Respiratory Physiology & Neurobiology 217 (2015) 17–24

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Hypoxic and hypercapnic ventilatory responses in rats with polycystic ovaries

Luis Henrique Montrezor[a,1], Débora de Carvalho[b,1], Mirela B. Dias[c], Janete A. Anselmo-Franci[d], Kênia C. Bícego[b], Luciane H. Gargaglioni[b,*]

[a] Department of Medicine, University Center of Araraquara-UNIARA, Araraquara, SP, Brazil
[b] Department of Animal Morphology and Physiology, Sao Paulo State University – UNESP, Jaboticabal, SP, Brazil
[c] Department of Physiology, Institute of Bioscience, Sao Paulo State University – UNESP, Botucatu, SP, Brazil
[d] Department of Morphology, Stomatology and Physiology, Dental School of Ribeirao Preto, University of Sao Paulo, Brazil

ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 20 June 2015
Accepted 21 June 2015
Available online 25 June 2015

Keywords:
Chemosensitivity
Hypercapnia
Hypoxia
Ventilation
Sex hormones

ABSTRACT

In female rats, a single injection of estradiol valerate (EV) results in effects that are similar to those observed in women with polycystic ovary syndrome (PCOS). We hypothesized that EV-induced PCOS affects breathing control based on evidence showing an influence of sex hormones on ventilation. To test this hypothesis, we studied the effects of EV treatment on the ventilation of female rats in air, in 7% CO_2 and in 7% O_2, at 30, 45 and 60 days after EV injection. The group examined 30 days after EV treatment showed a 61% reduction in the hypercapnic ventilatory response compared to the control group. Basal ventilation, hypoxic ventilatory response, and body temperature were not affected. These results, suggest that the hormonal changes observed in PCOS may result in a temporary inhibition of the central chemoreflex but do not influence basal ventilation or the hypoxic peripheral chemoreflex.

1. Introduction

Polycystic ovary syndrome (PCOS) is a clinical disorder that affects up to 10% of women of reproductive age (Goodarzi et al., 2011; Teede and Norman, 2006), and it is the most common endocrine disorder among pre-menopausal women in the United States (Tasali et al., 2008; Knochenhauer et al., 1998). PCOS is characterized by impairment of ovulation, reduced fertility, miscarriage, and imbalance of reproductive hormones (Goodarzi et al., 2011; Franks, 1995). In addition, women with PCOS often exhibit obesity, metabolic syndrome, hyperinsulinemia, insulin resistance, and dyslipidemia, which lead to increased risks of cardiovascular disease and type 2 diabetes (Pasquali et al., 2011; Lobo and Carmina, 2000). However, the mechanisms underlying such disorders remain unclear and may involve an array of factors including hormonal imbalances, epigenetic changes in fetal life, genetic abnormalities, lifestyle, and environmental factors (Goodarzi et al., 2011; Pasquali et al., 2011).

Previous studies have demonstrated that women affected by PCOS show respiratory disorders such as sleep disordered breathing (Vgontzas et al., 2001; Chatterjee et al., 2014). For instance, obstructive sleep apnea (OSA) has been reported to be higher in women with PCOS in comparison to the general population (Chatterjee et al., 2014; Vgontzas et al., 2001). It is possible that hormonal changes in women affected by PCOS such as hyperandrogenism, hyperestrogenism and variable levels of gonadotropins in the blood (Goodarzi et al., 2011), may be involved in the development of OSA. In fact, a previous study suggested that high free testosterone levels in women with PCOS may be a predisposing factor leading to OSA (Tock et al., 2014).

Additionally, evidences indicate that steroidal sex hormones such as progesterone (P4), testosterone (T) and estrogen (E2) are involved in the neural control of respiration (Behan and Kinkead, 2011). Not surprisingly, evidence also suggests that sex hormones influence the peripheral and central chemoreflex. For example, it has been demonstrated that hypoxic and hypercapnic responses differ between the sexes (Behan et al., 2003) and acute administration of testosterone in castrated cats increases the hypoxic and the hypercapnic ventilatory response and promotes enhanced sensitivity of the carotid body (Tatsumi et al., 1994). In addition,

* Correspondence to. Via de acesso Paulo Donato Castellane s/n, 14870-000, Departamento de Morfologia e Fisiologia Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista Júlio de Mesquita Filho, Jaboticabal, SP, Brazil. Fax: +55 16 32024275.
E-mail address: lucihel@fcav.unesp.br (L.H. Gargaglioni).
[1] L.H.M. and D.C. contributed equally to this study.

http://dx.doi.org/10.1016/j.resp.2015.06.009
1569-9048/

hypoxic and hypercapnic ventilatory responses increase during pregnancy (Moore et al., 1987). More recently, we have demonstrated that despite the hormonal fluctuations during the estrous cycle, the CO_2 ventilatory responses in female rats are similar along the cycle; however ovariectomized rats presented a reduced hypercapnic ventilatory response (Marques et al., 2015). Furthermore, hormonal replacement with estradiol or progesterone in those ovariectomized females did not restore the CO_2-drive to breathing, suggesting that other gonadal factors are possibly involved in this response.

Estradiol valerate (EV) treatment in pre-pubertal or adult female rats has been used as an experimental model to induce PCOS and promotes similar effects to those observed in women with this syndrome (Linares et al., 2013). Additionally, rodent models of PCOS have shown many characteristics of the human disorder, including elevated LH, disrupted cyclicity, the presence of follicular cysts/polycystic ovaries, and altered insulin sensitivity, which closely parallels the human condition (Walters et al., 2012).

The aim of the present study was to determine whether, PCOS affects breathing control. To this end, we induced PCOS in female rats by administering EV, and we examined the effects of EV on ventilation in air and in conditions of hypoxia and hypercapnia at 30, 45 and 60 days after EV treatment.

