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Sympathectomy Alters Bone Architecture in Adult Growing Rats

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Abstract Sympathetic nervous system (SNS) fibres and α- and β-receptors are present in bone, indicating that the SNS may participate in bone metabolism. The importance of these observations is controversial because stimulation or inhibition of the SNS has had various effects upon both anabolic and catabolic activity in this tissue. In this study we evaluated the effects of pharmacological sympathectomy, using chronic treatment of maturing male rats with 40 mg of guanethidine/kg i.p., upon various parameters in bone. Double labelling with tetracycline injection was also performed 20 and 2 days before sacrifice. Bone mass, mineral content, density and histomorphometric characteristics in different skeletal regions were determined. Bone metabolic markers included urinary deoxypyridinoline and serum osteocalcin measurements. Guanethidine significantly reduced the accretion of lumbar vertebral bone and of mineral content and density, compared to controls. Femoral bone mineral content and density were also significantly reduced, compared to controls. Histomorphometric analyses indicated these effects were related to a reduction of cortical bone and mineral apposition rate at femoral diaphysials level. Both markers of bone metabolism were reduced in controls as they approached maturity. Guanethidine significantly decreased serum osteocalcin compared to controls, while urinary deoxypyridinoline was unchanged. These data indicate that guanethidine-induced sympathectomy caused a negative balance of bone metabolism, leading to decreased mass by regulating deposition rather than resorption during modeling and remodeling of bone. J. Cell. Biochem. 104: 2155–2164, 2008.

Key words: guanethidine; bone mineral density; DXA; bone histomorphometry; osteocalcin; deoxypyridinoline

Several studies have shown that the nervous system participates in bone metabolism [Togari, 2002; Turner et al., 2002; Togari et al., 2005; Elefteriou, 2005]. Nervous system influence may be exerted centrally through the hypothalamus [Karsenty, 2000] and peripherally, through the release of neurotransmitters, as bones are richly innervated by fibres of sensory and sympathetic origin [Hukkanen et al., 1992; Duncan and Shim, 1977]. Bone cells have functional receptors for sensory peptides [Villa et al., 2003] as well as α- and β-adrenoreceptors for catecholamines [Togari, 2002]. The role of the sympathetic nervous system (SNS) on bone metabolism is not clearly understood, as conflicting effects, both catabolic and anabolic [Kondo et al., 2003; Lavasseur et al., 2003; Elefteriou, 2005], have been obtained after its stimulation or inhibition, which in turn may be context dependent.

Mechanical loading is one of the most influential regulators of bone cell activation. The SNS is thought to mediate effects of mechanical loading in bone; pharmacological blockade of β-adrenergic receptors prevents bone loss in the absence of mechanical loading [Lavasseur et al., 2003]. Kondo et al. [2005] reported that treatment with the $\beta_{(1,2)}$-blocker, propranolol, inhibits the reduction of bone mass in a model of disuse osteoporosis in mice, suggesting that the SNS mediates nonload-induced bone loss.

Clinical studies have reported contrasting results regarding the treatment of people with

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propranolol. In fact, some authors have reported a reduction of the risk of fractures [Schlienger et al., 2004; Bonnet et al., 2007] others have found no association between bone parameters and β-blocker(s) use after adjustment for weight [Reid et al., 2005a]. Activation of β-adrenergic receptors with isoproterenol reduces osteoblast proliferation, decreases the expression of Cbfa1, a transcription factor controlling bone formation, and of α1(I) collagen, a gene encoding the main component of bone extracellular matrix [Takeda et al., 2002]. These data indicate an anti-osteogenic function of the SNS, secondary to effects on osteoblasts. On the other hand, surgical sympathectomy by the removal of the superior cervical ganglion increases bone resorption [Sandhu et al., 1987]. Likewise, chemical sympathectomy of neonatal rats treated with guanethidine increases resorption in the mandibular bone surface [Hill et al., 1991]. In contrast, when guanethidine is administered to adult rats, the number of mononuclear pre-osteoclast(s), tartrate-resistant acid phosphatase-positive (TRAP+) cells [Cherruau et al., 1999], as well as bone resorption pits [Cherruau et al., 2003], are reduced in the mandibular cortex subjected to synchronized resorption by molar(s) extraction. Other authors [Haug and Heyeraas, 2003], using a similar experimental model, found that surgical sympathectomy increases osteoclastic activity in the mandible, but not in the maxilla suggesting a site-specific response of bones to sympathectomy. This assumption is in line with the data reported by Pataki et al. [1996] showing that, in ovariectomized rats, treatment with a β_2-agonist has anabolic effects only on cancellous bone. Recently, Bonnet et al. [2005] reported that treating rats with two β_2-agonists, clembuterol and salbutamol, lowered bone mineral content, femoral length and cortical width, but that only clembuterol altered bone microarchitecture.

