Experimental Gerontology 81 (2016) 19–27

Contents lists available at ScienceDirect

Experimental Gerontology

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

The transition to reproductive senescence is characterized by increase in A6 and AVPV neuron activity with attenuation of noradrenaline content

Angela Cristina Nicola [a,c], Cristiane Mota Leite [b], Mariane Mayumi Batista Nishikava [c], João Cesar Bedran de Castro [a,c], Janete Aparecida Anselmo-Franci [b], Rita Cássia Menegati Dornelles [a,c,*]

[a] Programa Multicêntrico de Pós-Graduação em Ciências Fisiológicas – SBFis/UNESP, Brazil
[b] Laboratory of Neuroendocrinology, School of Dentistry of Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
[c] Laboratory of Endocrine Physiology and Aging, Araçatuba Dental School, Universidade Estadual Paulista, Araçatuba, SP, Brazil

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 10 March 2016
Accepted 19 April 2016
Available online 21 April 2016

Section Editor: Christian Humpel

Keywords:
Reproductive aging
Locus coeruleus
Preoptic area
GnRH
Noradrenaline

ABSTRACT

During the course of life, cyclic females face a state of midlife transition that occurs in a fully functioning neurological system, and results in reproductive senescence. The authors' hypothesis was that changes in the activity noradrenergic neurons may be one of the factors involved in this phenomenon. The aim of this study was to investigate the activity of the neurons in the anteroventral periventricular nucleus (AVPV) and locus coeruleus (LC), to analyze their role in determining reproductive senescence. Adult female Wistar rats in the diestrus phase (4 months/cyclic) and old females (18–20 months/acyclic) in persistent diestrus, were decapitated or perfused at three different time intervals (10, 14 and 18 h) throughout the day. In acyclic rats, the gonadotropin-releasing hormone (GnRH) and noradrenaline (NE) content were reduced; *Fos*-related antigen (FRA) in AVPV and *Fos*-related antigen/Tyrosine hydroxylase (FRA/TH) in LC showed immunolabeling of a higher number of neurons in these animals. The 3-methoxy-4-hydroxyphenylglycol/noradrenaline (MHPG/NE) ratio was higher and plasma LH was lower in the acyclic rats. Furthermore, the estradiol level was higher, and the progesterone level was lower after 14 h of persistent diestrus. These findings suggested that during the periestropause, there was a higher level of POA/AVPV and NE neuronal activity in the LC of acyclic rats, associated with a lower capacity of synthesis and storage of neurotransmitters and neurohormones contributed to changes in the temporal pattern of neuroendocrine signaling, thereby compromising the accuracy of inhibitory and stimulatory effects, causing irregularity in the estrous cycle and determining reproductive senescence

1. Introduction

The state of transition in cyclic females, such as perimenopause, is characterized by an integrated series of phase-dependent transformations that involve sequential activation and deactivation of complex regulatory pathways (Petricka and Benfey, 2011). With advanced female aging, there are further reproductive changes, typically a loss of reproductive capacity with partial or complete reproductive senescence. During this process, each level of the hypothalamic-pituitary-gonadal (HPG) axis undergoes changes in structure, function, and hormone synthesis/release (Wang et al., 2015). All women who reach the age of 60 years with their reproductive organs intact will go through perimenopause to menopause with duration of 1–5 years from start to completion (Brinton, 2010; Harlow et al., 2012). These states of transition are associated with regulatory network restructuring that leads to reproductive senescence, and in the females of most species, this results from changes in the HPG axis (Brann and Mahesh, 2005; Downs and Wise, 2009; Neal-Perry et al., 2010).

Rodent and nonhuman primates, and humans share the common features of perimenopausal transition, with irregular cycling and fertility, steroid hormone fluctuations and lower sensitivity to estrogen (Diaz Brinton, 2012; Finch, 2014). Delayed onset and attenuated amplitude of the preovulatory LH surge, in rodents, occurs from 8–12-months of age and extends up to 18 months, when the persistent diestrus phase begins, after a brief period of constant estrus (Ferreira et al., 2015), and characterizes onset of reproductive senescence (Acuña et al., 2009; Cora et al., 2015). However, acyclic rodents are able to return ovarian function in response to stimuli in gonadotropin releasing hormone (GnRH) neurons (Campos and Herbison, 2014), suggesting that neuroendocrine components contribute to determining the reproductive aging (Cashion et al., 2003; Franceschini and Desroziers, 2013; MohanKumar and MohanKumar, 2004). Attenuated GnRH neurosecretion from the medial basal hypothalamus (MBH) (Rubin, 2000), and altered secretion of neurotransmitters such as glutamate (Neal-Perry et al., 2005); GABA (Mitsushima et al., 2002); noradrenaline (NE) (MacKinnon et al., 1983); and kisspeptin (Dungan et al., 2007;

