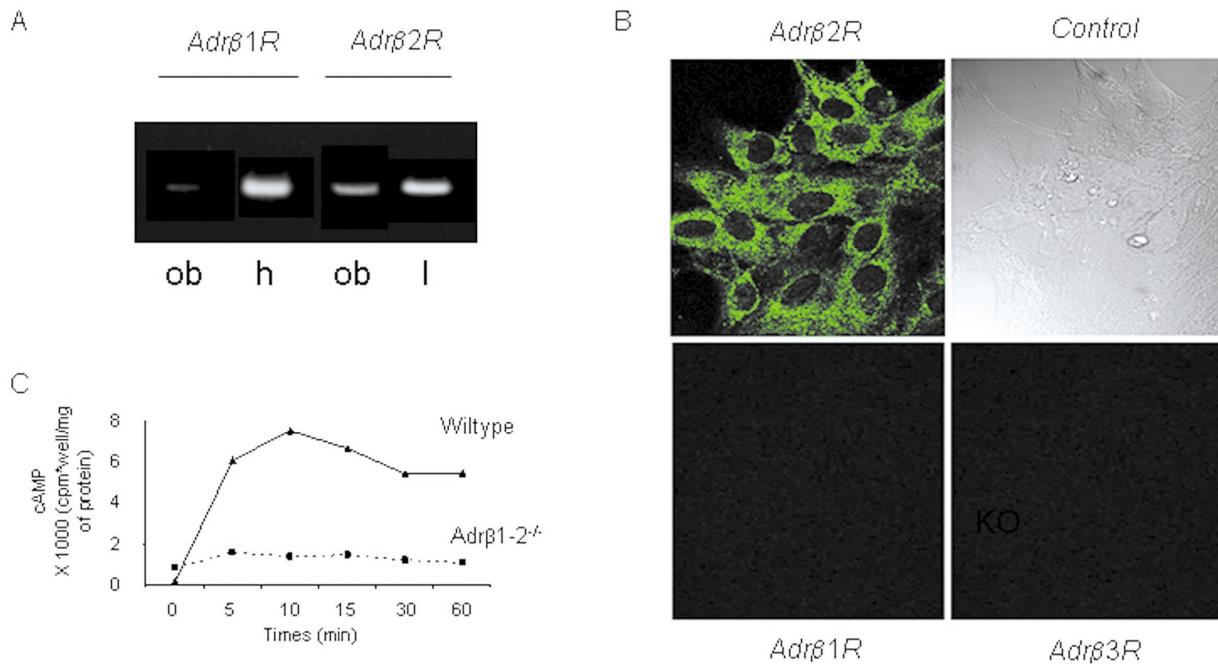
Figure 1. (A) Expression of β-adrenergic receptors (*Adr*β*1R*, *Adr*β*2R*) mRNA in osteoblasts (ob), heart (h) and liver (l) was probed by PCR. (B) Protein expression of *Adr*β receptors by osteoblast immunolabeling with affinity purified polyclonal anti- *Adr*β*1R*, anti- *Adr*β*2R* and anti- *Adr*β*3R* antibodies revealed by either FITC or TRITC and Cy5-tagged secondary antibodies. Note the negative expression of *Adr*β*1R* and *Adr*β*3R*. (C) Time-course of cAMP accumulation in response to isoproterenol in primary osteoblastic cells from WT and double *Adr*β*1β2R*^{-/-} mice. This figure was partially extracted from our article submitted to JCP[11].

β-adrenergic-stimulated production of Receptor Activator of NF-κB Ligand (RANKL) by osteoblasts may contribute to a negative bone mineral balance[6]. Despite this high level of evidence for an implication of the adrenergic system in the regulation of bone remodeling processes, both pharmacological and genetic experiments have produced contrasting, sometimes opposite, results. Here we review the effects of adrenergic stimulation and inhibition on bone cells and *in vivo*, and present the concept of a dual role of adrenergic signaling in the regulation of bone remodeling. In particular, we will examine the similarities and disparities that may exist in the effects of β-adrenergic and PTH stimulation on bone metabolism.