Despite the fact that adrenergic blockade may present a unique pharmacological alternative whereby bone resorption could be reduced without concomitantly decreasing bone formation [Elefteriou et al., 2005], the conflicting results reported above do not allow one to draw a definitive opinion on the importance of adrenergic signaling upon bone metabolism. We have therefore performed a detailed biochemical, densitometric and histomorphometric analysis of the appendicular and axial skeleton of growing rats which have undergone pharmacological sympathectomy in order to further characterize the importance of peripheral adrenergic neurotransmitters upon type of bone (i.e. appendicular vs. axial skeleton) and structural organization (i.e. trabecular vs. cortical).

MATERIAL AND METHODS

Animals

Male Sprague–Dawley rats weighing 275–300 g, 2 months old, were purchased from Charles River Laboratories (Calco, Italy). All rats were housed in single cages under controlled conditions ($22 \pm 2^{\circ}$C, 65% humidity, 12 h light/12 h darkness cycle). All experiments were carried out according to the European Union guidelines on laboratory animal care.

Experimental Protocol

Rats were allowed to acclimatize for 2 weeks and then were randomly divided into two groups: one group was treated daily with guanethidine sulphate (Sigma, Italy) dissolved in 0.9% saline, 40 mg/kg/ml intraperitoneally (i.p.) for 5 weeks. The other group was used as controls and received 1 ml of saline/kg i.p. The rats developed ptosis starting on the third day of guanethidine treatment, which persisted throughout the experiment, proving the success of the sympathectomy. No other systemic disorders were observed. Body weight was monitored at the start of the experiment (330–350 g) and every 2 weeks thereafter.

Bone areas, bone mineral content (BMC) and bone mineral density (BMD) were measured by DXA, and evaluated at the start of the experiment (t_0), after 30 days of treatment (t_{30}) and 60 days after the start of the experiment (t_{60}). Twenty-four hours urine samples were collected from rats housed in metabolic cages (Techniplast, Varese, Italy) at t_0, t_{30} and t_{60} for the measurement of deoxypyridinoline (DPD), a metabolite of collagen degradation and a marker of bone resorption. Samples were immediately frozen and stored at -20°C until assayed. Blood samples for the measurement of osteocalcin (OC), a marker of bone formation, were drawn at t_0, t_{30} and t_{60}, under light ether anaesthesia, by cardiac puncture. Plasma was stored at -80°C until assayed.

At the end of the experiment femora and vertebrae were excised for histomorphometric analysis.

Dual Energy X-Ray Absorptiometry (DXA)

All rats were anaesthetized with Zoletil, 40 mg/kg i.m. (Virbac, Italy) and scanned with a Hologic QDR-1000 instrument (Hologic Inc., Waltham, MA) in the ultra-high resolution mode. Longitudinal line spacing of 0.254 mm was used, implemented with a collimator 1.0 mm in diameter and with High Resolution Software (version 4.47) adapted for small animals. Four regions of interest were chosen: the lumbar vertebrae (L1–4), the entire femur, and the femoral diaphysis. The software provided the total area (cm^2) of the planar image of the selected segments, the BMC, in mg, and the BMD in mg/cm^2. Coefficients of variation were 3% for BMC and 1% for BMD. The precision and accuracy of DXA in small laboratory animals has been widely validated [Paniagua et al., 1988].

Biochemical Analysis

Total urinary DPD levels were measured in duplicate using an EIA kit (Quidel Corporation, San Diego, CA). Intra- and inter-assay variations were 5.5% and 3.1%, respectively. The total daily excretion of DPD was corrected for creatinine excretion. Urinary creatinine was measured colorimetrically using a commercial kit (Quidel Corporation, San Diego, CA). Total serum OC was determined using a commercial immunoenzymatic kit (Biomedical Technologies Inc., Stoughton, MA). The intra-assay variation was 4%, while the inter-assay variation was 7%.

Histomorphometry

The lumbar vertebra (L4) and the right femur of each animal were dehydrated and embedded in methylmethacrylate. Each vertebra was sectioned transversally and each femur was cut transversally in the central diaphyseal region and at the trochlear level of metaphyseal femur by means of a Leica SP 1600 diamond saw microtome (Leica SpA, Milano, Italy), obtaining 200-μm thick sections. Six central sections from the vertebral body and three sections from the femur were microradiographed (MicroXray, Italstructure, Como, Italy), scanned with an Epson Perfection 3200 PHOTO scanner and analyzed by means of image analysis software L.U.C.I.A. (Laboratory Imaging, Prague, CZ). The following parameters were measured in each vetebra and in metaphyseal femur: bone volume and total volume, trabecular thickness, trabecular number, trabecular separation and cortical thickness. Each diaphyseal femur was evaluated for: external area, internal area, cortical thickness and cortical bone volume (CBV%), according to the following formula: ((Ext area − Int area)/Ext area) × 100. Cortical thickness was measured at ten different points of the cortex, excluding margins.

To evaluate the bone formation rate, three controls and three guanethidine-treated animals were intraperitoneally injected with oxitetracycline (40 mg/kg) on the 40th and 58th day of the experimental period and were sacrificed on the 60th day. Periosteal and endosteal apposition growth was measured on seven serial cross sections, methylmethacrylate embedded, taken from mid-diaphyseal and metaphyseal levels of the right femur in all rats. The areas of bone between the two tetracycline labels at the periosteal and endosteal levels were measured.

