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Journal of the Autonomic Nervous System 78 (2000) 113–116

Journal of the Autonomic Nervous System

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Effect of unilateral superior cervical ganglionectomy on bone mineral content and density of rat's mandible

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Received 27 May 1999; received in revised form 12 August 1999; accepted 23 August 1999

Abstract

To assess the effect of a local sympathectomy on bone metabolism, the effect of a unilateral superior cervical ganglionectomy (Gx) on growth and bone mineral content and density of the ipsi- and contralateral mandibles was examined in female rats. A significant increase in the hemi-mandibular bone ipsilateral to Gx was found as compared to the contralateral, sham-operated side 30 days, but not 15 days, after surgery. Bone mineral content of the hemi-mandibular bones was significantly lower in the side ipsilateral to Gx in the group of rats killed on the 30th day after surgery. Since no difference in areas between innervated and denervated hemi-mandibles was found, bone mineral density was also significantly lower in the hemi-mandible ipsilateral to Gx. The results further support that a regional sympathectomy causes qualitative alterations in bone modeling and remodeling, leading to bone resorption.

Keywords: Bone sympathetic nerves; Bone mineral content; Bone mineral density; Mandible; Superior cervical ganglionectomy

1. Introduction

Bone metabolism is dependent on cells of the osteoblast and osteoclast lineage. These cells play a major role in the synthesis and degradation of osteoid and in its subsequent mineralization and demineralization, and are under the influence of various systemic and local auto/paracrine factors. The autonomic nervous system is one of these factors (Appenzeller, 1990).

Autonomic nerve fibers are found in the periosteum, endosteum, and cortical bone and, in many cases, the free-running fibers are associated with blood vessels that enter the bone through Volkmann's canals (Buma et al., 1995). Among the several neuropeptides identified in homogenates of bone, periosteum and bone marrow, neuropeptide Y, a co-transmitter with norepinephrine in peripheral sympathetic nerve fibers, consistently exhibited the highest concentrations in the different tissues (Ahmed et al., 1994). Apart from neural influences on bone mass and osteoclast/osteoblast coupling (activation–resorption–formation cycle), bone nerves may explain why various inputs/outputs are transformed in a meaningful way to altered mass and quality of bone (Chole and Tinling, 1998).

In a study on the effect of surgical sympathectomy on bone remodeling of rat incisor and molar root sockets (achieved by the unilateral surgical removal of the superior cervical ganglion, SCG), a decrease in periosteal and endosteal apposition and in the rate of mineralization, together with an increase in number of osteoclasts and in bone resorption surfaces were found (Sandhu et al., 1987). In another study, a unilateral cervical sympathectomy in rats brought about an increase in osteoclast surface and osteoclast number in the ipsilateral bulla (Sherman and Chole, 1996). Further supporting the stimulation by denervation of bone resorption mechanisms, guanethidine treatment of neonatal rats increased the percentage of periosteal surface of the mandible occupied by osteoclasts during induced remodeling (Hill et al., 1991). Potential mechanisms for the effects of sympathectomy may include disinhibition of resorption, secondary to the elimination of

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0165-1838/00/$ - see front matter
PII: S0165-1838(99)00067-3

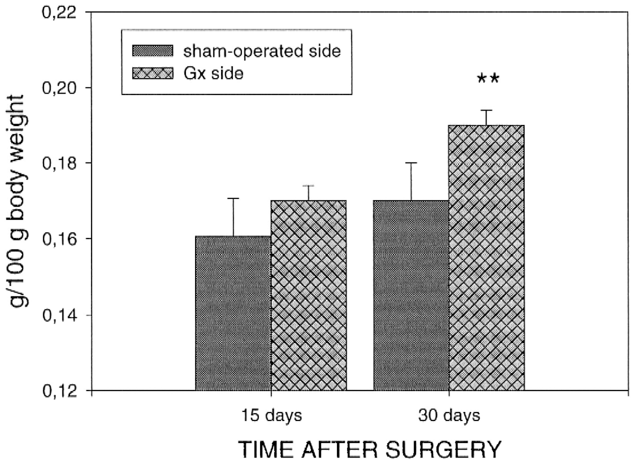

Fig. 1. Effect of a unilateral Gx on weight of hemi-mandibles of rats sacrificed 15 and 30 days after surgery. As a control, the contralateral sham-operated side was used. Shown are the means ± SEM ($n = 15$ for animals killed 15 days after surgery and $n = 13$ for animals killed 30 days after surgery). ** $p < 0.001$ as compared to the contralateral side, paired Student's t-test.

periosteal sympathetics, as well as indirect vascular effects.

