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

Zhang Hui a, Ma Xiaoyan a, Yang Mukun a, Wang Ke b, Yang Liyuan b, Zhu Sainan c, Jia Jing d, Qin Lihua b,*, Bai Wenpei a,***

a Obstetrics and Gynecology Department, Beijing University First Hospital, China
b Anatomy and Embryology Department, Beijing University Health Science Center, China
c Statistics Office, Beijing University First Hospital, China
d Department of Stomatology, General Hospital of Armed Police, China

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, and OVX+E groups were greater than the densities measured in the OVX groups in 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 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, SC, 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.

© 2012 Elsevier Ireland Ltd. All rights reserved.

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.
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 (Slujs 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 167 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 (2.0 points), and anxiety (2.0 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; Bachtel 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 (SCN) 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 ± 1 °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 & Brümmier 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
were resected. These rats were randomly divided into three groups: the ovariectomized group (OVX), OVX+E group (OVX group treated with estrogen), and the ovariectomized group of rats that was treated with the isopropanol extract of C. racemosa (OVX+ICR). Each group consisted of 50 rats. The remaining 50 rats comprised the sham-operated group (SHAM). The SHAM group underwent sham operations—i.e., an incision was made at the midline of the abdomen, their bilateral ovaries were revealed but not resected, and then the abdominal cavity was closed. The rats were given 2 weeks for wound healing after the operation.

2.2.2. Dosage and temperature experiments

The experimental drugs were prepared as follows. The estradiol and isopropyl alcohol extracts of the black cohosh tablets were dissolved in sterile saline after ultrasonic treatment to form a uniform cloudy liquid (Winterhoff et al., 2003). The concentration of the estradiol was 0.2 mg/mL; the concentration of the isopropanol extract of black cohosh was 12 mg/mL (as indicated by the concentration of the crude drug contained in a 20 mg tablet). Fifteen days after the operation, the rats underwent daily gastric lavage from 8:30–9:30 am for 7 day. These rats were treated as follows: SHAM group: 10 mL/kg normal saline; OVX group: 10 mL/kg normal saline; OVX+E group: 0.8 mg/kg estradiol (crude drug). All rats were weighed and the dosage was adjusted according to changes in body weight. Twenty-two days after the operation, the rats in each of the four groups were randomly divided into five temperature groups. Ten rats were assigned to each group and placed in five separate incubators for 2 h. Each incubator was kept at 4 °C, 10 °C, 25 °C, 33 °C, or 38 °C, respectively, at a relative humidity of 60%.

2.3. Section preparation and immunohistochemical staining

2.3.1. Section preparation

Following incubation, each rat was immediately anesthetized with an intraperitoneal injection of 1% sodium pentobarbital (80 mg/kg). The chest was opened to reveal the heart, the right atrium was cut open, a tube was inserted via the left ventricle to the ascending aorta, and the blood was quickly washed with 200 mL of physiological saline. The left ventricle was perfused with 300 mL phosphate-buffered solution (PB; pH 7.4, 4 °C, 4% paraformaldehyde in 0.1 mol/L PB), and then the tissues were fixed as the perfusion speed was slowed down. Following perfusion, the brain was immediately removed, placed in 4% paraformaldehyde containing 0.1 mol/L PB, and then fixed for 4–6 h. Next, the brain was placed in a solution of 30% sucrose containing 0.1 mol/L PB at 4 °C until it sank to the bottom. Coronal sections containing the hypothalamus were cut using a cryostat microtome (Leica 1900). Each section was 30-μm thick, and three sets of sections were made from each rat.

2.3.2. Immunohistochemical staining

Avidin–biotin complex immunohistochemical staining was then performed. First, each set of brain sections was washed three times in 0.01 mol/L phosphate-buffered saline (PBS) for 5 min each time, incubated in 0.5% Triton X-100 at 37 °C for 30 min, then washed three times in 0.01 mol/L PBS for 5 min each time. The antigen was placed in a boiling water bath for 10 min, cooled to room temperature, and then washed three times in 0.01 mol/L PBS for 5 min each time. Sections were then incubated in 3% hydrogen peroxide for 15 min at room temperature, washed three times in 0.01 mol/L PBS for 5 min each time, and then placed in goat serum for 2 h. The sections were then placed in 0.5% Triton X-100 at 37 °C for 5 min each time, incubated in 0.5% Triton X-100 at 37 °C for 30 min, then washed three times in 0.01 mol/L PBS for 5 min each time. The sections were then incubated in 3% hydrogen peroxide for 15 min at room temperature, washed three times in 0.01 mol/L PBS for 5 min each time, and then placed in goat serum for 2 h. The sections were then placed in rabbit anti-rat c-Fos monoclonal antibody (1:300 dilution; Santa Cruz) and incubated at room temperature for 48 h. The sections were washed three times in PBS for 5 min each time, then placed in biotinylated goat anti-rabbit IgG serum (Beijing Zhongshan Goldenbridge Biotechnology Co., Ltd.) and allowed to react at room temperature for 2 h. The sections were washed three times in PBS for 5 min each time, then placed in the avidin–biotin complex solution (Beijing Zhongshan Goldenbridge Biotechnology Co., Ltd.), marked with horseradish peroxidase, and allowed to react at room temperature for 2 h. The sections were washed three times in PBS for 5 min each time and stained with 3,3′-diaminobenzidine; the resulting immunoreactive product was brown. The sections were mounted, dried at room temperature, dehydrated, cleared, a cover slip was placed on the sample, and then the sample was placed in a Leica BX51 microscope for observation. The second set of sections served as the negative control and was processed in the same manner described above; however, the rabbit anti-rat c-Fos monoclonal antibody was replaced with 0.1 mol/L PBS. To localize the positive areas of the immunohistochemical stains, Nissl staining was used on the third set of sections.