2. Materials and methods

2.1. Animals

Experiments were performed on unanesthetized adult female Wistar rats (4 month old) weighing 280 ± 9.8 g in control group, 281 ± 5 g in 30 days, 252 ± 7 g in 45 days and 259 ± 11 g in 60 days after estradiol valerate treatment groups (mean ± SEM) on the day of experiment. Six animals were housed in each cage in a temperature-controlled chamber at 24–26 °C (ALE 9902,001; Alesco, Monte Mor, SP, Brazil) with a 12:12 h light/dark cycle (lights on at 7:00 A.M.), and rats had free access to water and food.

This study was conducted in compliance with the guidelines of the National Council of Control in Animal Experimentation (CONCEA-MCT-Brazil) and with the approval of the local Animal Care and Use Committee (CEUA-FCAV-UNESP, n# 000222-09).

2.1.1. Induction of polycystic ovary syndrome

Estradiol valerate (EV) (Sigma–Aldrich, MO, USA) was dissolved in mineral oil and administered (2 mg/0.2 mL/rat; intramuscular) to induce polycystic ovary syndrome through the formation of follicular cysts (Brawer et al., 1986; Pereira et al., 2014). EV was administered in a single dose at 30, 45 and 60 days before the experiments. The control group received no treatment and only those which were in diestrus at the time of the experiment were used because this phase of the cycle is longer lasting. Estrous cycle regularity was assessed, and only rats showing at least three consecutive regular 4-day cycles were included in the control group or received EV and then used in the experiment.

2.1.2. Surgery

For body temperature (Tb) measurements, a datalogger (SubCue, Calgary, AB, Canada) was implanted in all animals by abdominal cavity through a midline laparotomy 24 h prior to the experiment. The surgical procedure was performed under anesthesia using intraperitoneal ketamine (100 mg/kg, Agener, São Paulo, Brazil) and xylazine (10 mg/kg, Coopers, São Paulo, Brazil). After surgery, the rats were treated with subcutaneous antibiotic (10 mg/kg, Enrofloxacina, Flotril®, Schering-Plough, São Paulo, Brazil) and analgesic (2.5 mg/kg, Flunixina meglumina, Banamine®, Schering-Plough, São Paulo, Brazil) agents. The dataloggers were programmed to acquire data every 5 min.

2.1.3. Determination of pulmonary ventilation

Measurements of pulmonary ventilation (V_E) were performed using the whole body plethysmography method based on the Bartlett and Tenney, 1970 study, which has been described in detail previously (Biancardi et al., 2008; De Carvalho et al., 2010; Patrone et al., 2014). The flow rate of the inflow gas into the animal chamber was controlled by a flowmeter (model 822-13-OV1-PV2-V4, Sierra Instruments, Monterey, CA) and flow was maintained at 0.8 to 1 L/min.

2.1.4. Experimental protocol

At 30, 45 and 60 days after the EV injection each animal, including control groups, was individually placed in a Plexiglas chamber (5 L) and allowed to move freely while the chamber was flushed with humidified room air. After the animals remained calm (~30 min), control V_E was measured. A hypercapnic gas mixture (7% CO_2 in air, White Martins, Sertãozinho, São Paulo, Brazil) was flushed through the chamber for 30 min, and V_E was measured at the end of exposure. Subsequently, the chamber was ventilated for 60 min with humidified room air, and V_E was measured again after 60 min. A hypoxic gas mixture (7% O_2 and N_2 balance, White Martins, Sertãozinho, São Paulo, Brazil) was flushed through the chamber for 30 min, and V_E was measured at 30 min.

2.1.5. Hormone assay

After measurements of pulmonary ventilation rats were anesthetized and a blood sample of approximately 1 mL was collected from the heart into heparinized syringes. Plasma was separated by centrifugation at 3000 rpm for 20 min at 4 °C and stored at −20 °C for posterior analysis of progesterone, testosterone and estradiol levels by radioimmunoassay (RIA). Plasma progesterone, testosterone and estradiol concentrations were determined by double-antibody RIA with MAIA kits provided by Biochem Immunosystem (Bologna, Italy). The lower limits of detection for estradiol, progesterone and testosterone were 5.0 pg/mL, 0.02 ng/mL and 5.0 pg/mL, respectively. The intra-assay coefficient of variation was 4.3% for estradiol, 7.5% for progesterone and 4% for testosterone.

2.1.6. Ovarian morphology

The left ovary was removed, cleaned of adherent connective fat tissue, and fixed in 4% formaldehyde buffer for at least 24 h for use in morphological analysis. Thereafter, the samples were dehydrated and embedded in paraffin. The ovarian morphology characterization of the PCO was performed by histological analysis of 9-μm serial sections stained with hematoxylin and eosin (HE), and photographs were analyzed with Motic Live Imaging Module (format 1024 × 768) microscopy software. The number of follicles with hyperthecosis, type III follicles and cystic follicles were counted in every section of the ovary as described previously by Brawer et al. (1986). Briefly, antral follicles with hyperthecosis were defined as those medium-sized antral follicles that presented hypertrophied differentiated theca interna cells with increased thickness of the theca layer and a normal granulosa cell layer. Type III follicles were larger and contained four or five layers of small densely packed granulosa cells surrounding a very large antrum and thickened theca interna cell layer. Cystic follicles were defined as those follicles devoid of oocytes and displaying a large antral cavity, a thin granulosa cell layer and a thickened theca interna cell layer (Fig. 1).

2.1.7. Statistical analysis

The results are reported as the means ± SEM. The data were analyzed using two-way ANOVA for repeated measures, and Tukey's