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Plasma orexin A levels in recently menopausal women during and 3 years following use of hormone therapy

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ABSTRACT

Objective: Alterations in sleep quality and metabolism during menopause are improved by menopausal hormone therapy (MHT). The mechanisms mediating these effects remain unclear. Orexin A (OxA) is a neuro-peptide that regulates sleep/wakefulness, food intake and metabolism. This study examined changes in plasma OxA levels during and after treatment in women from the Kronos Early Estrogen Prevention Study (KEEPS).
Methods: KEEPS randomized women within three years of menopause to: oral conjugated equine estrogen (o-CEE, 0.45 mg/day), transdermal 17β estradiol (t-E2, 50 μg/day), or placebo pills and patches for four years. Plasma OxA levels were measured by enzyme immunoassays in fasting blood samples collected annually from KEEPS participants at Mayo Clinic during and three years after MHT. Changes in menopausal symptoms and plasma OxA levels were assessed for treatment differences.
Results: During treatment, OxA levels increased more in women randomized to o-CEE compared with the other groups. Women randomized to either form of MHT demonstrated smaller increases in BMI than those on placebo. Insomnia severity decreased similarly among treatment groups. However, neither changes in sleep nor changes in BMI correlated with changes in plasma OxA levels. Changes in waist circumference correlated positively with changes in plasma OxA levels three years after discontinuation of study treatments.
Conclusions: Although OxA levels increased only in women randomized to o-CEE, these changes did not correlate with changes in sleep quality or BMI. The modest correlation of OxA levels with waist circumference once study treatments were discontinued suggests that OxA may be modulated through multiple intermediary pathways affected by metabolites of 17β-estradiol.
Clinical Trial Registration for KEEPS: NCT00154180

1. Introduction

Orexin A (OxA or hypocretin 1) and Orexin B (OxB or hypocretin 2) are neuropeptides implicated in regulation of sleep/wakefulness, food intake and energy expenditure [1]. OxA and OxB are produced from a single protein precursor, prepro-orexin, in the lateral hypothalamus, and share 46% amino acid sequence homology [2]. Both peptides are endogenous ligands to G-protein coupled receptors; Orexin 1 (Ox1R) and Orexin 2 (Ox2R), with OxA having a 10 fold stronger affinity to the Ox1R [1]. Ox1R is distributed mainly in cortical regions and brainstem nuclei, with OxA neurons having dense projections to brain regions associated with control of arousal and metabolism [3].

Although the main focus of research concerning OxA has been in the central nervous system, OxA (and not OxB) is detected in the peripheral circulation. The Ox1R is expressed in peripheral tissues, through which OxA regulates insulin and glucagon secretion, affects intestinal motility and energy metabolism [4].

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The measurement of plasma OxA remains a challenge given that levels in the peripheral circulation are 1/5 to 1/8 those measured in the cerebrospinal fluid [5]. However, plasma OxA increased with age, with the highest levels measured in persons >60 years of age [6]. Sex differences in plasma OxA have yet to be explored in humans, although evidence in rats suggests a sexual dimorphic expression of orexin receptors [7].

When considering women-specific life events, circulating levels of OxA were lower in women with gestational diabetes mellitus [8] and polycystic ovarian syndrome [9] compared to age-matched women without these conditions. In addition, plasma OxA was lower in overweight and obese women than non-obese women, and showed a negative correlation with plasma leptin concentrations [10]. Leptin is a cytokine produced by adipose tissue that suppresses food intake and regulates sleep. Sleep restriction increased plasma leptin concentration and food intake in healthy young men [11]. These associations have yet to be examined in women.

Little is known about how plasma OxA changes with menopause and the association of plasma OxA with menopausal symptoms [12]. At menopause, women experience disturbances in sleep [13], declines in energy metabolism and expenditure and increases in central adiposity [14]. Menopausal hormone therapy (MHT) is beneficial in treating menopause-related symptoms. However, the impact of MHT on plasma OxA is unclear, and little is known about OxA fluctuations after MHT discontinuation.

The objective of this study was to compare the effects of two formulations of MHT with placebo on plasma levels of OxA in healthy, recently menopausal women during and following discontinuation of study treatment. In addition, the study explored the relationship of OxA with leptin, clinical and self-reported sleep and appetite/physical behaviors. Given findings of increased plasma OxA in patients with sleep restriction, narcolepsy or obstructive sleep apnea, and the reduction in sleep quality during menopause that is alleviated by MHT, we hypothesized that OxA would be reduced by MHT.

