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# Effects of black cohosh and estrogen on the hypothalamic nuclei of ovariectomized rats at different temperatures

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ABSTRACT

*Ethnopharmacological relevance:* *Cimicifuga racemosa* (L.) Nutt. (CR), known as black cohosh, has been used in Europe as a medicinal plant for more than a century and its roots have been widely used for the treatment of menopausal symptoms. Remifemin, the main ingredient in liquid or tablet medications prepared from isopropyl alcohol extracts of black cohosh rhizome, has also been evaluated in clinical studies.

*Objectives:* To observe changes in the expression of the c-Fos protein in the hypothalamic nuclei of four groups of rats—sham-operated group (SHAM), ovariectomized (OVX) group, ovariectomized group treated with estrogen(OVX+E), and ovariectomized group treated with the isopropanol extract of *Cimicifuga racemosa* (OVX+ICR)—and to investigate the mechanisms of black cohosh and estrogen that take place in the hypothalamic nuclei of ovariectomized rats.

*Methods:* Fifty rats were assigned to each of the four groups and placed in incubators at 4 °C, 10 °C, 25 °C, 33 °C, or 38 °C for 2 h. They were then anesthetized, and their brains were removed after heart perfusion. c-Fos expression in the hypothalamic nuclei was evaluated using immunohistochemical methods.

*Results:* In the median preoptic nucleus (MnPO), ventromedial preoptic nucleus (VMPO), and suprachiasmatic nucleus (SCh) of the SHAM group, in the anterior hypothalamic area (AH) and supraoptic nucleus (SO) of all four groups, and in the paraventricular nucleus (PVN) of the SHAM, OVX and OVX+E groups, the c-Fos-positive cell densities all changed in a similar manner: the cell density decreased when the temperature was less than 25 °C and the density increased when the temperature was greater than 25 °C, demonstrating a V-type curve. The c-Fos density was lowest at 25 °C. The other nuclei demonstrated irregular changes. The positive cell densities in the MnPO, AH, and PVN of the SHAM, OVX+E, and OVX+ICR groups were greater than the densities measured in the OVX group at all temperatures, except 25 °C. Positive cell densities in the SHAM, OVX+E, and OVX+ICR groups were greater than the densities measured in the OVX groups in the MPA at 25 °C, in the VMPO at 4 °C, 33 °C, and 38 °C, in the SO at 4 °C, 10 °C, and 38 °C, and in the SCh at 33 °C.

*Conclusion:* Regardless of the temperature, positive cell densities were lower in the MnPO, MPA, VMPO, AH, SCh, SO, and PVN of the OVX groups in comparison with the densities measured in the same sites in the SHAM group. Following the administration of black cohosh and estrogen, the positive cell densities in the OVX groups increased and became closer to, or exceeded, those measured in the SHAM group, suggesting that both drugs may act on the hypothalamic nuclei and have therapeutic effects on menopausal symptoms.



## 1. Introduction

The Perimenopausal syndrome begins with the recession of ovarian function and typically last 3–5 years, affecting the normal, societal, and family lives of most women. Around 80% of perimenopausal women develop clinical symptoms. About one-third of these women develop serious symptoms, and 9% require hospital treatment (Shen and Stearns, 2009). These symptoms include vasomotor, neuropsychiatric, urinary and reproductive system dysfunction. The most common symptoms are hot flashes during the day and sweating at night.

*Abbreviations:* AH, anterior hypothalamic area; MnPo, median preoptic nucleus; MPA, medial preoptic area; OVX, ovariectomized group; OVX+E, OVX group treated with estrogen; OVX+ICR, ovariectomized group of rats that was treated with the isopropanol extract of Cimicifuga racemosa; PB, phosphate-buffered solution; PBS, phosphate-buffered saline; PCD, c-Fos-positive cell density; POA, preoptic area; PVN, paraventricular nucleus; SCh, suprachiasmatic nucleus; SHAM, sham-operated group; SO, supraoptic nucleus; VMPO, ventromedial preoptic nucleus

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Hormone therapy is a specific treatment for short-term climacteric symptoms and the prevention for chronic disease (Seifert-Klauss et al., 2007). However, several clinical trials have indicated an increased risk of breast cancer in association with hormone therapy. Concerns about the safety of hormone therapy have led to more women seeking alternative treatments. A recent study conducted in Sydney found that approximately 54% of menopausal women used one or more forms of complementary and alternative medicine to alleviate symptoms, of which herbal treatment was the most common (Borrelli and Ernst, 2008) and very effective for relieving symptoms (Sluijs et al., 2007).

