The role of estrogen in hypothalamic regulation of hypothalamus-pituitary-adrenal axis activity, energy homeostasis and bone metabolism

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Chapter 6

Central estrogen signaling regulates bone formation in the rat
Central estrogen signaling regulates bone formation in the rat

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Abstract

Background: Inhibition of sympathetic signaling to bone prevents loss of bone mass after ovariectomy in rodents. As sympathetic activity is controlled by the central nervous system we hypothesized that an interaction between estrogen and the sympathetic nervous system (SNS), relevant for the regulation of bone remodeling, takes place in the brain.

Objective: To determine the effect of subcutaneous (SC) and intracerebroventricular (ICV) administration of 17β-estradiol (E2) on bone remodeling in ovariectomized rats.

Methods: Adult female Wistar rats were ovariectomized and an ICV cannula was placed in the lateral cerebral ventricle. After surgery rats were divided in three groups: 1) VEH: OVX plus ICV vehicle injections (sesame oil) every 4th day (n=6), 2) OVX-SC: OVX plus SC E2 injections (2.0 ug) every 4th day (n=5), 3) OVX-ICV: OVX plus ICV E2 injections (0.02 ug) every 4th day (n=10). After 4 weeks bone mass and bone remodeling was determined by histomorphometric analysis of the tibia. Data were analyzed by ANOVA.

Results: Bone volume was not different between the groups after 4 weeks (p=0.119), but bone formation rates were lower in OVX-SC and OVX-ICV as compared to VEH (p=0.019).

Conclusion: Both subcutaneous and intracerebroventricular administration of estrogen reduces bone formation rate after ovariectomy, suggesting that part of the effect of estrogen on bone remodeling is mediated via the central nervous system.

Key words: estrogen, bone volume, bone resorption, bone mass
Central estrogen reduces bone formation

Introduction

Osteoporosis is characterized by a decrease in bone mass and impaired bone integrity. Bone mass is maintained by the balance between osteoblastic bone formation and osteoclastic bone resorption [1]. Postmenopausal status is an important risk factor for accelerated bone loss [2, 3] and osteoporotic fractures in women [4, 5]. Similarly, ovariectomy (OVX) in rats increases bone resorption, and – although to a lesser extent- bone formation, leading to an overall loss of mainly cancellous bone [6, 7].

A decrease in serum levels of sex steroids, especially estrogen, is thought to induce bone loss, because several studies indicated that OVX induces osteopenia in rats by increasing the bone turnover [6, 7], while estrogen replacement decreases bone turnover [8] [9]. Thus, the bone loss during estrogen deficiency is attributable to an increase in bone resorption, together with a smaller increase in bone formation which results in a net bone loss. Indices of formation as well as resorption are suppressed by replacement of estrogen which results in a net gain, preventing further bone loss [6, 7, 10, 11].

It is generally thought that the effect of estrogen on bone metabolism is a direct local effect within the bone compartment. However, in vitro studies on cultured osteoblasts/oesteoclasts showed rather modest effects of estrogen on osteoblast proliferation, that were only observed when the cells were transfected with a high number of estrogen receptors [12]. These observations suggest additional pathways by which estrogen controls bone metabolism. Groundbreaking studies have revealed an important role for the brain in the regulation of bone metabolism via leptin, neuropeptide Y (NPY) and sympathetic signaling [13, 14].

Estrogen receptors (ER) are expressed throughout the brain. In the hypothalamus, ER expression has been reported in several nuclei, including the paraventricular nucleus (PVN), the ventromedial hypothalamus (VMH), the lateral hypothalamus (LH) and the arcuate nucleus (ARC) [15, 16]. In the brain estrogen interacts with leptin and NPY, both known to be involved in neural regulation of bone remodeling. However, whether estrogen signaling in the brain is also involved in the neural regulation of bone remodeling is not known. In the present study, we aimed to investigate the effect of centrally administered estrogen on bone metabolism.

