REVIEW ARTICLE

SKELETAL EFFECTS OF OXYTOCIN AND VASOPRESSIN

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ABSTRACT

Over the last decade, the relevance of the pituitary-bone axis has been recognized. Oxytocin (OXT), arginine vasopressin (AVP), growth hormone (GH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), adrenocorticotropic hormone (ACTH) and prolactin have a dominant action on the skeleton as demonstrated by the fact that genetically modified mice lacking their ligands or receptors exhibit a skeletal phenotype, although the primary target organs remain unaltered. Unraveling these new mechanisms of action of the pituitary hormones has paved the way for new therapeutic opportunities in the treatment of osteoporosis. Here, we summarize the action and interaction of OXT and AVP, closely related small peptides that modulate reciprocal secretion and receptor expression on bone cells, in the physiologic context of the skeletal homeostasis.

Introduction

Nonapeptides oxytocin (OXT) and arginine vasopressin (AVP) have a highly conserved structure and differ from each other by only two amino acids. They are produced as prepro-hormones in magnocellular neurons of the supraventricular and paraventricular nuclei in the hypothalamus, in association with carrier proteins, namely neurophysin I and II respectively.¹ It has long been believed that OXT only controlled parturition and milk ejection. However, several studies have shown that the biological function, the localization and regulation of OXT and its receptor (OXTR) were more widespread than expected.² OXT receptors are expressed in many tissues such as pituitary, kidney, ovary, testis, thymus, heart, vascular endothelium,
bone, muscle, pancreas, fat and in different types of tumor cells. Several results suggested important roles for OXT in pituitary function, fertility, T-cell function, cardiovascular control, muscle growth and the development of some tumor cells.

OXT is involved in a wide range of social behaviors. Its intranasal administration has proven effective in improving confidence, positive communication, socialization and positive response to stress caused by social interactions. OXT also controls food intake, mainly carbohydrates, through a centrally mediated action since intracerebroventricular OXT injection can reverse the overfeeding behavior observed in Oxt−/− and Otr−/− mice.

Arginine vasopressin (AVP) is well-known as the antidiuretic hormone which plays a key role in water balance by promoting reabsorption of H2O molecules through its action on AVPR2 receptor in the kidney. Physiologically, its synthesis and release are finely tuned by plasma osmolality, so that only 1–2% increases of Na+ concentration in serum strongly induce the transcription of AVP gene in the hypothalamus. The deficiency of AVP leads to the development of the diabetes insipidus, a disease characterized by an increase in water intake to maintain water balance, as long as the thirst sensation in these patients is not compromised. In addition to AVPR2, there are two other receptor subtypes that mediate AVP actions: namely AVP1a receptor (AVPR1a) and AVP1b receptor (AVPR1b), which are known to centrally mediate AVP effects on aggressive behavior. Studies in animals have found that oral administration of an AVPR1b antagonist produces a reduction in the number of defensive bites and in the duration of offensive aggression when mice face a threatening predator in the cage. Similar to the opposing centrally-mediated actions of OXT and AVP, the respective actions of the two hormones on bone are opposed.

**Anabolic Actions of OXT on the Skeleton**

There is a profound bone phenotype in mice in which the genes for OXT or its receptor had been deleted. These mice develop low turnover osteoporosis that worsens with age in both sexes. Histomorphometry and microCT analysis reveal a dramatic decrease in vertebral and femoral bone volume fraction (BV/TV) and reduction in bone formation rate. These defects are also noted in heterozygous mice. Furthermore, this effect is not centrally mediated since intracerebroventricular injection of OXT in mice did not affect bone remodeling.

Both osteoblasts and osteoclasts express OXTRs. Ex vivo cultures of osteoblasts from Oxt−/− and Otr−/− mice showed fewer mineralized nodules and decreased expression of several master genes involved in osteoblast differentiation compared with cells from wild type littermates. When osteoblasts were treated in vitro with recombinant OXT (rec-Oxt) they showed increased trend toward differentiation, particularly by upregulating Bmp2 and Atf4 expression.

The action of OXT is triggered by OXTR internalization and its translocation to the nucleus through β-arrestin (Arb). In osteoblasts, OXTR interacts with Rab5, consequently binds to the karyopherin transportin-1 (Tnpo1), which mediates the transport to the nucleus. Accordingly, Oxr intracellular trafficking is blocked by
knocking down Arrb or Tnpo1 and the up-regulation of osteoblast differentiation genes is blunted.

Rec-Oxt treatment down-regulates osteoprotegerin (Opg) and increases Rankl expression in osteoblasts, thus resulting in enhanced Rankl/Opg ratio which stimulates osteoclast differentiation. Rec-Oxt treatment also activates Nfkbeta and Mapk signaling, but inhibits bone resorption by triggering cytosolic Ca\(^{2+}\) release and nitric oxide synthesis. The increased osteoclastogenesis, despite the temporary decreases in bone resorption, physiologically serves as a reservoir for the cyclic availability of osteoclast precursors. This effect may lead to an increase in serum calcium and subsequent intergenerational transfer of calcium for mineralization of the offspring’s skeleton during the last phase of pregnancy and after parturition. In human, the skeleton of the mother loses ~120 g of calcium during the last phase of pregnancy and lactation, in favor of the fetal and postnatal bone growth, which corresponds to a reduction of 3–10% in bone mineral content in lumbar spine, hip, femur, and distal radius in trabecular and cortical bone. This rapid bone loss, at 1–3% per month, is also accompanied by high PTHrP and low estrogen levels to facilitate the maternal hyper-resorption and intergenerational calcium transfer. However, after a 6-month period of acute bone loss, the mother's skeleton is rapidly restored.

