Acta Histochemica 116 (2014) 1259–1269

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Effect of low estrogen on neurons in the preoptic area of hypothalamus of ovariectomized rats

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

[a] Anatomy and Embryology Department, Peking University Health Science Center, No. 38 Xueyuan Road, Haidian District, Beijing, China
[b] Obstetrics and Gynecology Department, Peking University First Hospital, No. 8 Xishiku Street, Xicheng District, Beijing, China
[c] Statistics Office, Peking University First Hospital, No. 8 Xishiku Street, Xicheng District, Beijing, China
[d] Department of Stomatology, General Hospital of Armed Police, No. 69 Yongding Road, Haidian District, Beijing, China

ARTICLE INFO

Article history:
Received 9 June 2014
Received in revised form 15 July 2014
Accepted 21 July 2014

Keywords:
Preoptic area of hypothalamus (POAH)
Temperature stimuli
c-Fos
Ovariectomized rats

ABSTRACT

The purpose of this study was to investigate the difference in neuronal activity in the preoptic area of the hypothalamus (POAH) under low estrogen condition induced by ovariectomy. One hundred and twenty sham-operated (SHAM) and ovariectomized (OVX) rats were placed in different temperatures for 2 h. Twelve rats from each group were stimulated by 4 °C, 10 °C, 25 °C, 33 °C and 38 °C, respectively. c-Fos expression in the POAH was detected by immunohistochemistry. Following exposure to warm and cold stimuli, there were markedly lower c-Fos-positive cell densities in the OVX group compared with the SHAM group in the median preoptic nucleus (MnPO) at 4 °C, 10 °C, 33 °C and 38 °C, in the medial preoptic area (MPA) at 25 °C and 38 °C, in the ventromedial preoptic nucleus (VMPO) at 4 °C, 10 °C and 38 °C and in the ventrolateral preoptic nucleus (VLPO) at 4 °C and 38 °C. Both temperature and surgery had an impact on c-Fos expression by two-way ANOVA method except in the lateral preoptic area (LPO). c-Fos expression differed within different nuclei of the two groups in the same and different temperature stimuli. This indicated that the temperature-sensitive nuclei in the POAH exhibited lower and different activities during temperature stimuli following ovariectomy, which possibly resulted in abnormal thermoregulation and menopausal symptoms.

Introduction

Menopause may be accompanied by symptoms of hot flashes in women, which can seriously affect normal life. The symptoms are closely related to hypothalamic thermoregulation disorders induced by low levels of estrogen (Santoro, 2008). The preoptic anterior hypothalamus (POAH) is the central area for thermoregulation in homeotherms and can be activated by heat stress induced by temperature changes (McKitrick, 2000; Bachtell et al., 2003). The POAH includes the median preoptic nucleus (MnPO), ventrolateral preoptic nucleus (VLPO), ventromedial preoptic nucleus (VMPO), medial preoptic area (MPA) and lateral preoptic area (LPO). All of these areas are involved in temperature regulation. In addition, some other hypothalamic areas, such as the paraventricular nucleus, the supraoptic nucleus, the dorsomedial nucleus, the ventromedial nucleus and the posterior and lateral hypothalamus also participate in thermoregulation (Lu et al., 2001; Cano et al., 2003).

It has been reported that increasing the temperature of POAH induces gasping and sweating in animals, while decreasing the local temperature causes an increase in heat production, indicating that the POAH itself can regulate both heat dissipation and heat production (Tang et al., 2012). Hypothalamic thermoregulation resembles a thermostat, so most researchers use the concept of a "set-point" to explain the importance of the thermoregulatory center. When the body temperature rises above the upper threshold of the set-point, the temperature receptor sends temperature signals to the center which then integrate these signals and send a final signal to induce less heat production and more heat dissipation, resulting in an action that will adjust the body temperature to the normal level (e.g., sweating). On the other hand, when the body temperature is below the lower threshold of the set-point, the center will send a signal to increase heat production and reduce heat dissipation, resulting in an action that will increase the body temperature

Abbreviations: AH, hypothalamic area; BLA, basolateral amygdaloid nuclei; CeA, central amygdaloid nucleus; DMH, dorsomedial hypothalamic nucleus; LPB, lateral parabrachial nucleus; LPO, lateral preoptic area; MnPO, median preoptic nucleus; MPA, medial preoptic area; OVX, ovariectomized; P5, peritrigeminal nucleus; POAH, preoptic area of the hypothalamus; SHAM, sham-operated; VMPO, ventromedial preoptic nucleus; VLPO, ventrolateral preoptic nucleus.

* Corresponding author.
E-mail address: qinlihua88@163.com (L. Qin).

http://dx.doi.org/10.1016/j.acthis.2014.07.010
0065-1281/

to the normal level (e.g., shivering) (Boulant, 2006). The set-point hypothesis has been widely accepted, but its physiological basis still lacks experimental research.

An elevated core temperature generally occurs before perimenopausal hot flashes, though the mechanism is unclear. The doctrine of the narrowed thermoneutral zone in the hypothalamic thermoregulatory center, as proposed by Freedman (2005) is the most convincing explanation for perimenopausal hot flashes, i.e., women with perimenopausal hot flashes have a narrower hypothalamic thermoregulatory range (temperature difference between hot flashes and shivering threshold) compared with women without perimenopausal hot flashes. The hypothalamic thermoregulatory center has a thermoneutral zone of about 0.4 °C (Rehman and Masson, 2005). Under normal physiological conditions, fluctuations in the ambient temperature that are within this range will not cause hot flashes, sweating, chills or other compensatory responses. However, if the thermoneutral zone is narrower, tiny fluctuations in ambient temperature can exceed the limit of this range, thus resulting in hot flashes and other symptoms of a narrow thermoneutral zone (i.e. thermoregulatory reactions).