Role of adrenergic signaling in the regulation of bone remodeling

Adrenergic receptors are mostly known for their role in the regulation of cardiovascular, uterine and airway smooth muscle functions. However, after the histological demonstration of NE fibers on bone, pharmacological studies have shown that both osteoblastic and osteoclastic cells possess receptors for NE. Gene expression of α1 and 2 receptors (*Adr*α*1R*, *Adr*α*2R*) and/or β2-adrenergic receptors (*Adr*β*2R*) has been detected by RT-PCR in human periosteum-derived osteoblastic cells (SaM-1)[7], human osteosarcoma-derived cells (SaOS-2, HOS, MG63), mouse primary osteoblasts[4] and human osteoclastic cells[8]. However, among all post-synaptic adrenergic receptors, *Adr*β*2R* appears to be the main, if not the only functional adrenergic receptor expressed in osteoblasts[4,6,9,10]. We further confirmed by PCR and confocal microscopy the presence of *Adr*β*2R* mRNA and protein, respectively, in rat calvarial osteoblasts, whereas neither *Adr*β*1R* nor Adrβ3R expression was detected in these cells (Figure 1)[11]. Furthermore, *Adr*β*2R* mRNA was expressed in whole extracts from mouse femur, whereas *Adr*β*1R* and Adrβ3R genes were not[12].

Adrenergic effects on osteoblasts and bone formation

Adrenergic receptors belong to the large family of seven transmembrane domain proteins that transduce signals through heterodimeric guanine-nucleotide-binding proteins (G proteins). Epinephrine was found to enhance DNA synthesis in proliferating cells and alkaline phosphatase (ALP) activity in differentiating MC3T3-E1 osteoblast-like cells by stimulating *Adr*α*1R* coupled to Gi proteins[13]. Furthermore, the extracellular signal regulated kinase (ERK) pathway was involved in cell proliferation, and the p38 mitogen-activated protein kinase (MAPK) pathway in ALP activity in response to epinephrine[14]. However, other studies did not confirm the expression of the *Adr*α*1R* in human osteoblasts nor *in vivo*[8].

Activated $Adr\beta 2R$ is coupled to Gsα and adenylyl cyclase (to produce cAMP). In turn, activated protein kinase A (PKA) will phosphorylate various protein targets, transcription factors, other kinases and cell surface receptors, including the $Adr\beta 2R$ itself. We and others confirmed a dose-dependent increase of cAMP in response to the β-adrenergic agonist isoproterenol in primary osteoblast cultures, which can be blocked by the non-selective β-blocker propranolol as well as in $Adr\beta 2R$-deficient cells (Figure 1)[6,15]. In osteoblast-like cells, $Adr\beta 2R$-mediated cAMP and PKA activation leads to the expression of the immediate early gene c-fos[9]. The c-fos protein forms heterodimers with jun proteins, which regulate the transcription of AP-1 responsive genes such as osteocalcin, ALP and type 1 collagen as shown in rat bone cells and human osteosarcoma cells[16]. Hence $Adr\beta 2R$ stimulation has the potential to induce osteoblast differentiation.

A bulk of *in vivo* observations however contrasts with these *in vitro* data. Hence osteoblast number and surface, bone formation rate (BFR) on trabecular surfaces, as well as cbfa1 and α1(l) collagen gene expression were decreased in wild-type mice receiving isoproterenol or a central infusion of leptin (which activates the sympathetic system); conversely, osteoblast number and activity was increased in propranolol-treated mice, as well as in $Adr\beta 2R^{-/-}$ mice[4,6,17]. Older studies in a fractured rat model had already shown that non-selective β-adrenergic blockade by propranolol increased BFR at the metaphysis, periosteum and endosteum[18]. More recently, Bonnet et al. confirmed that low dose propranolol improved bone formation indices (mainly mineral apposition rate, MAR) in ovariectomized (OVX) rats[19]. We reported similar results in OVX adult mice, including a higher osteoblast number and surface in mice treated with propranolol vs. vehicle[20]. Moreover low-dose propranolol increased BFR by nearly 40% in chronically (mildly) stressed mice[21].

In contrast, mice deficient for both β1- and β2-adrenergic receptors ($Adr\beta 1$-$2R^{-/-}$) present a marked inhibition of bone formation indices, particularly at periosteal surfaces (MAR: -61%; BFR: -79%, p<0.005 vs. wildtype)[12]. This suggests that the higher bone formation indices in $Adr\beta 2R^{-/-}$ mice may require functional β1 adrenergic receptors. Considering that $Adr\beta 1R$s do not appear to be well expressed in bone (see above), it is possible that β1 adrenergic signaling could stimulate bone formation indirectly (systemically), opposite to the direct inhibitory effects of β2 adrenergic signaling on osteoblasts. The molecular mechanisms by which β1-adrenergic receptors regulate bone remodeling remains to be elucidated but it has been suggested that it could be mediated by the growth hormone (GH)-IGF-1 axis[22].