* Corresponding author at: Department of Basic Sciences, Building 31, UNESP, Rodovia Marechal Rondon Km 527, 16018-805 Araçatuba, SP, Brazil.
E-mail address: rcdfisio@foa.unesp.br (R.C.M. Dornelles).

http://dx.doi.org/10.1016/j.exger.2016.04.015
0531-5565/

Gianetti and Seminara, 2008), may be associated with the female states of transition (Le et al., 2001).

The important action of the NE in controlling the preovulatory LH surge of reproductive-aged rodents occurs via the preoptic area (POA), principally the anteroventral periventricular (AVPV) nucleus, that receives projections of noradrenergic neurons from the locus coeruleus (LC), *A*1 and A2 (Campbell and Herbison, 2007; Szawka et al., 2009; Williams and Kriegsfeld, 2012). Electrolytic lesions in the LC decrease NE content in the medial POA and MBH, disrupt the estrous cycle and block the preovulatory surge of gonadotropin (Anselmo-Franci et al., 1997). Moreover, several studies have shown the participation of NE in the GnRH/LH surge during the reproductive period. Nevertheless, this has not been elucidated during the state of transition from the cyclic to acyclic stage, characterized by the non-occurrence of the LH surge. The authors hypothesized that in the aging female, changes in the activity noradrenergic neurons may be one of factors involved in determining the reproductive senescence.

Therefore, the authors analyzed and compared the activity of AVPV neurons and noradrenergic neurons of the LC; concentrations of the NE and 3-methoxy-4-hydroxyphenylglycol (MHPG)/NE ratio, in addition to the GnRH content in the MBH of cyclic adult rats, in diestrus, and in persistent diestrus of acyclic old rats, in order to elucidate their participation in reproductive aging. The authors performed in vivo experiments using rats in natural aging to analyze the real changes that occurred in this period and determined reproductive senescence.

2. Methods

2.1. Animals

Ninety-six female rats of the Wistar strain, aged 4 months (adult-cyclic) and 18–20 months (old-acyclic), were housed in plastic cages, in groups of four, in a temperature-controlled room (22 ± 2 °C) with a 12/12 h light/dark cycle (lights on at 7:00 h). Food and water were provided ad libitum. Vaginal smears were taken daily for analysis of the estrous cycle. Animal experiments were carried out in accordance with the laboratory principles of animal care (Council, 2011) and were approved by the local Ethics Committee for Research Involving Animals of the University Estadual Paulista (CEUA: 01829-2011). Only adult rats (4 months) showing normal estrous cycles, and old rats (18–20 months) in persistent diestrus, showing at least three consecutive cycles, were used in this study. The criteria for inclusion in this study were: the use of only multiparous female rats and those in the diestrus phase. All female rats were sacrificed at 10, 14 and 18 h on the diestrus day, in order to estimate neuronal activity of both the AVPV and LC, by using FOS protein expression as a marker of neuronal activity in the period important for change in the LH during the proestrus period of cyclic rats. The rats appeared to be healthy, and showed no anatomic pathological abnormality on the day of sampling.

3. Experimental design

3.1. Experiment 1: noradrenaline content and hormone profile

Forty-eight diestrus rats and those in persistent diestrus were decapitated (n = 8/group) for blood collection, removal and immediate freezing of their brains. Noradrenaline concentrations and GnRH content were measured in microdissections from the POA and MBH. NE, its metabolite and NE turnover rate, estimated by the MHPG/NE ratio, were determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED). Plasmatic concentration of the LH, FSH, estradiol and progesterone was determined by radioimmunoassay.