STATISTICAL ANALYSIS

Statistical analysis was performed using a statistical package (PRISM, version 2.01 GraphPad Software, San Diego, CA). Data are reported as the means ± SEM. Densitometric and biochemical results were analyzed by one-way repeated measures analysis of variance (ANOVA) followed by Bonferroni *t*-test. Differences between groups were analyzed by unpaired Student's *t*-test. Since the animals were still in the growing phase the results of DXA were analyzed both as absolute values and as a differences (Δ) between the values detected at t_{30} or at t_{60} minus that at t_0. Histomorphometric parameters were analyzed by unpaired Student's *t*-tests. A probability of $P < 0.05$ was considered significant.

RESULTS

Body Weight

Treatment with guanethidine did not affect rat body weights. The animals grew normally during all the experimental period and there was no statistical differences between controls and treated animals (Fig. 1).

DXA Analysis

In vertebrae (L1–4) the bone areas, BMC and BMD increased with time and the differences

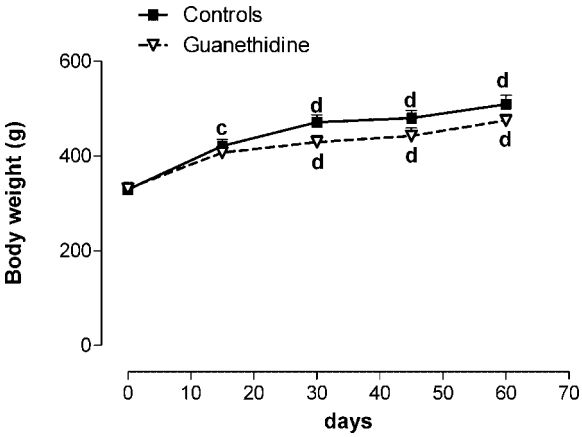


Fig. 1. Body weight of guanethidine-treated rats (40 mg/kg/ml i.p., daily for 5 weeks) and controls. Data are the mean ± SEM of seven rats per group. $^{c}P < 0.05$; $^{d}P < 0.01$ versus t_0 values.

compared to t_0 were significant in both the experimental groups. The effect of daily injection of guanethidine was a reduction of L1–4 vertebral bone areas compared to controls, that was significant at t_{60} (Fig. 2) and when comparing the absolute values at t_{30} ($P < 0.05$) and more so at t_{60} ($P < 0.01$; Table I). The extent of BMC accrual in the guanethidine group was significantly reduced, compared to controls at t_{30} ($P < 0.05$) and persisted until the end of the observation period, t_{60} ($P < 0.05$) as shown either in the results reported as changes relative to t_0 (Fig. 2) or as absolute values (Table I). BMD, which represents the ratio between BMC and the vertebral bone area, was significantly lower in the treated group at t_{30} but not so at t_{60} (Table I). However, when considering the changes, relative to baseline at t_0 BMD was significantly reduced at both times (Fig. 2).

Total femural areas in both controls and guanethidine-treated animals increased with time/age and were significantly different from those measured at t_0. Additionally, there were no differences between bone areas of the guanethidine-treated and control groups at t_{30} and t_{60} (Table II). BMC values and the changes in BMC relative to t_0, for example ΔBMC were reduced at t_{30} and at t_{60} compared to controls ($P < 0.05$), as well as BMD ($P < 0.01$) and ΔBMD ($P < 0.05$; Table II, Fig. 3). Bone areas of the diaphysis of femora increased with time/age and were significantly different from those measured at t_0 but they were not different between the two groups. The BMC accrual was significantly reduced in the guanethidine-treated group at t_{30} and at t_{60} ($P < 0.05$) as well as BMD ($P < 0.01$) compared to controls (Table III). A reduction was also shown for ΔBMC at t_{60} ($P < 0.05$; Fig. 4).

Histomorphometry

Histomorphometric analysis of L4 vertebral bodies showed a significant reduction ($P < 0.05$) of cortical thickness in the guanethidine-treated rats compared to controls, whereas no significant differences were detected between the two groups concerning all the trabecular bone parameters (trabecular bone volume, trabecular thickness, trabecular number and trabecular separation; Fig. 5). The histomorphometric analysis performed at the trochlear level of the femoral metaphysis showed no differences between the two groups in all the above-mentioned bone parameters (Fig. 6). The histomorphometric analysis performed at the mid-diaphyseal level of the femur showed that the internal area (area of the medullary canal) in the guanethidine group, resulted slightly increased although it did not reach the statistical significance. Cortical bone volume and cortical thickness were significantly reduced ($P < 0.05$) in the guanethidine-treated rats, showing that sympathectomy did not

TABLE I. Area, BMC and Planar BMD of Lumbar Vertebrae (L1–4) of Rats Treated With Guanethidine (40 mg/kg i.p. for 5 Weeks) Measured by DXA at the Start (t_0) and After 30 (t_{30}) and 60 (t_{60}) Days of the Experiment