Adrenergic effects on osteoclasts and bone resorption

Using osteoblast/osteoclast co-cultures from wild-type and $Adr\beta 2R^{-/-}$ mice, Elefeteriou et al. demonstrated that adrenergic agonists indirectly stimulate osteoclast differentiation by increasing RANKL expression in osteoblasts[6]. In addition to

its direct effects on osteoblasts, β-adrenergic stimulation may regulate osteoclastogenesis by enhancing the production of RANKL by T cells[23]. RANKL is a critical factor for osteoclast differentiation and activation, but it is antagonized by a soluble receptor, osteoprotegerin (OPG)[24-26]. In primary osteoblasts from mouse calvariae, we found that the RANKL/OPG mRNA ratio was significantly increased after isoproterenol stimulation compared to osteoblasts from double-deficient $Adr\beta 1\beta 2R^{-/-}$ mice[12]. In contrast, the RANKL/OPG response to PTH was similar in wild-type and $Adr\beta 1\beta 2R^{-/-}$ cells, indicating that the low levels of isoproterenol-induced RANKL expression in these cells are contingent to the absence of β-adrenergic signaling and not just a reflection of a poor level of osteoblast differentiation.

In addition to RANKL, other osteoclastogenic factors such as interleukin (IL)-6, IL-11 and PGE_2 are stimulated by β-adrenergic agonists in human and mouse osteoblastic cells[27], apparently through both PKA and p38 MAPK signaling[28]. Several authors have also suggested a direct stimulation of osteoclasts following activation of the $Adr\beta 2R$ and $Adr\alpha$[29,30]. In human osteoclast precursors (FLG 29.1 cells), catecholamines have been shown to induce maturation and to stimulate osteoclastic activity[30], whereas in human osteoclast-like multinucleated cells, isoprenaline ($Adr\beta$-agonist) and epinephrine ($Adr\alpha$ and β-agonist) upregulate typical markers of mature osteoclasts, such as cathepsin K, and increase resorbing activity[29].

At the tissue level, Elefeteriou et al. demonstrated that osteoclast surface and bone resorption markers were not increased after OVX in $Adr\beta 2R^{-/-}$ mice but in wild-type (WT) mice[6]. In adult OVX mice, we reported that propranolol treatment decreased TRAcP5b, a bone resorption marker[20]. Studies in rats further indicated a significant increase of bone resorption markers following the administration of β-adrenergic agonists[31,32]. Moreover, β-adrenergic agonists significantly decreased leptin levels, probably secondary to a decrease in fat mass, and this decrease was correlated with bone resorption parameters[33]. Of note, lower leptin levels in response to adrenergic agonists could contribute to inhibit cortical bone formation[34,35].

In summary, adrenergic stimuli increase osteoclast numbers and activity, and thereby potentially the remodeling space (although histomorphometrical evidence that adrenergic inhibitors reduce activation frequency is still missing, at least to our knowledge). β2-adrenergic stimulation may concomitantly inhibit osteoblasts functions, resulting in a relative uncoupling of bone formation from resorption that further contributes to generate a negative bone mineral balance (at the BMU level).

Adrenergic versus PTH effects on bone cells and turnover

It is interesting to compare the effects on bone of β-adrenergic agonists with parathyroid hormone (PTH) (Table 1). Both stimuli activate G protein-coupled receptors (GPCR) and recruit β-arrestin2, a GPCR regulatory molecule, to

	PTH	β-adrenergic agonist
GPCR	class B	class A
Signaling	cAMP-CREB & Ca/IP3 MAPKs (ERKs, mediated by β-arrestin and c-Src)	cAMP-ATF4 MAPKs (ERKs, mediated by β-arrestin and c-Src)
Signaling desensitization and receptor internalization by agonist	++ (by β-arrestin)	βAdr2R<βAdr1R (by β-arrestin)
Effects on osteoblast	proliferation + differentiation/activation +++ survival +	proliferation-differentiation/activation +++ survival +
Effects on bone formation	+++	--
Cytokine production	RANKL, IL-6, (±PGE2)	RANKL, IL-6, PGE2
Effects on osteoclast	+++, indirect (RANKL)	++, direct and indirect (RANKL)
Effects on bone resorption	intermittent PTH + continuous PTH +++	++

Table 1. Similarities and differences between PTH and β-adrenergic signaling in bone.

desensitize cAMP signaling[36] and provide a molecular switch towards MAPKinase signaling[37,38]. Of note, several β-adrenergic antagonists, including propranolol, exert partial agonist activity on the activation of MAPKinases ERKs through the recruitment of β-arrestins[39]. This recent finding may further complicate the interpretation of studies on the skeletal effects of β-blockers (see below).