3.1.1. Brain microdissection

Coronal brain sections were obtained by using a cryostat at —15 °C (Microm® HM 505) according to the rat brain atlas (Paxinos and Watson, 2007). One section of 1500 μm and two consecutives sections of 1000 μm were obtained by microdissection by using the *punch* technique (Palkovits, 1973). The POA was microdissected from the first section (approximately +0.48 mm from the bregma until −1.08 mm), in one punch obtained with a 2.0 mm diameter needle, centered immediately above to the optic chiasm. The MBH was microdissected from the second and third sections, starting at approximately −1.8 and −2.8 mm from the bregma, respectively, in one punch bilaterally obtained with a 1 mm 'square puncher', centered on the third ventricle.

3.1.2. HPLC-ED

To analysis of concentrations of NE and metabolites in POA, by HPLC-ED, the samples were mixed with 100 μL of perchloric acid (PCA 0.15 M) and ethylene diaminetetraacetic acid (EDTA 0.1 mM) containing 10 pg/μL of isoproterenol (ISOP) as internal standard. The homogenate was centrifuged (13.000 rpm/5 min/4 °C), filtered (0.22 μm membrane; Durapore, Millipore) and hydrolyzed by heating to 94 °C for 5 min (Lookingland et al., 1991) before being injected into the HPLC-ED system by means of an autoinjector (SIL-10Advp; Shimadzu, Kyoto, Japan). Separation was performed at 35 °C in a 250 × 4-mm RP-18e column (Purospher, 5 μm; Merck, Darmstadt, Germany), preceded by a 4 × 4-mm RP-18e guard column (Lichrospher, 5 μm; Merck). The mobile phase consisted of 100 mM sodium dihydrogen phosphate, 15 mM sodium citratum, 10 mM sodium chloride, 0.1 mM EDTA, 0.4 mM sodium 1-octanesulphonic acid (Sigma-Aldrich) and 16% methanol (Omnisolv; EMD Chemical Inc., Gibbstown, NJ, USA) (pH 3.5). The pump (LC-10Advp; Shimadzu) flow rate was set at 0.4 mL/min, and the detector potential was 0.75 V (Decade; Antec, Leyden, The Netherlands). Chromatography data were plotted with the Class-VP software program (Shimadzu). Noradrenaline and MHPG were identified by their peak retention time and quantified by the internal-standard method based on the area under the peak. All samples from each brain area were measured in the same analysis. The intra-assay coefficient of variation was 0.96% for NE and 4.6% for MHPG. The NE level was considered to estimate the neurotransmitter contained in synaptic vesicles, whereas the MHPG level reflected the amount NE released in the sample (Lookingland et al., 1991). The MHPG/NE was used as a measure of neurotransmitter turnover. In the remaining pellet, the protein content was determined by the Bradford method (Bradford, 1976). The catecholamine content was expressed in pg/μg protein.

3.1.3. Radioimmunoassay

MBH samples were sonicated (50 μL HCl), and centrifuged (12,000 rpm/20 min/4 °C; Eppendorf®, Model 5417 R) to determine the GnRH content. Antibody GnRH R1245 was used, produced by Terry Nett (Department of Biomedical Sciences, Colorado State University, CO, USA); standard INC was supplied by Peninsula Laboratories (Bachem Inc., CA, USA), and hormone marked (^{125}I) by PerkinElmer (NEX-10 μCi 1630, PerkinElmer Life and Analytical Sciences, MA, USA). All dilutions were made in a phosphate buffer gel containing EDTA (pH 7.4), at 4 °C. The complex was precipitated with cold absolute ethanol and the lowest limit of detection was 0.24 pg/mL. The radioactivity of the precipitate was determined by using a gamma counter (Wizard 1470 Automatic Gamma Counter, Perkin Elmer) and the results were expressed as pg/mg protein in the MBH determined by protein assay (Bradford, 1976).

Plasma LH and FSH levels were determined by double-antibody RIA using the specific kit provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK, Bethesda, MD, USA). The antibodies used were anti-rat LH-S10 and FSH-S11 diluted with normal rabbit serum and standard preparation LH-RP3 and FSH-RP2 diluted in phosphate buffered gel 0.1% (0.01 M, pH = 7.5). The minimum detectable dose was 0.16 ng/mL for LH, and 0.09 ng/mL for FSH and the intra-assay coefficient of variation was 4%.

Progesterone was determined using a kit from MP Biomedicals LLC (Divisions Diagnostics, New York, USA); and for estradiol, the Siemens kit was used (Siemens Medical Solutions Dignostics, Los Angeles,