When this sequence is out of balance, pregnancy- and/or lactation-induced osteoporosis ensues. The mechanism through which OXT orchestrates the process of intergenerational calcium transfer has remained unknown until the pivotal role of OXT in maintaining high rate of bone cell activity, but controlling the amount of bone resorbed, was revealed.

**OXT and OXTR Synthesis are Regulated by Estrogen**

As in several tissues, 17β-estradiol induces OXT production in osteoblasts via a membrane receptor and Extracellular Signal-regulated Kinase (Erk) phosphorylation, a different pathway that does not involve genomic actions through the estrogen-responsive element (ERE). This was proven by using a relatively cell-impermeant analog of 17β-estradiol, the 17β-estradiol-BSA-conjugate, which was equally effective in increasing Oxt mRNA within 2 h and Erk phosphorylation within 3 min. Moreover, by using the Mapk kinase (Mek1/2) inhibitor PD98059, which prevents Erk phosphorylation, the 17β-estradiol-dependent Oxt mRNA increase was blunted. Overall, these results suggest a non-genomic, Erk-dependent pathway for the induction of OXT by estrogen. On the contrary, only 17β-estradiol, but not the cell-impermeant estradiol-BSA-conjugate, increased Otxr mRNA within 6 h, suggesting that the estrogen-mediated OXTR induction follows the classical genomic mechanism with a slower time course. Accordingly, Otxr mRNA up-regulation was not affected by the PD98059 that blocks Erk phosphorylation.

Similar to OXT, other studies showed that some pituitary hormones, namely TSHβ and ACTH, are synthesized in non-pituitary tissues, such as in bone, suggesting that a local autocrine circuit may serve to coordinate the activity of neighboring bone cells.
AVP Negatively Regulates Osteoblasts and Stimulates Osteoclasts

Osteoblasts and osteoclasts express both AVP receptors, AVPR1α and AVPR2. To prove their functionality, Erk phosphorylation (pErk) was analyzed in response to AVP treatment in the presence or absence of SR4905, an Avpr1a inhibitor. In both osteoclasts and osteoblasts, AVP stimulated pErk activation, but this intracellular signal was only attenuated and not completely blunted by SR49059, suggesting that AVPR2 was also expressed in bone cells. Treatment with AVP in osteoblasts from Avp1α+/− mice inhibited the expression of several master genes regulating osteoblast differentiation, confirming functionality of AVPR2. In vivo experiments further showed that SR49059 injection in wild type mice resulted in increased BV/TV, mineral apposition rate and bone formation rate, whereas resorption parameters, including osteoclast surfaces and serum C-telopeptide levels were significantly reduced compared with untreated mice. By analyzing the bone phenotype of Avp1α−/− mice, a significant increase of bone mass was observed, mimicking the pharmacologic inhibition of AVPR1α by SR49059.

Moreover, when wild type mice were treated with SR49059, a significant decrease in osteoclast surface was observed. Accordingly, ex vivo culture of bone marrow stromal cells from Avpr1α−/− showed a dramatic reduction in osteoclast formation in the presence of Rankl, accompanied by decreased expression of key master genes for osteoclast differentiation, namely Cfms, Rank, Nfatc1, and Integrin β3 (Intb3), compared with wild type mice. Overall, these data suggest that AVP has a pro-resorptive action, since the ablation of AVPR signaling, pharmacologically or genetically, inhibits osteoclast formation and bone resorption. The direct action of AVP on the skeleton had never been explored before, despite evidence showing that hyponatremia, which is invariably accompanied by elevated levels of circulating AVP, is associated with low bone mass and high risk of fracture. Therefore, a new therapeutic strategy based on AVPR inhibitors could target hyponatremia, also resulting in prevention of bone loss.

OXT and AVP are Potent Regulators of Skeletal Integrity and Cooperate to Control the Formation of New Bone

OXTR and the three AVPR isoforms, namely AVPR1α, AVPR1β, and AVPR2, belong to the G protein-coupled receptor family, and their ligands, OXT and AVP, are both nonapeptides with a single disulfide bridge, differing only by two substitutions in the amino acid sequence. Their similarity led to the question: could these ligands cross-react with the respective receptors expressed on osteoblasts? Consistent with its function, AVP inhibited osteoblast differentiation in bone marrow stromal cell from Oxtr+/- mice. However, this inhibitory action was also observed in osteoblast cultures from Oxtr−/− mice, suggesting that OXTR was not necessary for AVP to exert its anti-osteoblastic function. To investigate whether deleting Oxtr could modify the bone phenotype of Avpr1α−/− mice, which display high bone mass, we analyzed double-mutant Avpr1α−/−/Oxtr−/− mice. Histomorphometry of spinal trabecular bone showed that the increase in BV/TV was less pronounced
in the double-mutant mice than that observed in Avpr1α−/− mice, suggesting that the absence of OXTRs can rescue the Avpr1α−/− bone phenotype. This also confirmed that the two receptors have opposing effects in regulating bone mass (Figure 1). Taken together, these studies suggest that it might be clinically relevant to measure plasma OXT and AVP in patients in whom osteoporosis accompanies hyponatremia.

![Figure 1. Oxytocin and Vasopressin have opposing effects in regulating bone mass.](image)

**Acknowledgments**

This study was supported by NIH Grant AG40132, AG23176, AR06592, DK80459, DK113627, and AR67066 (all to M.Z.). A.Z. is supported by the Italian Space Agency and the Italian Ministry of Education, Universities and Research.
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