PTH and adrenergic signaling pathways diverge downstream of cAMP: whereas PTH activates cAMP response element-binding protein (CREB), *Adr*β*2R* stimulation leads to the phosphorylation of ATF4, known to control osteoblast terminal differentiation, collagen synthesis and to stimulate RANKL expression[6,40]. Taken together with the variable expression of *Adr*β*2R* and parathyroid hormone receptor 1 (PTH1R) at different stages of osteoblast differentiation, these observations suggest that adrenergic and PTH signaling regulate RANKL expression and bone resorption by acting on different populations of osteoblasts[17]. Moreover, PTH stimulates osteoblast proliferation, differentiation and/or survival[41], whereas β-adrenergic agonists may also exert anti-apoptotic effects on osteoblasts *in vitro*[42] but inhibit bone formation *in vivo*[6]. Therefore, PTH increases bone turnover, whereas adrenergic signaling potentially induces a negative bone mineral balance by uncoupling the functions of osteoblast and osteoclast at the BMU level. Of note, the net effect of PTH on the bone mineral balance depends on the pharmacodynamic profile of the hormone (or hormone analog[43]), and it could therefore be speculated that brief or prolonged exposure to adrenergic agonists, as encountered during stress (spikes of neuroendocrine stimulation) or in the case of algoneurodystrophy, respectively, may as well have different effects on bone.

Influence of the adrenergic system on bone mass, structure and strength: Animal studies

The main studies describing the effects of adrenergic receptor activation on the skeleton were initiated following the discovery that leptin, a pleiotropic adipocyte-derived hormone, was a central inhibitor of bone formation[44]. The sympathetic nervous system (SNS) was identified as the neuronal pathway controlling osteoblast function and bone mass. The involvement of SNS in the regulation of bone mass has been demonstrated both pharmacologically and genetically.

Genetic studies

Based mostly on the analysis of the trabecular bone compartment, mouse models with low SNS activity, such as leptin-deficient *ob/ob* mice and mice deficient for dopamine β-hydroxylase (DbH^{-/-}), the step-limiting enzyme responsible for catecholamine synthesis, have first been reported to have a high bone mass phenotype[44]. However, both models suffer from multiple endocrine dysregulations, including hypercorticism, hyperinsulinemia and hypogonadism. Hence, their bone phenotypes are necessarily more complex than a mere increase in trabecular bone volume[34,35,44]. To delineate more precisely the role of β-adrenergic signaling on bone mass and microstructure, mice deficient for β-adrenergic receptor (s), i.e., *Adr*β*2R*[-/-], *Adr*β*1R*[-/-] and *Adr*β*1-2R*[-/-] mice, were subsequently investigated.

*Adr*β*2R*[-/-] mice have a normal body weight, a normal hormonal status but an increased trabecular bone volume fraction (BV/TV) in the vertebrae and distal femur[6]. Furthermore, bone mass did not decrease in *Adr*β*2R*[-/-] mice following OVX[6], highlighting the importance of the SNS
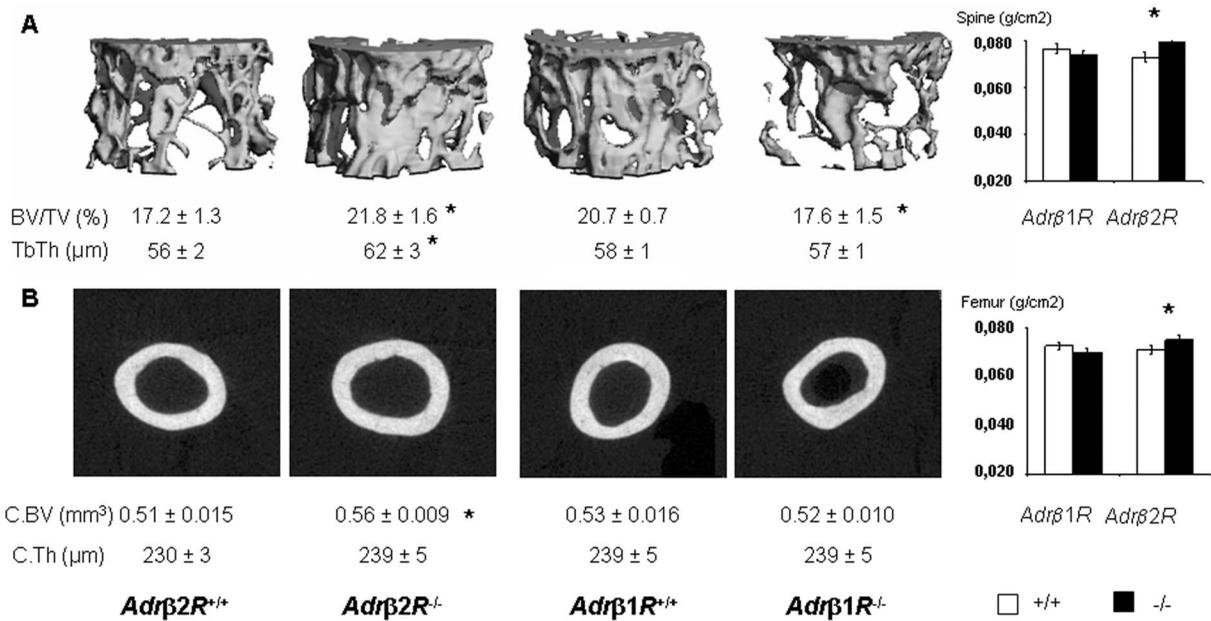
Figure 2. Bone mineral density (BMD) and microcomptuted tomography analysis of the trabecular microarchitecture at the lumbar spine L5 (A) and of the cortical bone at femoral mid-shaft (B) for 6-month-old $Adr\beta2R^{-/-}$ and $Adr\beta1R^{-/-}$ mice. BV/TV: trabecular bone volume fraction, TbTh: Trabecular thickness, C.BV: Cortical bone volume, C.Th: Cortical thickness. Mean±sem; *$p<0.05$ versus WT littermates.