Materials and Methods

Animals

Female Wistar rats (Harlan, Horst), housed in a 12-h light–12-h dark schedule (lights on at 0700 h) were used for all experiments. Body weight was between 220 and 280 g. Food and drinking water was available ad libitum. The experiments were conducted with the
approval of the Animal Experimental Committee of the Academic Medical Center (AMC) in Amsterdam.

All animals underwent a bilateral ovariectomy (OVX) or sham operation under anesthesia by a mixture of ketamine/xylazine (100:10 mg/kg IP). With a standard Kopf stereotaxic apparatus, a single intracerebroventricular (ICV) probe was placed into the lateral ventricle. The coordinates for the ICV probe were AP: 0.6mm, lateral: 1.5mm and ventral 3.5mm from the surface of the dura. The animals were divided into three groups: OVX plus ICV vehicle treatment (VEH) (n=5), OVX plus subcutaneous estradiol (OVX-SC) (n=5) and OVX plus ICV estradiol treatment (OVX-IVC) (n=7). Estradiol (20ug/ml; Sigma, St. Louis, USA) was dissolved in sesame oil (Sigma, St. Louis, USA) for injection. Estradiol was injected subcutaneously or ICV, 100 μl (i.e. 2 μg) and 1 μl (i.e. 0.02 μg) respectively, every fourth day for four weeks. The same volume of sesame oil was injected ICV in the vehicle animals. Nine and three days before sacrifice, a single dose of tetracycline was administered by i.p. injection (20 mg/kg, Merck Millipore, Amsterdam, The Netherlands).

Tissue preparation
The right tibia was dissected and freed of soft tissue, and one end of the tibia was cut off and flushed by cold PBS. The tissue was fixated in 4% formaldehyde for 24 hours at room temperature, washed twice with phosphate buffer solution for 30 minutes, dehydrated through graded ethanols and embedded in methylmethacrylate. Five μm thick undecalcified sections were cut with a Polycut 2500S microtome (Reichert Jung, Nussloch, Germany) and stained with Goldner’s trichrome. Tetracycline labels were measured on unstained sections.

Histomorphometry
Histomorphometry was performed automatically using NIS-Elements AR2.10 (Nikon GmbH) at x100 magnification. All histomorphometric indices were defined and calculated according to the ASBMR nomenclature [17]. Trabecular bone volume and bone surface, single and double labeled surface, interlabel thickness and osteoclast number was measured. We calculated labeled surface, mineral apposition rate, bone formation rate and osteoclast surface and volume.

Statistical analysis
Data are presented as mean ± SEM. All results were initially analyzed by analysis of variance (ANOVA). When appropriate, further post-hoc tests were performed with Fisher LSD test.
Results

As expected, subcutaneous and ICV E2 treatment reduced body weight gain compared to vehicle, although the effect was less pronounced for the E2-ICV than for the E2-SC group (P=0.017, ICV vs VEH; P<0.001, SC vs VEH) (Figure 6.1).

Bone volume was not significantly different between the groups after 4 weeks of treatment (p=0.119) (Figure 6.2A). However, bone formation rate was lower after E2-SC and E2-ICV compared to OVX (E2-SC 0.2082±0.117 μm3/μm2/day, E2-ICV 0.2998±0.071 μm3/μm2/day, OVX 0.7358±0.168 μm3/μm2/day, p=0.019) (Figure 6.2B). Post-hoc analysis showed that both SC and ICV treatment with E2 significantly decreased bone formation rate when compared to the vehicle treatment group, and there was no significant difference between the two E2 treatment groups (OVX vs E2-SC p=0.014, OVX vs E2-ICV p=0.015, E2-SC vs E2-ICV p=0.618). Mineral apposition rate (MAR) was not significantly affected by treatment, either in the trabecular bone or in the cortical bone (P=0.141 and P=0.108, respectively) (Figure 6.2C and 6.2D). In addition, we did not find significant changes in osteoclast number, whether expressed per surface or per volume (P=0.198 and P=0.214, respectively) (Figure 6.2E and 6.2F).
To exclude leakage of estradiol from the cerebroventricular compartment to the systemic circulation, we measured plasma estradiol concentration 15 min, 45 min, 75 min, 135 min, 1 day, 2 days and 3 days after an ICV bolus of estradiol. No significant differences were found between the estradiol and vehicle treated groups (\textit{time} effect p=0.001, \textit{group} effect P=0.436, \textit{time} *\textit{group} effect P=0.72) (Figure 6.3).