2.4. Data collection and statistics

The sections were observed under a light microscope (BX51; Olympus, Japan), and the images were collected using an image analysis system (Quanti met 5700; Leica). For the anatomical locations of the cells, please see Paxinos and Watson’s method (Paxinos and Watson, 1998). Cell counts were performed on a computer from the digital images of the respective brain structures by an examiner blind to the study protocol. First, the number of c-Fos-positive hypothalamic nuclei cells was determined, followed by the area of each nuclei and the density. Data were fed into a computer, but only the sharpness, contrast, and brightness were adjusted. Each temperature group consisted of eight rats, and the number of c-Fos-positive cells in each of the five sections was counted and averaged for each rat.

The data were calculated as the mean ± standard deviation and analyzed using SPSS 14.0. The independent t test was used to analyze the data regarding the number of cells expressing c-Fos in the nuclei of the POA of the hypothalamus: one-way ANOVA was used to compare c-Fos-positive cell densities between groups. P < 0.05 was considered statistically significant.

3. Results

To verify whether the ovariectomies were successful, vaginal exfoliated cells were examined for 7 day in succession starting at 3 day after the operation according to Gold’s method (Gold and Josimovich, 1980).

Brown c-Fos expression was mainly distributed throughout the cell nuclei by immunohistochemistry. At both high and low temperatures, these nuclei and PCDs of the SHAM, OVX+E, and OVX+ICR groups in the MnPO (Fig. 1A–H), PVT (Fig. 2A–H) and AH demonstrated higher expression levels than the OVX group. The PCDs of the SHAM, OVX+E, and OVX+ICR groups in the SO nucleus (Fig. 3A–D), demonstrated higher expression levels than the OVX group after 38 °C temperature stimulation.

The PCDs decreased as the temperature increased until 25 °C, at which point the density began to increase demonstrating a V-type curve, and lowest at 25 °C in the MnPO of the SHAM group. The PCD had no regular change in other three groups (Fig. 4). c-Fos PCDs at the same temperatures were also compared. The PCDs of the SHAM, OVX+E, and OVX+ICR groups were higher than the OVX group except 25 °C. At 4 °C, the PCD of the
OVX group was significantly lower than the PCDs of the OVX + E and SHAM groups ($P < 0.01$). The PCD had irregular change as the temperature changed in the MPA of four groups. (Fig. 5). c-Fos PCDs at the same temperatures were also compared. The PCDs of the four groups showed no significant differences at 4 °C, 10 °C, and 33 °C. The PCDs of the SHAM, OVX + E, and OVX + ICR groups were significantly higher than that of the OVX group at 25 °C ($P < 0.05, 0.01$), and the PCDs of the SHAM and OVX + ICR groups were higher than that of the OVX group at 38 °C ($P < 0.01$).

The PCD showed a V-type curve as temperature increased in the VMPO of the Sham group, and no regular change in other three groups (Fig. 6). The c-Fos PCDs at same temperatures were also compared. The PCDs of the SHAM, OVX + E, and OVX + ICR groups were significantly lower than the PCDs of the OVX + E and SHAM groups ($P < 0.01$). The PCD had irregular change as the temperature changed in the MPA of four groups. (Fig. 5). c-Fos PCDs at the same temperatures were also compared. The PCDs of the four groups showed no significant differences at 4 °C, 10 °C, and 33 °C. The PCDs of the SHAM, OVX + E, and OVX + ICR groups were significantly higher than that of the OVX group at 25 °C ($P < 0.05, 0.01$), and the PCDs of the SHAM and OVX + ICR groups were higher than that of the OVX group at 38 °C ($P < 0.01$).
were higher than those of the OVX group at 4 °C, 33 °C, and 38 °C (P < 0.05, 0.01). The PCDs of the SHAM and OVX+ICR groups were higher than the PCD of the OVX group at 10 °C (P < 0.01). There was no significant difference between the four groups at 25 °C.