integrity for estrogen-deficient bone loss. In contrast, $Adr\beta1R^{-/-}$ mice were briefly reported to have a normal BV/TV at 6 months of age (ibidem). Using both DXA and microcomputed tomography, we confirm here that $Adr\beta2R^{-/-}$ mice have higher vertebral and femoral BMD, trabecular BV/TV and thickness (TbTh) compared to WT littermates, as well as a greater cortical bone volume (BV) and thickness (C.Th) at the femoral mid-shaft (Figure 2). On the opposite, in $Adr\beta1R^{-/-}$ mice, we found lower vertebral BV/TV compared to WT littermates, whereas femoral BMD, cortical BV and C.Th were similar to WT (Figure 2).

Of note, single gene deletion in the β-adrenergic receptor system may result in phenotype(s) that represent, at least partially, an upregulation/overstimulation of the remaining β-adrenergic receptor subtypes, inasmuch as the loss of a specific receptor. For instance, $Adr\beta1R$ is upregulated in the fat tissue of $Adr\beta3R^{-/-}$ mice[45], which may contribute to a limited increase of adiposity in these mice. The analysis of double-deficient $Adr\beta1-2R^{-/-}$ mice is therefore of interest. These mice have a slightly decreased body weight (-16%), and a significantly lower BMD at total body (-15%), spine (-21%) and femur (-26%) compared to WT[46]. Their femur cross-sectional area (CSA), bone area and C.Th. were all significantly decreased vs. WT (-25% to -33%). Vertebral trabecular number (TbN) was higher, but BV/TV was not significantly increased, compared to WT[46]. TbTh indeed was decreased in $Adr\beta1-2R^{-/-}$ mice. Moreover, and in contrast to $Adr\beta2R^{-/-}$, we observed no protection of trabecular or cortical bone microstructure against ovariectomy in double-deficient $Adr\beta1-2R^{-/-}$ mice[47].

Hence, the "high bone mass" phenotype observed in adult $Adr\beta2R^{-/-}$ mice results from both a preservation of trabecular number (consistent with a lower bone resorption) and thickening of the trabeculae and cortex. The latter is consistent with an increased bone formation in these mice, but appears to require functional β1-adrenergic receptors. Taken together with the histological analysis presented above (see above 'Adrenergic effects on osteoblasts and bone formation'), these observations suggest that β1- and β2-adrenergic receptors-mediated signaling acts like the yin and yang for the regulation of bone remodeling. Several questions however remain to be elucidated, including the precise role of adrenergic nerve fibers projecting into the periosteum and the influence of the SNS on bone mass growth (structure modeling) in addition to maintenance (remodeling).

Pharmacological studies

Adrenergic agonists

Studies on the effects of adrenergic agonists on bone have also led to controversial, sometimes opposite, results. On one side, isoproterenol (ISO), a β-agonist, markedly decreased trabecular BV/TV in mouse vertebrae (-34% versus placebo) due to a lower BFR and osteoblast number[4]. We confirmed that ISO administered to growing mice for 8 weeks reduced BMD gain at total body (-33.5%), and spine (-64%), and modestly decreased femur lengthening (-5%)[47]. Vertebral TbN decreased by 24% with ISO, with a consequent decrease of trabecular connectivity (-45%) and

increase in trabecular separation (+127%). Similar results were observed at the distal femur. At the midshaft femur, C.Th and bone area were also both significantly decreased by ISO[47]. Of note, isoproterenol increased the percentage of lean mass and markedly decreased the percentage of fat (-77%), without major alterations in body weight gain compared to vehicle-treated mice. These important changes in body composition were accompanied by a decrease of leptin levels. Similar results were reported using a number of β-adrenergic agonists, namely clenbuterol and salbutamol[31,33,48]. Moreover, the biomechanical properties of rat bones treated with clenbuterol were significantly reduced, as shown by the loss of ultimate force (-21% versus placebo) and total energy (-22% versus placebo) using vertebral body compression and femoral three-point bending tests, despite the fact that clenbuterol had anabolic effects on muscle[31].