	Area (cm^2)		BMC (mg)		BMD (mg/cm^2)	
	Controls	Guanethidine	Controls	Guanethidine	Controls	Guanethidine
t_0	1.87 ± 0.02	1.81 ± 0.03	351.3 ± 13	356.0 ± 09	195.4 ± 6	196.2 ± 3
t_{30}	$2.25 \pm 0.05^{\ddagger}$	$2.10 \pm 0.04^{*,\ddagger}$	$518.7 \pm 25^{\ddagger}$	$449.5 \pm 11^{*,\ddagger}$	229.7 ± 7	$213.5 \pm 3^{*}$
t_{60}	$2.50 \pm 0.02^{\ddagger}$	$2.24 \pm 0.06^{\dagger,\ddagger}$	$601.2 \pm 24^{\ddagger}$	$502.0 \pm 23^{*,\ddagger}$	243.2 ± 8	224.0 ± 7

Data are the mean ± SEM of seven rats.
$^{*}P < 0.05$.
$^{\dagger}P < 0.01$ versus controls.
$^{\ddagger}P < 0.01$ versus t_0.

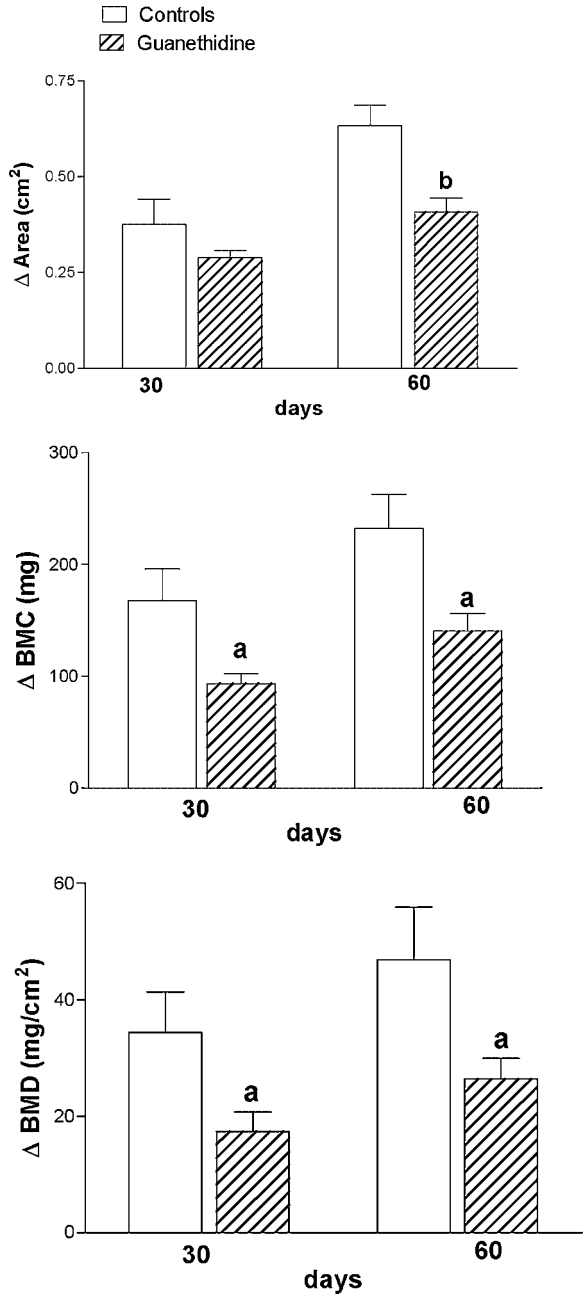


Fig. 2. Area, BMC and BMD measured at lumbar vertebrae (L1–4) in guanethidine-treated animals (40 mg/kg/ml i.p., daily for 5 weeks) and controls. Data are expressed as the differences between $t_{30}-t_0$ and $t_{60}-t_0$ (Δ). Results are the mean ± SEM of seven rats per group. $^{a}P < 0.05$; $^{b}P < 0.01$ versus controls.

change bone size but decreased cortical bone (Fig. 7). The periosteal and endosteal appositional growth, measured with double tetracycline labeling, showed a lower bone formation rate in guanethidine-treated animals compared to controls at the mid-diaphyseal level of femur whereas no statistical differences were detected at the metaphysisial level (Table IV).

Biochemical Markers

Serum OC and urinary DPD decreased with time in the guanethidine-treated rats and in the controls, as expected, because rats were reaching maturity. In the guanethidine-treated animals serum OC levels, were significantly reduced at t_{30} ($P < 0.01$) and at t_{60} ($P < 0.05$) compared to controls (Fig. 8). No statistically significant changes were found in the DPD excretion between the two groups (Fig. 9).

DISCUSSION

This study provides evidence that pharmacological sympathectomy, after long-term treatment with guanethidine, impairs the normal process of bone mass acquisition of the appendicular and axial skeleton in growing rats. BMC and BMD accruals, both in vertebrae and in femura, as well as vertebral bone area, were lower in the guanethidine-treated rats compared to controls.

