

COMMENTARIES

Is Menopause Getting on Your Nerves?

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Commentary on: Burt-Pichat B, Lafage-Proust MH, Duboeuf F, Laroche N, Itzstein C, Vico L, Delmas PD, Chenu C. Dramatic decrease of innervation density in bone after ovariectomy. *Endocrinology* 2005 Jan;146(1):503-10.

That ovariectomy and the estrogen depletion it causes induce bone loss may be one of the best described phenomena in bone physiology. However, the molecular mechanisms underlying this pillar of both clinical and experimental biology are still elusive. Although much awaited, studies of mouse models with disrupted estrogen receptors have not shed as much light as expected, because they often report conflicting data and emphasize the complexity of this hormonal system rather than clarifying its role in bone pathophysiology (1,2). Most importantly, such studies have failed to explain how estrogen deficiency can increase the number of osteoclasts and induce bone loss, leaving our field in search of more complex/indirect mechanisms. In a recent paper, Burt-Pichat et al. (3) proposed that the link between gonadal failure and bone loss could be mediated, at least in part, by a neural-dependent mechanism, opening yet another realm of investigation.

Over the past few years, a growing body of evidence has established that the nervous system and neuromediators are involved in the regulation of bone homeostasis. In most cases, this area of bone biology is still in its infancy. For instance, some regulatory loops, among them the regulation of bone formation by neuropeptide Y receptors, have been defined centrally and are now in search of a direct effector on bone cells (4,5). Others have identified specific

neuropeptide receptors and/or the effect of neuropeptides or neuromediators on bone cells *in vitro* or *in vivo*, but still miss several links of the corresponding regulatory loops (6,7). Lastly, one physiologic cascade has been defined that regulates bone formation by osteoblasts following leptin-dependent central control of sympathetic signaling via β 2-adrenergic receptors present on these cells (8,9). The biomedical relevance of this cascade was recently illustrated by retrospective studies showing that treatment of postmenopausal women with β -blockers decreased risk of fracture (10,11).

Drawing on these observations and on the well-described fact that bone is among the best innervated tissues of the body, Burt-Pichat *et al.* (3) hypothesized that ovariectomy-induced bone loss might be associated with a decrease in bone innervation. To test this hypothesis, the authors compared the density of the nervous network in tibiae of ovariectomized (OVX) and sham-operated rats, using quantitative image analysis of immunostaining for two general nerve markers, NF200 and SY, which mark the neurofilaments present in all nerve cells and synaptic vesicles, respectively. In both primary and secondary spongiosa, the presence of these two markers was significantly decreased in OVX rats, compared with sham-operated rats. This was a tissue-specific effect (*i.e.*, such a decrease was not observed in skin or muscle). In an effort to further characterize the population of nerve fibers affected following ovariectomy, the authors then used the same approach to monitor glutamate (Glu) expression, as this neuromediator is preferentially released by sensory nerves and has been shown to affect bone cell activity (6,12). Again, OVX rats showed a

decreased presence of Glu positive nerves, compared with Sham-operated rats. These experiments thus establish that ovariectomy is indeed associated with a decrease in bone innervation, and more specifically, with a decrease in sensory nerve fibers.

At this point, one must remain cautious, however, and acknowledge that the conclusions of this study leave many questions to answer. One of the most important will be to determine whether ovariectomy-induced decrease in innervation is indeed a cause for bone loss or a parallel event. That ovariectomy and sciactomy both decrease nervous network density in bone, as well as bone mass, is certainly a strong correlative argument; however, formal experimental evidence of a causal effect on bone cells is needed. As a matter of fact, addressing this particular point might also be critical in determining whether one or both sides of bone remodeling are affected by this process, to what extent, and how. Indeed, a simple mechanism would be that depletion in one type of neuronal network affects bone

resorption, whereas another regulates bone formation; however, many other combinations are possible. One way to address this question would be to better define whether nervous connections other than glutamatergic innervation are also affected by ovariectomy. In particular, it would be interesting to monitor the response of β -adrenergic neurons, as this network is known to control bone homeostasis *in vivo* (9). Lastly, it will also be important to define the mechanism leading to nerve disappearance and to determine whether the decreased nerve density observed results from a direct or indirect effect of estrogen depletion. Not only, as the authors rightly indicate, is it not known whether and which estrogen receptors are expressed in the nerve affected, but the question will be whether estrogen depletion acts directly on these cells or via other relays. Reexamining mouse models of estrogen receptor deficiency in this new light might well reveal some unexpected answers to these questions.

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