On another side, in a preliminary study Pataki et al. showed a beneficial effect of salbutamol in OVX rats[49]. Their hypothesis was based on the theory of the mechanostat of Frost, where an increase in the mass or force of the muscle is accompanied by an anabolic effect on bone tissue[50]. Clenbuterol also prevented the reduction of mineralization caused by lower limb denervation in young rats[51]. Formoterol and salbutamol both reduced the loss of dry weight and BMD of the trabecular fraction of rat distal femora in ovariectomized rats[49,52]. Of note, in 1980 the countries of Eastern Europe had proposed clenbuterol as a treatment against the muscular and osseous atrophy observed in certain diseases or during a long period of microgravity or immobility[51,53]. These studies indicate that, under certain conditions, β2-specific adrenergic agonists have the potential to exert positive effects on the skeleton, mainly through the preservation and/or increase of muscle mass (doping effect). Alternatively, we may hypothesize that non-specific β-adrenergic stimuli that would preferentially activate β1-adrenergic signaling could exert systemic (indirect) positive effects on the skeleton (see above).

Adrenergic antagonists

Several studies have investigated the implication of the SNS on bone by using β-adrenergic antagonists, particularly propranolol, a non-specific β-blocker. The beneficial effect of propranolol on bone was first demonstrated by Minkowitz et al. in a fractured rat model[18]. They showed that 9 weeks of low-dose propranolol treatment (0.1 mg/kg/day) increased bone formation parameters both at the periosteum and endosteum compartments[18]. They concluded that propranolol increased bone formation and consolidation in fracture cases. Moreover, in non-fractured animals, they showed by the torsion biomechanical test that propranolol improved the ultimate force by 33%[18]. Others observed the same preventive effect of propranolol on BMD and trabecular bone microarchitecture in suspended or immobilized rats[54,55]. Takeda et al. demonstrated that propranolol could prevent the loss of vertebral trabecular bone induced by central lep-

tin infusion and OVX in young/growing mice[4]. In contrast, propranolol increased trabecular thickness in the tibial metaphysis, but had no effect on BV/TV at the tibia and the vertebrae and no effect on the cortical compartment in intact mice[56,57]. Thus, several authors suggested that β-blockers could have site-specific effects, by postulating a differential innervation between loaded and unloaded bones[35,58,59].

In adult mice, we reported that propranolol only partially rescued the bone loss following OVX, by preventing BMD decrease at total body, but not specifically at spine nor femur[20]. In the lumbar vertebral body, propranolol did not significantly improve microarchitectural parameters, such as BV/TV, whereas at the femoral midshaft, propranolol increased CSA and medullary area. Thus, propranolol appeared to have limited effects on OVX-induced changes of bone microarchitecture in the trabecular and cortical bone compartments by influencing both bone forming and bone resorbing processes (see above 'Adrenergic effects on osteoblasts and bone formation'). However, when propranolol was combined to intermittent PTH, these drugs exerted synergistic effects on trabecular BV/TV and number as well as on bone formation indices (BFR, mineralizing perimeter), indicating that by uncoupling bone formation from bone resorption, propranolol has the potential to favor a positive bone mineral balance induced by PTH.

An important caveat in interpreting results from these studies resides in the variable degree of inhibition of β1- and β2-adrenergic receptor-mediated signaling obtained at various concentrations of antagonists. To approach this question, we used increasing doses of propranolol (0.1, 5 or 20 mg/kg/day, five days a week during 10 weeks) in adult rats. By hemodynamic monitoring, we demonstrated as Yaoita et al. a dose-dependent effect of propranolol in these rats[60]. Yet, in intact animals, we observed no significant difference on bone mineral density, microarchitecture and biomechanical properties of bone at any propranolol dose compared to placebo[61]. We then tested the efficiency of these various doses of β-blockers to prevent OVX-induced bone loss in rats. Trabecular BV/TV was decreased 30 to 50% in long bone metaphysis and vertebral bodies in the OVX compared to sham rats. Likewise, TbN and TbTh were decreased while osteoclast number was increased in OVX animals. In contrast, the bone mass of OVX rats treated with the lowest dose (0.1 mg/kg/day) of propranolol was not significantly different from sham-operated animals. Moreover, low-dose propranolol prevented the increase in osteoclast number in OVX rats and improved their bone formation parameters (mainly MAR) compared to rats treated with either placebo or higher dose (5 mg/kg/day) of propranolol[19], consistent with the uncoupling effects of β2-adrenergic inhibition (see above 'Adrenergic effects on osteoblasts and bone formation'). In the OVX rat model, Zhang et al. also demonstrated that propranolol treatment moderated the decrease of BMD and trabecular area, increased TbN, lowered trabecular separation to some extent as well as significantly depressing the urinary deoxypyridinoline levels compared to placebo[62].