*Cimicifuga racemosa* (L.) Nutt. (CR), known as black cohosh, is a perennial herb that is native to North America and a member of the buttercup family (Ranunculaceae). Its roots have been widely used for treating menopausal symptoms for more than 50 years in Europe (Margaret, 2009). *American Herbal Pharmacopoeia* states that liquid or tablet forms of medication that are derived from isopropyl alcohol extracts of black cohosh rhizome (remifemin) have been investigated in clinical studies (Herbalust, 2002). Considerable clinical research suggests that both black cohosh and estrogen are equally effective for relieving hot flashes and emotional symptoms in menopausal women, especially anxiety and depression(Nappi et al., 2005; Wuttke, 2006), and the benefit-risk balance about black cohosh is acceptable (Briese et al., 2006). Another study examined the changes in the subjective symptoms of menopause in 2016 Hungarian women who had been treated with an isopropanol extract of black cohosh(Vermes et al., 2005). The average decrease in the Kupperman index after 12 weeks of therapy was 17.64 points ($P<0.001$). Based on the weighted symptom scores, the most favorable changes were decrease in the occurrence of hot flashes ($-6.31$ points), sweating ($-2.86$ points), insomnia ($-2.27$ points), and anxiety ($-2.00$ points; $P<0.001$ for each symptom). In China, our parallel, double-blind, randomized, controlled trial confirmed that remifemin is as good as 2.5 mg/day tibolone for the treatment of climacteric complaints (Bai, 2007). These data show that the isopropyl alcohol extract of black cohosh can effectively relieve menopausal symptoms. Black cohosh can be used as a substitute for sex hormones, particularly by those who are unwilling to accept hormone therapy. However, information regarding the primary mechanisms of black cohosh and estrogen for relieving menopausal symptoms is incomplete. Clarifying the mechanistic action of this drug might help determine the mechanisms that result in perimenopausal symptoms.

As a key area involved in thermoregulation, the hypothalamus is sensitive to local temperature changes in the brain. The preoptic area (POA) and anterior hypothalamic area (AH) in the hypothalamus are the central areas involved in the thermoregulation of homeothermic animals (Hissa, 1990; Bachtell et al., 2003). The the median preoptic nucleus (MnPO), ventromedial preoptic nucleus (VMPO) and the medial preoptic area (MPA) in the the preoptic area of the POA,, and hypothalamic areas, the supraoptic nucleus (SO) and the paraventricular nuclei (PVN) in the hypothalamus also participate in thermoregulation (Cano et al., 2003). The suprachiasmatic nucleus (SCh) is the central structure of the mammalian circadian rhythm system and is responsible for regulating sleep, arousal, hormone levels, metabolism, and reproductive and biological rhythms (Guo et al., 2006; Sujino et al., 2007). Sleep disorders and hormonal and behavioral circadian rhythm disorders are also common in menopausal women. These nuclei are associated with the physical and psychological symptoms of perimenopausal syndrome. Understanding the central mechanisms of adaptability following external temperature changes in ovariectomized rats could help explain menopausal symptoms. At present, no studies have examined the changes that occur in hypothalamic nuclei activity following hormone deficiency.

The Fos protein is widely recognized as a marker of neuronal activity and morphology (Rehman and Masson, 2005). It has become an important way to localize thermoregulating neurons and cells that are activated by acute temperature changes. Therefore, c-Fos immunohistochemistry was used to observe the responses of the hypothalamic neurons in ovariectomized rats in response to different thermal stimuli. The indicated temperatures were selected for the following reasons: 25 °C is the normal room temperature at which both rats and humans feel comfortable, 33 °C is the normal room temperature during the summer, and 38 °C is generally the highest room temperature that is tolerated in the summer and is close to the normal body temperature of rats. Konishi, M. found that rats have a central body temperature of 10 °C or 4 °C that can activate the POA(Konishi et al. 2003) and other thermoregulatory central nervous system neurons (Bratincsak and Palkovits, 2005). In addition, 4 °C and 10 °C are normal temperatures in winter. The duration of stimulation was set to 2 h, during which time most nuclei achieve maximum c-Fos expression (Bratincsak and Palkovits, 2004. The aim of this study was to observe the sensitivity of hypothalamic nuclei to external temperature stimuli and changes in the densities of sensitive cells that occurred in drug- and nondrug-treatment ovariectomy groups in order to determine the mechanisms involved in hypothalamic regulation of body temperature and the possible mechanisms of drug action. These results could provide the theoretical basis for choosing the best treatment.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Animal models

Two hundred healthy adult female Sprague Dawley rats aged 8–10 weeks (purchased from the Laboratory Animal Science Department of Peking University Health Science Center) were used in this study. The rats weighed 210–230 g and were housed in the laboratory at a temperature of $25\pm1$ °C, relative humidity of 40–50%, and a light/dark cycle of 12 h. The rats were exposed to direct light and given free access to water and soy-free feed for 2 weeks, as previously described (Rachon et al., 2008). This study was performed with the approval of the local ethics committee, and all of the experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

#### 2.1.2. Reagents and instruments

Rabbit anti-rat c-Fos monoclonal antibody was purchased from Santa Cruz (USA), and the avidin–biotin complex staining kit for immunohistochemistry was purchased from Beijing Zhongshan Goldenbridge Biotechnology Co, Ltd. The remifemin tablets were produced by Schaper & Brümmer Ltd & Co KG (batch number 824821; Germany). Each tablet contained 20 mg of the crude drug that was extracted using 40% isopropyl alcohol (equivalent to 0.018–0.026 mL), producing an average of 2.5 mg of dried extract. Estradiol (1 mg per tablet) was produced by Bayer Health Care Co, Ltd. (batch number 169 A 11; produced in Guangzhou, China). SPX-80BS-II incubators were purchased from Shang Hai CIMO Medical Instrument Co, Ltd. A Leica 1900 microtome and an Olympus BX51 microscope were also used in this study.

### 2.2. Methods

#### 2.2.1. Establishment of the ovariectomized rat model

After the ovariectomized rats were anesthetized, an incision was made at the midline of the abdomen and the bilateral ovaries