Since the static histomorphometric analysis performed on the excised bone samples showed that cortical bone volume and thickness were significantly lower in the treated animals while trabecular bone was unaffected, it can be inferred that pharmacological sympathectomy exerts an inhibitory action on cortical bone development during growth. The structural modifications observed by histomorphometric analysis fit with the observed changes of DXA parameters, since bone cortex accounts for most of the X-rays absorbance of the appendicular and axial skeleton.

By assuming that the bone modeling process induces a progressive outward displacement of the cortex from the neutral axis during bone growth [Parfitt et al., 2000], the lower cross-sectional vertebral area of the treated animals as well as their lower bone cortical thicknesses suggests the process of bone modeling itself is impaired in vertebrae after pharmacological sympathectomy. However, the same did not hold for the appendicular skeleton where the bone areas, measured by DXA on the frontal plane or by histomorphometry on the cross-sectional plane, did not differ between groups. This suggests that following guanethidine treatment there is a region specific alteration of the outward drift, due to modeling, for the

TABLE II. Area, BMC and Planar BMD of Total Femur of Rats Treated With Guanethidine (40 mg/kg i.p. for 5 Weeks) Measured by DXA at the Start (t_0) and After 30 (t_{30}) And 60 (t_{60}) Days of the Experiment

	Area (cm^2)		BMC (mg)		BMD (mg/cm^2)	
	Controls	Guanethidine	Controls	Guanethidine	Controls	Guanethidine
t_0	1.48 ± 0.01	1.50 ± 0.03	369.8 ± 10	380.1 ± 7.0	249.4 ± 6	252.4 ± 4
t_{30}	$1.80 \pm 0.03^{\ddagger}$	$1.74 \pm 0.05^{\ddagger}$	$556.1 \pm 15^{\ddagger}$	$505.4 \pm 17^{*,\ddagger}$	$308.1 \pm 4^{\ddagger}$	$283.6 \pm 4^{\dagger,\ddagger}$
t_{60}	$1.92 \pm 0.04^{\ddagger}$	$1.85 \pm 0.07^{\ddagger}$	$615.6 \pm 18^{\ddagger}$	$550.2 \pm 15^{*,\ddagger}$	$319.0 \pm 4^{\ddagger}$	$296.0 \pm 1^{\dagger,\ddagger}$

Data are the mean ± SEM of seven rats.
$^{*}P < 0.05$.
$^{\dagger}P < 0.01$ versus controls.
$^{\ddagger}P < 0.01$ versus t_0.

axial skeleton. In the appendicular skeleton bone grew normally but the decreased thickness of cortical bone in the guanethidine-treated animals, is probably due to changes of the bone apposition in the inner corticeps. It is interesting to note that the inhibitory action on bone, due to peripheral sympathectomy, is confined to the cortical bone compartment, the trabecular compartment being insensitive. This observation is in line with previous data showing regional and structural differences in bone after the central infusion of leptin, which is known to stimulate the SNS [Takeda et al., 2002]. In growing rats central leptin was seen to reduce trabecular bone in femura and tibiae but not in vertebrae, moreover to increase cortical bone in tibiae [Guidobono et al., 2006]. These observations support the view that the SNS might be more involved in a modulatory role in controlling bone mass as a function of the mechanical environment rather than affecting the trabecular compartment which is more involved in the organism metabolic needs.

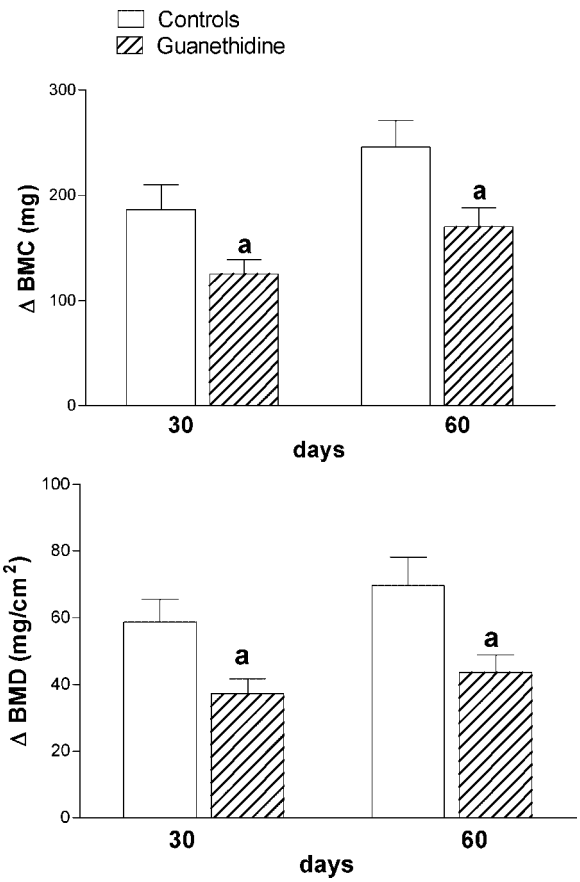


Fig. 3. BMC and BMD measured on total femur in guanethidine-treated animals (40 mg/kg/ml i.p., daily for 5 weeks) and controls. Data are expressed as the differences between $t_{30}-t_0$ and $t_{60}-t_0$ (Δ). Results are the mean ± SEM of seven rats per group. $^{a}P < 0.05$ versus controls.