Study design	Case/control or cohort size	Subjects n= number of beta blocker	BMD associate with beta blocker	OR for any fracture	References
Observational	163/978	N=38, perimenopausal women	No association	3.3 (95% CI, 1.1-9.4) adjusted for medication, weight, activity	Rejnmark et al.[68]
Observational	569/775	N=569, postmenopausal women	Higher BMD (total hip, UD forearm) S after adjustments for weight, age and thiazide	0.68 (95% CI, 0.49-0.96) adjusted for age, weight, height	Pasco et al.[66]
Observational	18441/72778	N=640, women aged 30 to 79	No measurement	0.85 (95% CI, 0.77-0.93) adjusted for medication, BMI	Schlienger et al.[67]
Observational	12160/48041	N=292, men aged 30 to 79	No measurement	0.66 (95% CI, 0.58-0.75) adjusted for medication, BMI	Schlienger et al.[67]
Observational	8412	N=838, postmenopausal women	Higher BMD (total hip, os calcis) NS after adjustments for weight, thiazide, statin, estrogen use	0.92 (95% CI, 0.81, 1.05) unadjusted	Reid et al.[70]
Prospective	7598	N=283, mean age 80.5±3.8 women	Higher BMD (femoral neck, ward triangle) NS after adjustments for weight, thiazide, statin, estrogen use	1.2 (95% CI, 0.9–1.5) adjusted for medication, weight	Levasseur et al.[75]
Prospective	100	N=50, aged men & women	Higher BMD (total hip, spine)	No measurement	Turker et al.[77]
Observational	124655/373962	N= 35 838, aged men & women	No measurement	0.91 (95% CI, 0.88–0.93) adjusted for medication, weight	Rejnmark et al.[69]
Meta-analysis			No measurement	0.86 (95% CI, 0.70–0.98) adjusted for medication, weight	Wiens et al.[74]
Observational	156/944	N=158, women aged 41 to 96	Higher BMD (femoral neck, spine) S after adjustments for weight, age, statin and thiazide	0.58 (95% CI, 0.36-0.94) adjusted for medication, weight	Bonnet et al.[73]
Observational	22247/22247	N=2013 men & women UK study short use of beta blocker	No measurement	0.82 (95% CI, 0.74-0.91) adjusted for medication, weight	De Vries et al.[76]
Observational	6763/26341	N=4447 men & women Dutch study short use of beta blocker	No measurement	0.87 (95% CI, 0.80-0.95) adjusted for medication, weight	De Vries et al.[76]
Observational	22247/22247	N=1446 men & women UK study long use of beta blocker	No measurement	1.06 (95% CI, 0.94-1.20) adjusted for medication, weight	De Vries et al.[76]
Observational	6763/26341	N=2116 men & women Dutch study long use of beta blocker	No measurement	0.97 (95% CI, 0.87-1.09) adjusted for medication, weight	De Vries et al.[76]
Studies (S=significant, NS=non-significant).					

Table 2. Odds ratio (OR) for prevalent fractures and BMD association in β-blocker users in observational, prospective and meta-analysis.

In an attempt to reconcile results from pharmacological and genetic studies, we will therefore suggest that, similar to the situation in $Adr\beta2R^{-/-}$ mice, partial blockade of the adrenergic system by low doses of antagonists may be beneficial to the skeleton, mostly by limiting bone resorption and the remodeling of the cancellous bone compartment, whereas non-selective inhibition of $Adr\beta R$-mediated signaling, as obtained with high doses of propranolol and in double-deficient $Adr\beta1-2R^{-/-}$ mice, will also affect bone formation and fail therefore to maintain a positive bone mineral balance.

Effects of adrenergic system on bone in humans

There are several clinical situations in which overstimulation of the adrenergic system has been associated with a low bone mass and/or increased bone fragility: 1) users of β2-agonists as bronchodilators (for asthma for instance) have 2-fold increased risk of hip/femur fracture. This risk remains significantly higher after adjustment for disease severity, but may be confounded by concomitant use of glucocorticoids[63]; 2) in reflex sympathetic dystrophy, characterized by a hyper-

activity of the adrenergic pathway, localized osteopenia is induced by an increased bone resorption[64]; 3) athletes doped with β2-agonists for their anabolic effect on muscle and catabolic effect on fat mass show bone loss and may be at increased risk of fatigue fractures. Hence, Collomp et al. reported a 4% decrease of the bone mineral content at the lumbar spine in both sedentary and athletic men receiving 12 mg per day of oral salbutamol during 3 weeks[65].

