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Postmenopausal osteoporosis is associated with the regulation of SP, CGRP, VIP, and NPY

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ABSTRACT

Estrogen deficiency is the main factor underlying postmenopausal osteoporosis. A large number of neuropeptides, which regulate skeletal metabolism, potentially represent a regulatory pathway for the pathogenesis of osteoporosis. The aim of this study was to explore factors involved in the regulation of bone-related neuropeptides and their association with estrogen deficiency and bone metabolism. Thirty adult female Sprague-Dawley (SD) rats were randomly divided into a control group with sham surgery (n = 15) and an ovariectomy group with bilateral oophorectomy (n = 15). After 16 weeks, serum estrogen was reduced,CTX-1 was increased and P1NP was not significantly affected in the ovariectomy group and a model of osteoporosis was established. We then investigate the gene expression and protein levels of a range of neuropeptides and their receptors, including substance P (SP) and tachykinin receptor 1 (TACR1), calcitonin gene-related peptide (CGRP) and calcitonin receptor-like (CALCRL), vasoactive intestinal polypeptide (VIP) and receptor 1 and 2 (VPAC1, 2), neuropeptide Y (NPY) and receptor Y1 and Y2, in the brain and femora. Ovariectomy reduced TACR1, CGRP, CALCRL, NPY, NPY Y2 in the brain, but increased TACR1 and decreased SP, CALCRL, VIP, VPAC2 in the bone. Collectively, our data revealed that the pathogenesis of postmenopausal osteoporosis is associated with the regulation of SP, CGRP, VIP, and NPY. These novel results are of significant importance in the development of neuropeptides as therapeutic targets.

1. Introduction

Characterized by a loss of bone mass and quality with a greater risk of fragility fractures [1], osteoporosis afflicts a significant proportion of the aged population, particularly postmenopausal women. Osteoporosis is responsible for over 1,500,000 fractures each year in the United States of America, with most cases occurring in postmenopausal women [2]. These fractures, from an individual perspective, bring about a poor quality of life, as well as a heavy personal economic burden [3,4]. Furthermore, these fractures, from a social perspective, consume substancial social resources for direct medical costs and an indirect increased need for life care [5].

Postmenopausal osteoporosis is mainly caused by an abrupt cessation of estrogen after the menopause [6]. Estrogen deficiency leads to series of metabolic alterations that break the balance between bone formation and bone reabsorption, and eventually result in a rapid reduction in bone mineral density [6]. While, many regulatory mechanisms have been proven to be involved in the complex process of postmenopausal osteoporosis, none of these have allowed us to come up with strategies that can efficaciously prevent and cure hormone-deficient osteoporosis [7]. Since the pathogenesis of postmenopausal osteoporosis has not been fully elucidated, there is a very real need to explore the development of new pharmacological targets for the treatment of postmenopausal osteoporosis.

Neuropeptides are neuronal signaling peptides involved in brain activity and other parts of the body which modulate a wide range of physiological functions, including skeletal metablism [8]. Neuropeptides regulating skeletal metabolism repreesnt a potential regulatory pathway for the pathogenesis of postmenopausal osteoporosis.

The central and peripheral regulation of neuropeptides in the skeleton is mediated by their receptors. There are four types of bone-related neuropeptides: substance P (SP) suppresses osteoclasts and promotes osteoblasts through the tachykinin receptor 1 (TACR1) in the cell membrane and cytoplasm [9,10]; calcitonin gene-related peptide

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Table 1
Details of PCR primers for real-time quantitative PCR.

Gene symbol	Product length (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
SP	220	ATGAAAATCCTCGTGGCGGT	CAGCATCCCGTTTGCCCATT
TACR1	82	CCAACAGGACTTATGAGAAAGCGTA	GCGTAGCCGATCACCAGTAGAG
CGRP	134	AGTTCTCCCCTTTCCTGGTTGT	CAGTAGGCGAGCTTCTTCTTCA
CALCRL	325	GATGGGCTGTAACTACTTTTGGA	GAGATTGGATTCTGCTTGGTGT
VIP	278	CAGATAGGCTGCCGTGTTACA	TGGAAATCAAGCACTCCGTTAG
VAPC1	217	AAACTACGGCCACCCGACAT	CACCATTGAGGAAGCAGTAGAGGA
VAPC2	203	AGTACAAGAGGCTCGCCAAGT	CCTTCTTTTCAGTTCACGCTGT
NPY	232	GTGGACTGACCCTCGCTCTAT	GGGCATTTTCTGTGCTTTCTC
NPY Y1	299	CCACAATCTGCTGTTCCTGCTCT	CACAGATGTAGCCTGGGACCGTA
NPY Y2	183	AGCCTTTCCACCCTGCTAAT	GCTGACTGCAAACACCACTACC
GAPDH	96	CAACGGGAAACCCATCACCA	ACGCCAGTAGACTCCACGACAT