In order to further assess the effect of local sympathectomy on bone physiology, we examined the effect of unilateral superior cervical ganglionectomy (Gx) on growth and bone mineral content (BMC) and bone mineral density (BMD) of the ipsi and contralateral mandibles. Results to be described support the existence of a significant effect of the sympathetic input in the regulation of bone mineral mass.

2. Materials and methods

Female Wistar rats (1 month old) raised in our colony were kept under a 12:12 h light–dark cycle (lights off at 1800 h) with access to food and water ''ad libitum''. A unilateral superior cervical Gx and a contralateral sham-operation were performed under pentobarbital anesthesia (50 mg/kg i.p.) as described earlier (Romeo et al., 1985). The experiment was conducted in accord with the principles and procedures outlined in the NIH guide for the care and use of the laboratory animals.

At 15 and 30 days after surgery, groups of rats were sacrificed and both hemi-mandibles were excised, put in hot 1 M sodium hydroxide for 10 min and carefully cleaned of soft tissues. The hemi-mandibles were then dried for 24 h at ambient temperature and were weighed in an analytical Mettler balance.

BMC, bone area and BMD of every hemi-mandible were determined by using a total body scanner and a specifically designed software for small animals (DPX Alpha 8034, Small Animal Software, Lunar Radiation, Madison, WI). A constant potential X-ray source of 76 kVp with effective energies between 38 and 70 keV was employed. Collimation (i.e., size of X-ray beam at the source) was 0.84 mm. Results were expressed as g (BMC) or g/cm^2 (BMD). The coefficient of variation (expressed as a percent standard deviation of the mean) was 3.0% for BMC and 0.9% for BMD (Zeni et al., 1997). Results were statistically analyzed by a paired Student's t-test.

3. Results

Fig. 1 shows the differences in weight between hemi-mandibles of rats sacrificed 15 and 30 days after a unilateral Gx. A significant increase in the weight of the hemi-mandibular bone ipsilateral to SCG removal was found as compared to the contralateral, sham-operated side 30 days after surgery ($t = 3.256$, $p < 0.001$, paired Student's t-test).

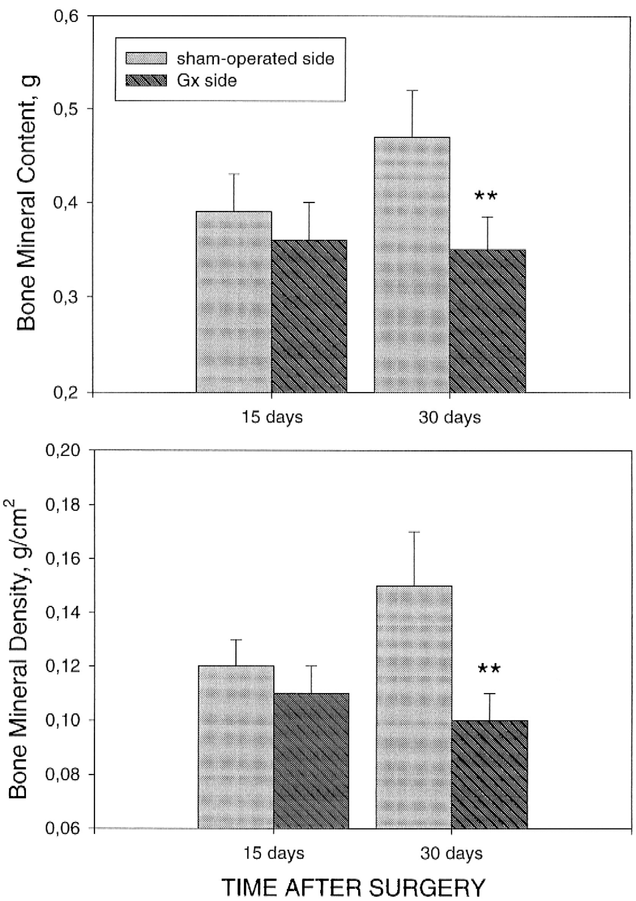

Fig. 2. Effect of a unilateral Gx on BMC and BMD of hemi-mandibles of rats sacrificed 15 and 30 days after surgery. As a control, the contralateral sham-operated side was used. Shown are the means ± SEM ($n = 15$ for animals killed 15 days after surgery and $n = 13$ for animals killed 30 days after surgery). ** $p < 0.001$ as compared to the contralateral side, paired Student's t-test.