The PCD demonstrated a V-type curve as the temperature increased in the AH of the four groups (Fig. 7). The c-Fos PCDs at the same temperatures were also compared. The PCDs of the SHAM, OVX+E, and OVX+ICR groups were higher than the PCDs of the OVX group at 4 °C, 10 °C, 33 °C, and 38 °C (P < 0.01), but were similar at 25 °C.

The PCD showed the same V-type curve as temperature increased in the SO of four groups (Fig. 8). PCDs at the same temperatures were also compared. The PCDs of the SHAM, OVX+E, and OVX+ICR groups were higher than the PCDs of the OVX group at 4 °C, 10 °C, and 38 °C (P < 0.05). The PCD of the OVX+ICR group was lower than that of the SHAM group at 4 °C (P < 0.05). There was no difference between the four groups at 25 °C. The PCDs of OVX+ICR and OVX+E groups were higher than the PCDs of the OVX group (P < 0.01) and than that of the SHAM group at 33 °C (P < 0.01).
There was a V-type curve as temperature increased in PCD of PVN except OVX+ICR group (Fig. 9). c-Fos PCDs at same temperatures were also compared. The PCDs of the SHAM, OVX+E, and OVX+ICR groups were higher than the PCDs of the OVX group at 4 °C, 10 °C, 33 °C, and 38 °C \( (P < 0.05, 0.01) \) but similar at 25 °C. The PCD of the OVX+ICR group was higher than that of the SHAM group at 33 °C \( (P < 0.05) \).

The PCD had a V-type curve as temperature changed in the SCh of the SHAM group, and irregular change in other three groups (Fig. 10). c-Fos PCDs at the same temperatures were also compared. The PCDs of the SHAM and OVX+E groups were higher than the PCDs of the OVX group \( (P < 0.01) \), and the PCD of OVX+ICR group was lower than that of the SHAM group at 4 °C \( (P < 0.01) \). There were no differences between the four groups at 10 °C and 25 °C in four groups. The PCDs of the SHAM, OVX+E, and OVX+ICR groups were higher than the PCDs of the OVX group \( (P < 0.01) \), and the PCDs of the OVX+E and OVX+ICR groups were higher than the PCD of the SHAM group at 33 °C \( (P < 0.05 \text{ and } 0.01, \text{ respectively}) \). The PCDs of the OVX+E and OVX+ICR groups were lower than the PCD of the SHAM group at 38 °C \( (P < 0.05 \text{ and } 0.01) \).

4. Discussion

The POA plays an important role in detecting temperature changes, organizing thermal information, and is considered an integrator of the thermal signals from the peripheral and central nervous systems. It receives thermal information from the peripheral and central nervous systems, and the outputs are delivered to the other brain areas responsible for thermoregulation (Nakamura and Morrison, 2007; Romanovsky, 2007). We found that under different thermal stimuli, c-Fos expression in the nuclei of the POA of the SHAM group was similar to the results reported by Bratincsak (Bratincsak and Palkovits, 2004)—that is, most nuclei in this area demonstrated a V-type curve, the lowest reactivity of neurons at 25 °C, and indicated that nuclei were more sensitive to warm stimuli than cold stimuli. The c-Fos protein expression in the hypothalamus of the ovariectomized rats changed in response to different temperatures and, as far as we know, these differences before and after drug intervention have not been previously reported.

Our result showed that the PCD in the MnPO, VMPO, and AH nuclei of the four groups of rats were not significantly different, except the PCD in the MPA of the OVX group was lower than that of other groups at 25 °C. After cold and warm temperature stimulation, the PCD in the hypothalamic nuclei of the ovariectomized rats was significantly and irregularly decreased, suggesting that the decrease in estrogen had a major effect on the adaptability of the hypothalamic neurons to hot and cold temperature stimuli. The dysfunction of these neurons was improved after treatment with black cohosh and estrogen, resulting in PCD values that were similar to those of the SHAM group. However, the effects of these two drugs on different nuclei at different temperatures were dissimilar. Under hot and cold stimuli, black cohosh and estrogen both demonstrated positive effects on the MnPO. Both drugs also had positive effects on the MPA at 25 °C, but only black cohosh demonstrated a positive effect at 38 °C. Both black cohosh and estrogen demonstrated positive effects on VMPO at 4 °C, 33 °C, and 38 °C, but only black cohosh demonstrated positive effects at 10 °C. Under hot and cold stimuli, black cohosh and estrogen both demonstrated positive effects on the AH, suggesting that both of these drugs improved the response of the POA/AH neurons at different levels; however, their responses were most effective at different temperatures and in different nuclei. This may explain why the effects of these two drugs for treating menopausal symptoms are not exactly the same.