The c-Fos gene is a kind of immediate early gene. A variety of stimulating factors, such as oxygen, mechanical stimuli, light stimulus and pain can induce the expression of c-Fos gene within the central nervous system. After stimulation, the c-Fos gene within the nucleus is activated and mRNA transcription forms. Then the mRNA passes through the nuclear membrane into the cytoplasm to synthesize c-Fos protein. After phosphorylated modification, c-Fos protein returns to the nucleus and participates in several brain functions, such as signal transduction and regulation (Jisheng, 1999). The c-Fos protein is widely recognized as a marker of neuronal morphology and acute neural activation in the central nervous system (Bachtell et al., 2003) and can be used to localize warm- and cold-sensitive neurons and estimate the functional status of neurons. In the case of warm stimuli, enhanced c-Fos expression in the hypothalamus indicates warm-sensitive neurons and vice versa. The higher the expression of c-Fos, the more active the neuron is. After warm stimulus by 34 °C, expression of c-Fos protein is observed in the MnPO, LPA (lateral preoptic area), AH (hypothalamic area) and DMH (dorsomedial hypothalamic nucleus) (Harikai et al., 2003). After warm stimulus by 37 °C, a strong expression of c-Fos protein is observed in the MPA, CeA (central amygdaloid nucleus), BLA, LPB and P5 (Plessis et al., 2006). After cold stimulus by 4 °C, a strong expression of c-Fos protein is observed in MPA, LPB (lateral parabrachial nucleus), P5 (peritrigeminal nucleus) and few expressions in BLA (basolateral amygdaloid nuclei) (Bratincsak and Palkovits, 2004). Apart from being involved in thermoregulation, MnPO and VLPO in POAH are also related to sleeping (McGinty and Szymusiak, 2001). Changes in the circadian rhythm and sleep deprivation can result in increased c-Fos expression in the POAH of the hypothalamus. While during normal exposure to sunlight, c-Fos expression is generally distributed throughout the MnPO and VLPO. Ovariectomy can reduce c-Fos expression in the VLPO and anteroventral periventricular area (AVPV), thereby resulting in the absence of the circadian rhythm in the VLPO and AVPV (Peterfi et al., 2004). However, there have not been any reports on c-Fos expression in POAH under temperature stimulation after ovariectomy. Compared with men, women are more vulnerable to sleep disorders (Dzaja et al., 2005; Liu and Liu, 2005) and the sleeping patterns of women are closely related to their menstrual cycles (Mashoodh et al., 2008). Sudden fluctuations in the estrogen level during menopause can lead to changes in the POAH and may result in menopausal symptoms such as hot flashes and sleep disturbance.

The functions of warm- and cold-sensitive neurons in the hypothalamus are critical to hypothalamic thermoregulation in response to different ambient temperature stimuli. Confirmation of the inherent relationship between warm- and cold-sensitive neurons and thermoregulation under low estrogen levels will greatly improve the study of menopausal symptoms, but no such report has been published. Therefore, we selected five ambient temperatures (4 °C, 10 °C, 25 °C, 33 °C and 38 °C) according to normal temperature variation between the four seasons in this study. c-Fos expression was detected using immunochemistry in order to determine the expression densities of various nuclei and their temperature-sensitive regional changes, to assess their responses to different temperature stimuli in SHAM and OVX rats, and determine if these nuclei were involved in hypothalamic thermoregulation and their effects on thermoregulation. 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's guide for the care and use of laboratory animals.

Experimental procedures

Animals

A total of 120 healthy adult female Sprague-Dawley rats (8 – 10 weeks of age; purchased from the Laboratory Animal Science Department of Beijing University Health Science Center) were used in this study. These rats weighed 210 – 230 g and were housed in an animal room at a temperature of 25 ± 1 °C, relative humidity of 40 – 50%, and under a 12-h light/dark cycle. They were exposed to direct light and given free access to water and soy-free feed for 2 weeks (Rachoń et al., 2008). Before surgery, they were kept in a warm and quiet environment for 48 h.

Experimental design

Establishment of the ovariectomized rat model and grouping

To establish the OVX group, 60 rats were selected and anesthetized using an intraperitoneal injection of 1% sodium pentobarbital (40 mg/kg), then an incision was made in the midline of the abdomen and the bilateral ovaries were removed. The remaining 60 rats underwent a sham operation (SHAM group), i.e., they had an incision made at the midline of the abdomen, their bilateral ovaries were exposed, but not resected, and then the abdominal cavity was closed. These 120 rats were given two weeks for healing after the operation.

Criteria of successful ovariectomy

To verify that the ovariectomy had been successful and the level of estrogen had decreased, exfoliated vaginal cells were examined for seven consecutive days beginning on the third day after the operation. The vaginal cells of the SHAM group exhibited the signs of the estrous cycle, while the vaginal cells in the OVX group indicated continuous diestrus (Gold and Josimovich, 1980).

Section preparation and immunohistochemical staining

Temperature stimulation to establish warm and cold stimuli models

The 60 ovariectomized rats were randomly divided into five temperature groups (12 rats per temperature group) and placed in five separate incubators (SPX-80BS-II incubators; ShangHai CIMO Medical Instrument Co., Ltd.) for 2 h. The incubators were kept at 4 °C, 10 °C, 25 °C, 33 °C or 38 °C, respectively, and a relative humidity of 60%. The control group comprised 60 SHAM rats that were maintained under the same groupings and temperature stimuli as the OVX group. After receiving different temperature stimuli in the