BMC of the hemi-mandibular bones in the same series of animals is shown in Fig. 2 (upper panel). This parameter was significantly lower in the hemi-mandible ipsilateral to Gx in the group of rats killed on the 30th day after surgery ($t = 3.607$, $p < 0.001$, paired Student's t-test). Since no differences in bone area between innervated and denervated hemi-mandibles were found (results not shown), BMD (i.e., mineral content/area) was also significantly lower in the hemi-mandible ipsilateral to Gx in rats 30 days after surgery ($t = 3.950$, $p < 0.001$, paired Student's t-test, Fig. 2, lower panel).

4. Discussion

Previous studies indicated that regional sympathectomy leads to qualitative alterations in localized bone modeling and remodeling, leading to bone resorption (Sandhu et al., 1987; Hill et al., 1991; Sherman and Chole, 1996). Our foregoing results show that 30 days after a unilateral Gx the weight of the ipsilateral mandible augmented, and its BMC and BMD decreased. This effect is presumably dependent on the chronic deprivation of adrenergic neurotransmitter input at a local level. Indeed, there is evidence that bone tissue is responsive to stimulation with β-receptor agonists (Garbarino and Greene, 1984; Moore et al., 1993).

The modifications in bone mass caused by denervation could be due to changes in preexisting tissue as well as to modification of developmental growth, since young growing rats were used in these studies. The disparate effect of Gx on bone weight and BMC and density indicates the occurrence of dissimilar effects of sympathetic nerve endings on matrix and mineral content of bone. Further studies, including the histological examination of bone tissue, may help to clarify this point.

Generally, the data herein presented are in agreement with previous studies on sequela of sympathectomy in bone. For example, Sandhu et al. (1987) examined the effect of a unilateral Gx on bone remodeling at rat incisor and molar root sockets. Following sympathectomy, periosteal and endosteal apposition as well as the rate of mineralization were significantly lower and an increase in the number of osteoclasts and in active and inactive bone resorption surfaces was seen. Since independent, sham-operated rats were used as controls, rather than the contralateral sham-operated side of the same animal, a possible systemic effect of hemi-Gx could not be excluded.

In another study, Hill et al. (1991) reported an increase in percentage of periosteal surface of the mandible occupied by osteoclasts during induced remodeling in rats treated neonatally with guanethidine. It must be noted that one of the major criticisms to studies employing drugs that interfere with sympathetic autonomic function is that systemic effects on hormonal mechanisms controlling bone mineral cannot be excluded. Collectively, the present and previous results support a significant stimulatory effect of the sympathetic input in regulation of bone mass.

It is interesting that the administration of β-adrenoceptor agonists (e.g., clenbuterol, dobutamine) was found to reduce net bone loss in denervated hindlimbs (Zeman et al., 1997) and to decrease the scoliosis seen after a partial transection of rat spinal cord (Zeman et al., 1991). Generally, the proposed explanation for β-adrenergic effects on bone physiology was an indirect effect mediated by the muscles. However, in view of the present and previous results, direct effects of adrenoceptor agonists on the bone cannot be excluded.

The proposed effect of sympathetic nerves in bone is not without experimental background in other tissues. For example, the autonomic nervous system affects the amplitude of proliferative responses like that occurring during the immune reaction or after a reduction of the erythrocyte mass (for reference, see Cardinali and Esquifino, 1998). In turn, the activity of sensory fibers (e.g., substance P-containing fibers) in the vicinity of proliferating bone cells may help to serve to build up in the brain a viscerotopic map not dissimilar from that of dermatomes as far as the cutaneous structure. That sensory innervating fibers in bones can be important for bone growth is supported by a number of studies in which work load was decreased by surgical denervation, tenotomy, immobilizing casts, or weightlessness. For example, rats with sciatic nerve resection had a higher tibial mineral content and a defective organization of the large callus in fractured tibiae (Nordsletten et al., 1994) as well as a lower bone mineralization (Madsen et al., 1998). A link between bone peripheral sensory and autonomic fibers was indicated by the efficacy of capsaicin, a neurotoxin that eliminates substance P-containing sensory fibers, to blunt sympathectomy-induced bone resorption (Sherman and Chole, 1995).

Summarizing, bone modeling and remodeling are highly regulated processes in the mammalian skeleton. Disruption of the control of modeling may lead to various bone diseases (e.g., osteogenesis imperfecta). One possible mechanism for such delicate control may be related to the ubiquitous and rich sympathetic innervation of all periosteal surfaces and to effects like those reported in these and previous studies.

Acknowledgements

This work was supported in part by grants from the University of Buenos Aires (TM 07), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina (PIP 4156), Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 2350) and Fundación Argentina de Osteología. Drs. Ladizesky, Cutrera, Mautalen and Cardinali are members of the Carrera del Investigador, CONICET.

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