The lack of measurement of osteoblasts and osteoclasts numbers does not allow us to determine which cellular phase is affected by the depletion of the adrenergic neurotrasmitters. However the lower bone deposition in femur diaphysis and the greater decrement of OC levels in the treated animals with absence of significant changes in DPD excretion, suggest that the acquired bone phenotype is the result of a greater reduction of osteoblasts reaching maturity, rather than a prolonged activated state of bone resorption. This non-parallel change of both phases of bone (re-)modeling might be responsible for a relatively greater rate of bone resorption over formation, which can explain the thinning bone cortex and the reduced cortical thickness. Consistent with this interpretation is the observation that the medullary area of the femoral bone diaphysis is enlarged while the total cross-sectional area is comparable to controls.

Cherruau et al. [1999] showed that guanethidine treatment of rats significantly reduced the resorption phase during bone remodeling as

TABLE III. Area, BMC and Planar BMD of Femoral Diaphysis of Rats Treated With Guanethidine (40 mg/kg i.p. for 5 Weeks) Measured by DXA at the Start (t_0) and After 30 (t_{30}) and 60 (t_{60}) Days of the Experiment

	Area (cm^2)		BMC (mg)		BMD (mg/cm^2)	
	Controls	Guanethidine	Controls	Guanethidine	Controls	Guanethidine
t_0	0.355 ± 0.007	0.357 ± 0.010	99.7 ± 3.2	98.6 ± 1.7	280.3 ± 5	270.4 ± 5
t_{30}	$0.405 \pm 0.006^{\ddagger}$	$0.401 \pm 0.009^{\ddagger}$	$140.4 \pm 2.3^{\ddagger}$	$132.4 \pm 2.0^{*,\ddagger}$	$347.0 \pm 2^{\ddagger}$	$330.5 \pm 4^{\dagger,\ddagger}$
t_{60}	$0.418 \pm 0.005^{\ddagger}$	$0.414 \pm 0.010^{\ddagger}$	$151.3 \pm 3.85^{\ddagger}$	$137.1 \pm 3.7^{*,\ddagger}$	$359.0 \pm 6^{\ddagger}$	$331.1 \pm 3^{\dagger,\ddagger}$

Data are the mean ± SEM of seven rats.
$^{*}P < 0.05$.
$^{\dagger}P < 0.01$ versus controls.
$^{\ddagger}P < 0.01$ versus t_0.

well as the number of osteoclasts, by inhibiting preosteoclast differentiation. Those experiments were performed four days after 21 days of guanethidine treatment. Other authors [Sherman and Chole, 2000] showed that pharmacologically induced sympathectomy in gerbils increased resorption in the membranous ossification but not in the endochondral ossification in the long bones. Hill et al. [1991] have shown that guanethidine treatment had no effect on cortical area, medullary area, and periosteal apposition rate in neonatal rats. Kondo et al. [2003] reported that guanethidine and propranolol did not affect cancellous bone volume whereas Takeda et al. [2002] showed that mice treated with propranolol had an increase in trabecular bone volume. Takeda et al. [2002] did not investigate the effect of sympathectomy on cortical bone and on biochemical markers of bone metabolism.

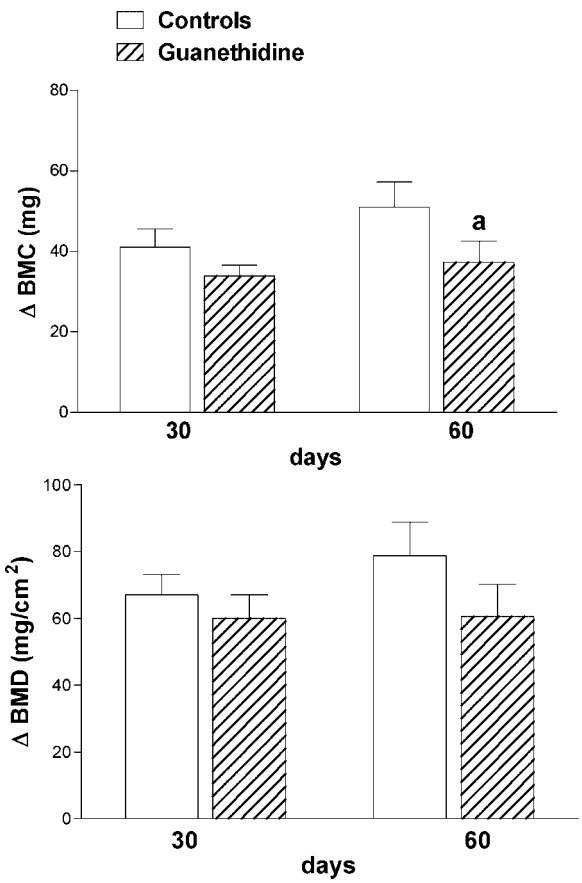


Fig. 4. BMC and BMD measured at femoral diaphysis in guanethidine-treated animals (40 mg/kg/ml i.p., daily for 5 weeks) and controls. Data are expressed as the differences between $t_{30}-t_0$ and $t_{60}-t_0$ (Δ). Results are the mean ± SEM of seven rats per group. $^{a}P < 0.05$ versus controls.