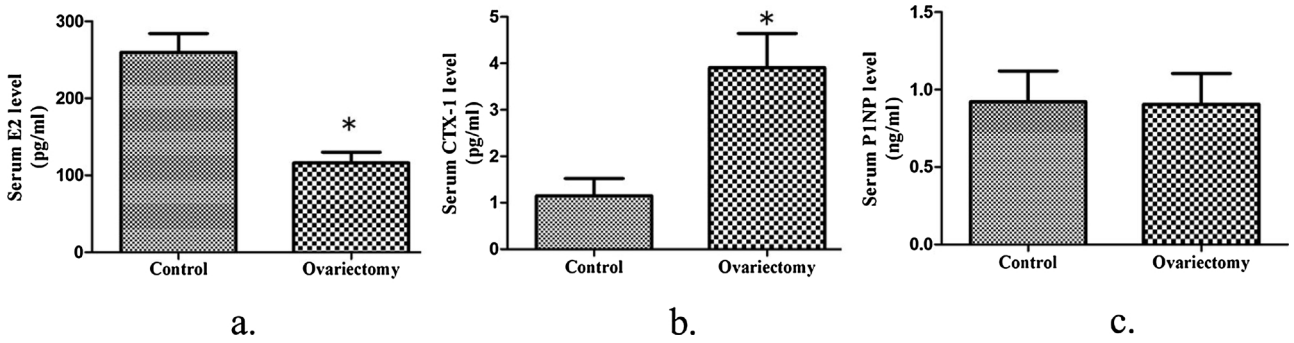

Fig. 1. Effects of ovariectomy surgery on serum estrogen, CTX-1 and P1NP levels. Serum was measured by ELISA assay. Compared with the control group, serum estrogen levels in the ovariectomy group were significantly reduced (a.). Ovariectomy also remarkably increased CTX-1 levels in serum (b.), but failed to affect serum P1NP (c.). Control group *vs.* ovariectomy group $^{*}P < 0.05$.

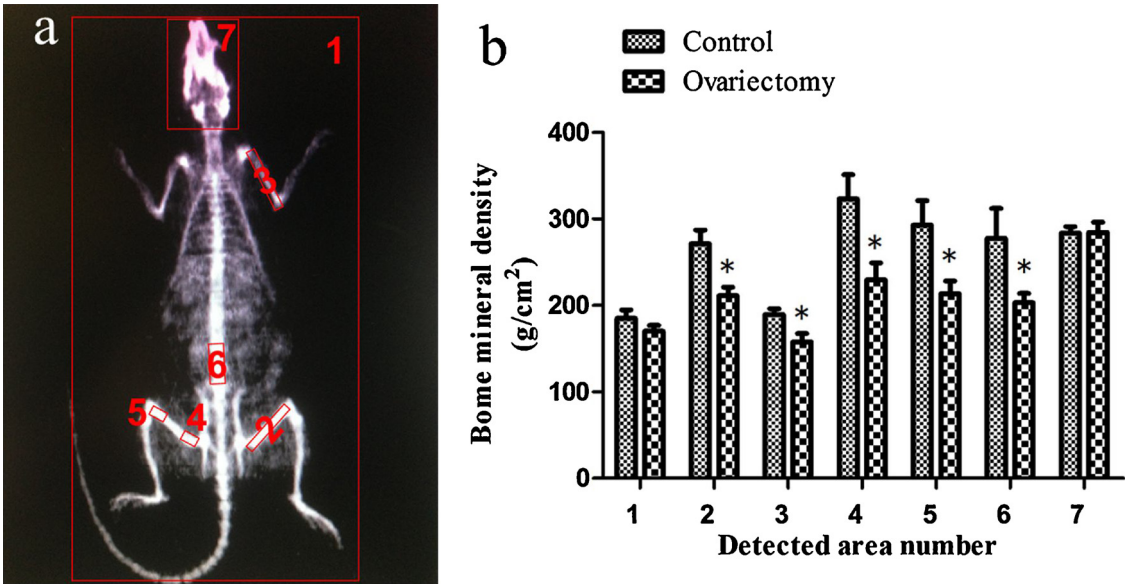

Fig. 2. Effects of ovariectomy surgery on bone mineral density. Bone mineral density (BMD) was determined by dual X-ray absorptiometry in seven areas (Fig. 2a), including (1) whole body, (2) thighbone, (3) arm bone, (4) epiphyseal of proximal thighbone, (5) epiphyseal of distal thighbone, (6) fourth and fifth vertebra lumbalis and (7) head. Data (Fig. 2b) revealed that compared with the control group, the BMD of the thighbone, arm bone, epiphyseal of proximal thighbone, epiphyseal of distal thighbone, and the fourth and fifth vertebra lumbalis were lower in the ovariectomy group. In the whole body and head area, however, there was no significant difference in terms of BMD. Control group *vs.* ovariectomy group $^{*}P < 0.05$.

(CGRP) stimulates osteogenesis and restrains bone reabsorption by the combination and modification of calcitonin receptor-like (CALCRL) and the regulated expression of runt-related transcription factor 2 (RUNX2) and the Osterix gene [11–13]; vasoactive intestinal polypeptide (VIP) regulates the activity of osteoblasts and controls the movement of osteoclasts through VIP receptor 1 (VPAC1) and 2 (VPAC2) [14] and neuropeptide Y (NPY) regulates cAMP synthesis in osteoblasts to affect osteogenesis through NPY receptor type 1 (NPY Y1) and type 2 (NPY Y2) [15,16]. Many studies also suggested that SP, CGRP, VIP and NPY not only directly affect different types of osteocytes, but also play pivotal roles in controlling bone mass centrally [17,18].

The hypothalamic-pituitary-ovarian axis is the female sex gland axis in which the nervous system and estrogen secretion interact with each other [19]. Previous research into the interplay of the nervous system