\textbf{Figure 6.2} The effect of SC and ICV E2 treatment on several parameters of bone metabolism in ovariectomized rats. (A) bone volume; (B) bone formation rate (BFR); (C) and (D) mineral apposition rate in trabecular and cortical bone; (E) and (F) osteoclast number expressed per surface or per volume. N = 5 – 7 animals per group.
Discussion

In the present study, we found that not only systemic, but also central treatment of OVX animals with estrogen decreases bone formation rate. Although the dose used for the ICV administration was 100 times less than that of the SC injections the ICV effect on BFR was similar to that of the SC injections. This result clearly indicates that estrogenic modulation of bone formation is at least partly mediated by estrogen signaling in the central nervous system. Previous studies indicated that the reduction in bone formation was caused by a suppression of bone resorption which was not fully compensated for by an increased bone formation rate [18-21]. Thus, the estrogen treatment, either peripherally or centrally, most likely decreases OVX induced bone resorption.

Estrogens are important endocrine regulators of skeletal growth and maintenance, as demonstrated in both animal models and human studies [12, 22, 23]. A decline of endogenous estrogen, induced either by OVX in animal models or by menopause in women, reduces bone mass, whereas estrogen replacement reverses it [7] [24, 25]. The physiological effects of estrogen are mainly exerted via the two classic nuclear estrogen receptors (ERs), ERα and ERβ, which are ligand-activated transcription factors. Previous studies using transgenic mouse models showed that signaling via ERα protects against OVX-induced trabecular bone loss [24, 26, 27]. ERα is widely expressed in the hypothalamus, especially in the ventromedial-hypothalamus (VMH) and arcuate nucleus. Estrogen and leptin receptors are co-localized in neurons within these areas [28], while leptin signaling is well known to modulate bone metabolism [29]. These findings highlight the VMH and ARC as potential brain sites for estrogenic modulation on bone formation. Ducy et al [29] first provided evidence that the central nervous system regulates bone mass by showing that leptin-deficient mice have a higher bone mass, and that this
phenotype could be reversed by ICV injections of leptin [29]. Furthermore, in mice lacking the β2-adrenergic receptor, the central bone-reducing effects of leptin were blunted, demonstrating that the central effects of leptin on bone mass may be mediated by the sympathetic nervous system [30]. Of interest, both efferent and afferent fibers have been shown to innervate bone tissue [31].

We did not detect any change in bone volume, mineral opposition rate or osteoclast indices, neither after systemic nor after ICV E2 treatment. The lack of SC effects seems inconsistent with previous studies. However, the dose, group size and treatment period in the present study were different from previous studies. For example, Wronska et al [1988] found that estradiol treatment (10ug, 25ug and 50ug/kg) for 35 days increased trabecular bone volume while it decreased bone formation rate [32]. Yeh et al [1997] showed bone density and total bone volume to be changed by treatment with 10ug/kg estradiol for 6 weeks [33]. In order to unmask more subtle effects of SC and/or ICV estrogen signaling on bone metabolism, probably longer periods of treatment and/or higher doses will be needed in future experiments.

To conclude, the estradiol-induced decrease in bone formation rate is at least partly mediated by the central nervous system.
Central estrogen reduces bone formation

Reference


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