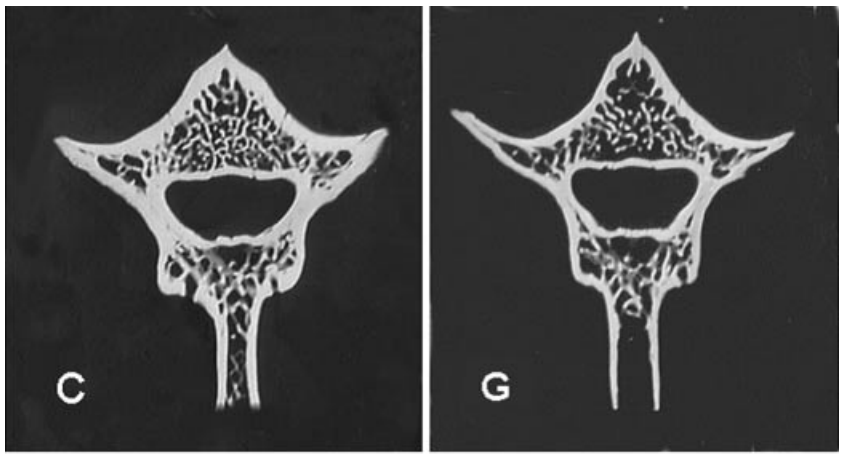
In our experiment DPD excretion did not differ between guanethidine-treated animals and controls so the observed reduction of bone mass appears to involve a decrease of bone formation rather than an increase of bone resorption supported by decreased serum levels of OC in sympathectomized rats compared to controls. In line with what we found in rats, serum OC was seen to decline by almost 20% in postmenopausal women after propranolol treatment [Reid et al., 2005b] which is in contrast with the results obtained in mice, where treatment with β-blockers increased bone mass [Takeda et al., 2002].

The slight decrement, in the serum marker of bone formation observed after sympathectomy

TABLE IV. Areas of Bone (mm^2) Between the Double Tetracycline Labelling at Periosteal and Endosteal Sites

	Femur metaphysis	Femur diaphysis
Controls	0.58 ± 0.08	0.58 ± 0.027
Guanethidine	0.70 ± 0.07	0.43 ± 0.035*

Data are the mean ± SEM of three animals evaluated from seven cross sections.
$^{*}P > 0.05$ versus controls.

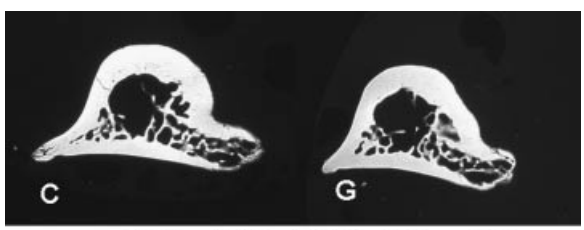


	BV/TV%	TbTh μm	TbN mm^{-1}	TbS μm	WTh μm
Controls	35.8±2.5	79.6±3.6	4.4±0.2	144.7±11.8	297.8±22.1
Guanethidine	35.4±3.5	75.5±5.9	4.6±0.1	140.1±11.1	231.5±13.7[a]

Fig. 5. Representative histomorphometric image of vertebrae at L4. C, control; G, guanethidine treated rat (40 mg/kg/ml i.p., daily for 5 weeks). Values are the mean ± SEM of seven rats per group. [a]$P < 0.05$ versus controls. BV/TV, bone volume/total volume; TbTh, trabecular thickness; TbN, trabecular number; TbS, trabecular separation; WTh, cortical wall thickness.

could be a consequence of the reduced resorption surface of the osteogenic compartment [Cherruau et al., 1999] that, by reducing the osteoclast activity it could reduce osteoblast activity as well. However, this assumption seems to be ruled out since in our experiment we could not detect any reduction in bone resorption by the measurement of DPD urinary excretion.