2. Methods

2.1. Protocol approval

This study was approved by the Mayo Clinic Institutional Review Board (IRB protocol # 2241). All participants gave written informed consent.

2.2. Participants

This study evaluated a subgroup of white participants (n = 74) from the Kronos Early Estrogen Prevention Study (KEEPS; NCT00154180) Cognitive and Affective Ancillary study (KEEPS-Cog) at Mayo Clinic, Rochester MN [15]. KEEPS was a randomized, double-blind, placebo-controlled trial studying the effects of MHT, started within 3 years of menopause, on progression of cardiovascular disease. [16] Briefly, women were included in KEEPS-Cog if their last spontaneous menses occurred between 42 and 58 years of age with >6 months to <3 years of amenorrhea, plasma follicle-stimulating hormone $\geq$35 ng/mL and/or E2 < 40 pg/mL. Women were excluded if they had a coronary arterial calcification (CAC) score of >50 Agatston Units, a history of cardiovascular disease, a body mass index (BMI) > 35 kg/m^2, low-density lipoprotein cholesterol (LDL) > 190 mg/dL, triglycerides (Tg) > 400 mg/dL, blood glucose > 126 mg/dL, uncontrolled hypertension (systolic blood pressure >150 mmHg and/or diastolic blood pressure >95 mmHg), current or recent (6 months) use of cholesterol-lowering medications (statins, fibrate, or >500 mg/day niacin) and if they smoked more than 10 cigarettes per day.

2.3. Study design

Participants were randomized to one of the following: oral conjugated equine estrogens (o-CEE; Premarin, 0.45 mg/day) plus a placebo transdermal patch, transdermal 17β-estradiol (t-E2; Climara 50 μg/day) plus a placebo pill, or placebo (PL) pills and patch. Women in the active treatment groups also received oral micronized progesterone (Prometrium, 200 mg) for the first 12 days of each month. A random number table was used to assign treatment. Study drugs were supplied to centers identified only by the women's ID number, with both research participants and investigators blinded to treatment.

Treatment was given for four years with visits prior to randomization (baseline) and then annually at 12, 24, 36 and 48 months. In a follow-up study, participants were studied three years after the cessation of study treatment and were compared to their final on-treatment measurements (from 48 to 84 months).

2.4. Blood collection and plasma preparation

At each visit (baseline, 12, 24, 36, 48, and 84 months), morning fasting venous blood was collected in tubes containing 7.5% ethylenediamine tetra-acetic acid tri-potassium salt [EDTA (k3)] anti-coagulant. Aliquots of plasma were stored immediately at −80 °C until analysis. The range of storage time for plasma samples was from 2 to 7 years.

2.5. Measurements of plasma orexin a and leptin

OxA was measured in diluted (1:2 in standard diluent supplied by manufacturer) plasma using a commercially available extraction free peptide enzyme immunoassay kit (EIA, Catalog # S-1374; Peninsula Laboratories, California, USA). Each plasma sample was measured in duplicate and the mean of these two measurements was used for analysis. The lowest detection limit of the assay was 0.5 ng/mL with intra- and inter- assay coefficients of variation of <6% and <15%, respectively. The cross-reactivity of OxA with OxB was reported to be 18%.

Leptin was measured in plasma samples collected at baseline and at 48- and 84-month follow-up visits using the human leptin double antibody radioimmunoassay kit (Linco Research, Inc. St. Louis, MO 63304). Intra −assay coefficients of variation were 6.1%, 7.7%, and 6.3% at 39.7, 21.6, 3.8 ng/mL, respectively; inter-assay coefficients of variation of 11%, and 13% at 20.4, and 3.0 ng/mL, respectively.

2.6. Sleep and energy metabolism outcomes

BMI and waist circumference (WC), were used as surrogates for changes in energy metabolism. In addition, information regarding appetite and physical well-being were obtained through the Health Quality of Life (QOL) domain within the Utian Quality of Life Scale. The Utian Quality of Life Scale incorporates sense of well-being into an assessment tool that is specific to the menopausal population [17]. To address changes in sleep quality, participants completed a questionnaire reporting menopausal insomnia symptoms over the last 3 months, ranking them on a 4-point numerical scale as none, mild, moderate or severe (0–3). BMI and WC were measured at baseline and at end of the intervention (48 months), and at 3 years following discontinuation of treatments (84 months). Health QOL responses were collected at baseline, at 18-, 36- and 48-months during study treatment, and at the three year discontinuation follow up visit (84 months). The insomnia severity scale was collected at baseline, 6 months, and then annually during study treatment.