On the opposite, in a population of 1,344 postmenopausal women with a mean age of 70, Pasco et al. found that β-blockers were associated with a reduced risk of any fracture (OR 0.68 [95% CI, 0.49-0.96])[66]. BMD at the total hip and ultradistal forearm was positively correlated with the use of β-blockers in the global population and in the fracture group after adjusting for age and weight, but not in the subgroup of women without fracture (Table 2). Schlienger et al. also suggested a protective effect of β-blockers against osteoporotic fractures[67]. Their work was performed on the UK General Fracture Research Database, and included 30,601 cases, in which the OR of fractures was 0.77 among β-blockers users [95% CI, 0.72-0.83][67]. In contrast, Rejnmark et al. found no BMD differences between β-blocker users and non-users[68]. Furthermore, they observed a higher fracture risk in subjects treated by β-blockers compared to a non-user group. However, they had a low statistical power for detecting differences in BMD due to a small number of subjects (n=38) and their population was younger than in previous studies (50 years old)[68]. In a larger study, these authors recently reported that β-blockers were actually associated with a slightly reduced fracture risk (OR 0.91 [95% CI, 0.88-0.93])[69]. Reid et al. also found no association between bone parameters and β-blocker use after several adjustments, particularly for weight[70]. Despite the general conclusion of this study that there was no association between fracture risk and β-blocker use, OR was 0.66 [95% CI, 0.49-0.90] for hip fractures after adjustments for weight, age, thiazide, glucocorticoid and alcohol[70]. Intriguingly, a similar significant reduction in hip and wrist fracture risk was observed in a subpopulation of patients using cardio-selective agents (β1-blocker) (relative risks 0.70 and 0.51, respectively)[70].

The relationship between bone markers and β-blockers has been analyzed in two studies. One was a randomized, placebo controlled trial comparing propranolol 160mg/day vs. placebo over 3 months, in which both osteocalcin and free deoxypyridinoline declined in the β-blocker group, but no change of alkaline phosphatase or C-terminal telopeptide of type I collagen (CTx) occurred. Consequently these results provided no firm evidence that β-blockers may alter bone turnover in humans[71]. In the second observational study, Pasco et al. demonstrated that CTx is lower in β-blocker users versus non-users and that after 2 years of follow-up CTx was predictive of the adjusted bone loss[72].

We analyzed a cohort of 944 women, including 158 women who were taking β-blockers and 341 age- and sex-matched controls. BMD at L1-L4 and femoral neck as well as calcaneus H mean (microarchitecture index) remained significantly higher in the β-blocker group compared to control after adjustments for age, weight, thiazide and statins, although adjustment for weight attenuated the difference in bone parameters between groups[73]. Moreover, β-blockers were associated with BMD, cortical width and H mean parameter in both women with and without fracture[73]. Consistent with previous studies, the OR for fracture was 0.64 [95% CI, 0.45-0.83], excluding the subject treated with other medications, and the OR was 0.58 [95% CI, 0.36-0.94] after adjustments for weight, age, HRT, thiazides, statins, corticosteroids as well as anti-osteoporotic treatments[73]. As also noted by Reid et al., a substantial proportion (71%) of our population was using cardio-selective β1-blockers[70,73].

Two meta-analyses have been conducted to analyze the relationship between β-blockers and fractures and/or BMD. A meta-analysis of seven studies assessing fracture risk in patients using β-blockers concluded that their use was associated with a 28% reduction in hip fracture risk [95% CI 19-37% reduction] and a 14% reduction in the risk for any fracture [95% CI 2-24][74]. In contrast, another meta-analysis did not find any effect of β-blockers on fracture risk[75]. In the latter study, however, patients treated with β1-blockers were excluded, further suggesting that in humans the protective effects of β-blockers would be mediated through the β1 adrenergic pathway.

Conclusion

Studies on the influence of β-adrenergic signaling on bone metabolism and the resulting effects of β-adrenergic stimulation and inhibition on bone mass, microstructure and ultimately bone strength have underscored the complexity of the regulation of bone remodeling by the SNS. Further studies will be required in both animal models and humans to definitely characterize the role of β1AR, β2AR and their interaction on the regulation of bone turnover. In turn, results from these studies could prompt the development and use of selective β-adrenergic agonists and antagonists for the treatment of osteoporosis.

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