It is well known that adrenergic receptors are present in osteoblastic cells [Togari, 2002]. Suzuki et al. [1999] demonstrated that epinephrine stimulates osteoblast-like cell proliferation and increases alkaline phosphatase activity by stimulating α1-adrenergic receptors coupled to G proteins. Treatment with epinephrine induces RANKL and OPG mRNAs expression [Tacheuchi et al., 2000] but different adrenergic receptors mediate RANKL and OPG expression. Activation of β-receptors increases RANKL expression and causes osteoclastogenesis in mouse bone marrow cells, whereas activation of α-receptors increases OPG expression in MC3T3-E1 osteoblast-like cells [Suzuki et al., 1998; Tacheuchi et al., 2000]. Thus it is possible that a bimodal receptor-specific model



	BV/TV%	TbTh μm	Tb N mm^{-1}	TbS μm	WTh μm
Controls	29.1±0.3	114.7±0.5	2.7±0.16	264.3±27.1	724.3±32.6
Guanethidine	30.7±0.4	120.9±2.2	2.5±0.08	274.0±10.6	687.0±2.8

Fig. 6. Representative histomorphometric image of trochlear femoral metaphysis. C, control; G, guanethidine-treated rat (40 mg/kg/ml i.p., daily for 5 weeks). Values are the mean ± SEM of three rats per group. BV/TV, bone volume/total volume; TbTh, trabecular thickness; TbN, trabecular number; TbS, trabecular separation; WTh, cortical wall thickness.

	Ext Ar mm^2	Int Ar mm^2	CBV %	Ct Th μm
Controls	11.1±0.5	3.9±0.3	64.6±1.1	711.9±39.7
Guanethidine	11.1±0.5	4.3±0.3	61.0±2.2[a]	659.2±27.7[a]

Fig. 7. Representative histomorphometric image of femoral diaphysis. C, control; G, guanethidine treated rat (40 mg/kg/ml i.p., daily for 5 weeks). Values are the mean ± SEM of seven rats per group. [a]$P < 0.05$ versus controls. Ext Ar, total area of the section; Int Ar, area of the medullary canal; CBV%, cortical bone volume expressed as % of total area; Ct Th, cortical thickness.

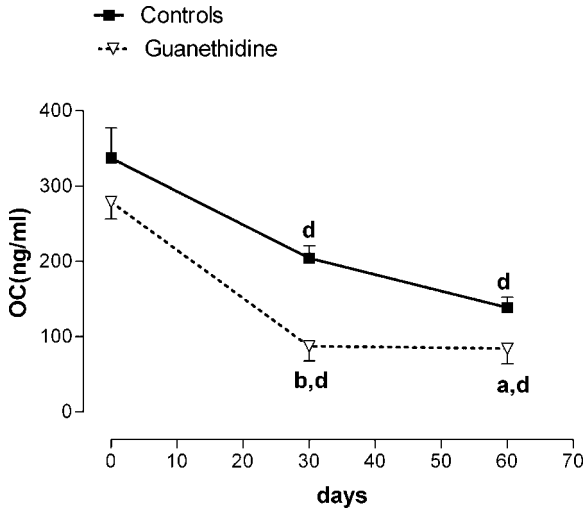


Fig. 8. Serum osteocalcin (OC) levels in guanethidine-treated rats (40 mg/kg/ml i.p., daily for 5 weeks) and controls. Values are the mean ± SEM of seven rats per group. $^{a}P < 0.05$; $^{b}P < 0.01$ versus controls; $^{d}P < 0.01$ versus t_0.

for catecholamine-mediated modulation of resorption may operate in bone, with β-receptors (in particular β_2-receptors) [Togari et al., 2005] stimulating osteoclastogenesis and α-receptors, by increasing OPG secretion, keeping this effect under control in order to maintain bone homeostasis. The fact that guanethidine depletes catecholamine vesicles, thus leading to decreased stimulation of both α- and β-receptors, may account for our results as opposed to the results of other authors [Takeda et al., 2002]

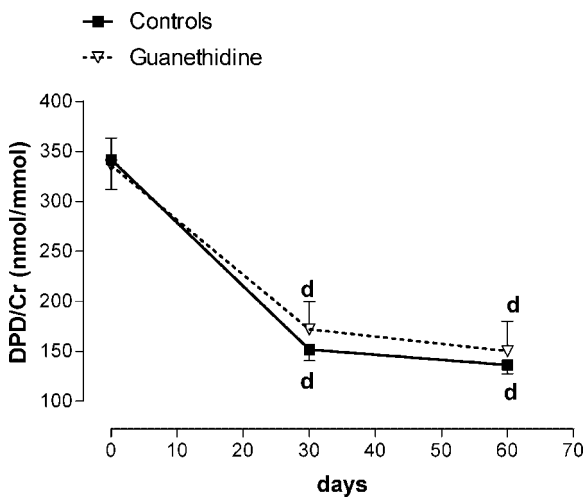


Fig. 9. Urinary excretion of deoxypyridinoline (DPD) in guanethidine-treated animals (40 mg/kg/ml i.p., daily for 5 weeks) and controls. Data are expressed as a ratio to 24 h creatinine urinary excretion. Values are the mean ± SEM of seven rats per group. $^{d}P < 0.01$ versus t_0.

who used antagonist or agonist drugs specific for one or the other receptors.

In conclusion, our results obtained in an animal model, support the hypothesis that sympathectomy alters bone accrual by acting on the cortical compartment via region specific pathways, still undefined, but potentially linked to the putative modulatory control of nerve derived signals on the response of bone to its mechanical environment.

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