POA/AH plays a critical role in the regulation of the internal environment. Electrophysiological studies have identified the presence of two thermally classified populations of neurons within POA/AH(warm-sensitive neurons and cold-sensitive neurons). These cells can detect their own and peripheral temperature changes, and adjustments can be made by resulting in the production or loss of heat. An increase in the firing rate of warm-sensitive neurons might activate the body’s cooling processes and inhibit the production and storage of thermal energy, resulting in a decrease in body temperature, and an increase in the firing rate of cold-sensitive neurons, which might inhibit the body’s heat dissipation and increase the production and storage of thermal energy, thereby resulting in a rise in body temperature. Estrogen levels rapidly decreased in the ovariectomized rats, and temperature-sensitive neuron activities in these rats were significantly decreased in comparison with normal rats when the outside temperature was changed, the PCD in the POA/AH of the OVX group was lower than that of the SHAM group, indicating that the adaptability of the OVX group was obviously reduced. This also indicates that the thermoregulatory center does change during menopause and that the abnormal central nervous system cannot sufficiently regulate body heat in order to maintain a normal body temperature. This might lead to fluctuations in body temperature and menopausal hot flashes. After 1 week of drug treatment, the curves of the OVX+E and OVX+ICR groups were closer to that of the SHAM group, indicating that black cohosh and estrogen may directly act on the neurons in the POA/AH. Both drugs increased the excitability of these neurons, played an important role in the functional recovery of the POA/AH, and remedied abnormalities in central body temperature function and other menopausal symptoms.

After ovariectomy and drug treatment, other hypothalamic nuclei also demonstrated changes in addition to those seen in the POA/AH. When there was a sudden change in the outside

---

**Fig. 9.** c-Fos-positive cells in the PVN. The PCD in the PVN at different temperatures demonstrated significant differences at 4 °C, 10 °C, 33 °C, and 38 °C \( (P = 0.000, 0.014, 0.000, \text{ and } 0.000, \text{ respectively}) \), but there was no significant difference at 25 °C \( (P = 0.285) \).

**Fig. 10.** c-Fos-positive cells in the SCh. The PCD in the SCh at different temperatures demonstrated significant differences at 4 °C, 33 °C and 38 °C \( (P = 0.000, 0.000, \text{ and } 0.000, \text{ respectively}) \), but there was no significant difference at 10 °C and 25 °C \( (P = 0.144 \text{ and } 0.580) \).
temperature, the density of active neurons is reduced in the SO and PVN of OVX group, indicating that adaptability to the external environment was reduced. Dysfunction of the SO and PVN can induce emotional irritability, chest pain, palpitation, deterioration of learning and memory skills, ataxia, tachypnea, digestive dysfunction, and abnormal energy, glucose, and cholesterol metabolism, all of which are common problems in climacteric women. After 1 week of drug treatment, both black cohosh and estrogen increase their excitability and alleviated dysfunction to some extent, thereby improving the symptoms of menopause.

The SC is the central structure that generates and regulates the circadian rhythm in mammals. On one hand, SC has an autonomous circadian rhythm, similar to its electrophysiological properties, that affects sugar and protein synthesis; on the other hand, SC integrates outside information so that the intrinsic biological rhythms can be synchronized with the external environment. In addition, the SC may have some effect on glucocorticoid secretion (Balsalobre et al., 2000). One study found that the SC regulates daily activities, the core temperature, and is involved in the regulation of corticosterone secretion together with the hypothalamus (Angeles-Castellanos et al., 2010). In this study, the excitatory neuron density in the SC of the OVX group was lower than that in the SHAM group, indicating that activity in the neuronal nuclei decreased as estrogen was reduced. These neurons may have an automatic effect on circadian pacemaker functions. Therefore, some perimenopausal women may develop diseases related to disorder of the hormone circadian rhythm and biological clock rhythm, such as insomnia and obesity. Estrogen and black cohosh can increase the excitability of neurons in the SC, but the former has a greater effect than the latter.

5. Conclusion

Further investigations need to be carried out on the mechanisms that lead to menopausal hot flushes and other symptoms. In the case of an ovariectomy and the subsequent decrease in estrogen levels, the dysfunction of temperature-sensitive neurons in the POA and other hypothalamic nuclei may explain the reduced ability to adapt to changes in ambient temperature, resulting in hot flushes, sweating, and other menopausal symptoms. Estrogen and black cohosh can improve the functions of the hypothalamic nuclei, but the effects achieved with the two drugs in different nuclei were dissimilar, suggesting that the drugs have different effects on menopausal symptoms.

Acknowledgments

We are grateful to all the colleagues for their assistance. We also thank Dr Zheng for her financial assistance. This study was supported by a grant provided by the Natural Science Foundation of China (grant no. 81070462) and Natural Science Foundation of Beijing (grant no. 7113168).

References