Acta Histochemica 117 (2015) 528–535

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Testosterone application decreases the capacity for ACTH and corticosterone secretion in a rat model of the andropause

Vladimir Ajdžanović*, Ivana Jarić, Jasmina Živanović, Branko Filipović, Nataša Ristić, Marko Miler, Verica Milošević

Department of Cytology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Despot Stefan Blvd. 142, 11060 Belgrade, Serbia

ARTICLE INFO

Article history:
Received 19 March 2015
Received in revised form 9 April 2015
Accepted 14 April 2015

Keywords:
Andropause
Rats
Testosterone
ACTH
Corticosterone

ABSTRACT

The culminating phase of ageing in males–andropause is characterized by enhanced activity of the hypothalamic–pituitary–adrenal axis and frequent glucocorticoid excess. In parallel, free testosterone deficiency provides the baseline hormonal milieu for the ageing male. The aim of this study was to illustrate (using diverse microscopic and biochemical methodologies) the effects of testosterone application on the capacity for adrenocorticotropic hormone (ACTH) and corticosterone secretion in a rat model of the andropause. Middle-aged Wistar rats were divided into sham-operated (**SO**; $n = 8$), orchidectomized (**Orx**; $n = 8$) and testosterone treated orchidectomized (**Orx + T**; $n = 8$) groups. Testosterone propionate (5 mg/kg b.w./day) was administered for three weeks, while **SO** and **Orx** groups received the vehicle alone. ACTH cells and the adrenal cortex were stained using immuno-histochemical, immuno-fluorescent and histochemical procedures. Circulating concentrations of testosterone, estradiol, ACTH and corticosterone, as well as the adrenal tissue corticosterone levels were measured by immunoassays. Testosterone application led to increased ($p < 0.05$) serum concentrations of sex steroids. Consequently, in **Orx + T** rats the ACTH cell nuclei volume increased ($p < 0.05$) by 34%, while the volume density of ACTH cells and their relative intensity of fluorescence decreased ($p < 0.05$) by 46% and 21%, respectively, in comparison with the corresponding parameters in the **Orx** group. Testosterone also induced vasodilatation in the adrenocortical *zona fasciculata*, and decreased ($p < 0.05$) the ACTH concentrations and adrenal tissue corticosterone levels by 38% and 31%, respectively, compared to the **Orx** group. In conclusion, testosterone administration caused a decrease in the capacity for ACTH and corticosterone secretion in a rat model of the andropause.

© 2015 Elsevier GmbH. All rights reserved.

Introduction

Ageing in males represents a progressive psycho-physiological process which is characterized by a gradual entry into its culminating phase—the andropause (Chahal and Drake, 2007; Morales, 2004) *i.e.* the specific multi-symptomatic syndrome that *inter alia* implies an enhanced activity of the hypothalamic–pituitary–adrenal (HPA) axis and frequent stress-related disorders (Hatzinger et al., 2000; Vance, 2003). It is well known that HPA axis circadian rhythmicity and pulsatile neurohormone secretion largely define the life-sustaining adjustments to various stressors (Bergendahl et al., 2000), while ageing-caused morpho-functional disruptions ultimately affect the delicate mechanisms coordinating the axis (Chahal and Drake, 2007). Namely, the loss of hippocampal, hypothalamic and limbic neurons and synapses impairs the central regulation of adrenocortical secretion (Ferrari et al., 2001; Mani et al., 1986; Nichols et al., 2001), which in parallel with some age-related changes occurring in the target, adrenal cortex tissue (Hatzinger et al., 2000) establishes glucocorticoid excess, consistent with the pathogenesis of metabolic syndrome and psychological disturbances (Ajdžanović et al., 2012; Akbaraly et al., 2011). It should not be overlooked that free testosterone deficiency, essentially related to a myriad of andropausal symptoms (sexual dysfunction, loss of bone and muscle mass, fatigue, depression, etc.) (Chahal and Drake, 2007; Gray et al., 1991), provides the baseline hormonal milieu of the ageing male.

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, one-way analysis of variance; CV, intra-assay coefficient of variation; DAB, diaminobenzidine; ECLIA, electrochemiluminescence immunoassay; HPA, hypothalamic–pituitary–adrenal; Orx, orchidectomized; Orx + T, orchidectomized and testosterone treated; PAP, peroxidase-antiperoxidase; PBS, phosphate buffered saline; POMC, proopiomelanocortin; RIF, relative intensity of fluorescence; SO, sham-operated.

* Corresponding author.
E-mail address: avlada@ibiss.bg.ac.rs (V. Ajdžanović).

http://dx.doi.org/10.1016/j.acthis.2015.04.002
0065-1281/© 2015 Elsevier GmbH. All rights reserved.

In view of the abovementioned data, the idea of some therapeutic, hormonal intervention during andropause seems logical and legitimate. More precisely, the irreversible, age-related changes at the organic level largely exclude the causal mode of therapy, so testosterone supplementation appears to be the mainstay of a symptomatic approach (Beg et al., 2008). Such supplementation should inevitably imply some potential effects on the andropausal glucocorticoid secretion. Furthermore, it was shown that testosterone application in ageing males may prevent or reverse sexual dysfunction, bone loss, muscle weakness or depressive symptoms (Amore et al., 2009; Haren et al., 2006; Myers and Meacham, 2003), but due to the possible side effects (cardiovascular complications, prostate carcinogenesis, etc.) close monitoring for efficacy and safety is advised and there is still no consensus about this therapeutic approach (Myers and Meacham, 2003; Theodoraki and Bouloux, 2009). On the other hand, it was observed that the HPA axis functioning and responsiveness to stress in male rats are significantly dependent on testosterone levels, proposed to have a central, inhibitory influence on the axis (Evuarherhe et al., 2009; Handa et al., 1994). Also, some studies in mice have shown that the local aromatization of applied androgen in estrogens enables its inhibitory effect through estrogen receptors in the hypothalamic paraventricular nucleus, the crucial HPA axis driving force (Lund et al., 2004). Testosterone has long ago been found to negatively affect the pituitary proopiomelanocortin (POMC–adrenocorticotropic hormone (ACTH) precursor) derived peptide content, either in ovariectomized female or orchidectomized male rats (Wardlaw, 1988). It could be said that testosterone application in males is promising from the perspective of HPA axis/glucocorticoid excess attenuation.

We assumed that the capacity for ACTH and corticosterone secretion (reflecting the specific operative level of HPA axis) may decrease after testosterone application to ageing male rats, while the idea was to illustratively explain a phenomenology of the potential decrease using diverse microscopic and biochemical methodologies. Accordingly, we focused on the immuno-histomorphometric, -fluorescent and secretory characteristics of pituitary ACTH cells together with adrenocortical *zona fasciculata* vascular profile, corticosterone production and serum levels in a rat model of the andropause. Orchidectomized middle-aged rats, in which both testicular testosterone and estradiol remain eliminated from the hormone milieu, were used in order to mimic the andropause. This experimental set-up also corresponds to an established approach for examining the potential effects of synthetic steroids/steroid-like compounds on endocrine homeostasis (Ajdžanović et al., 2009a,b, 2011, 2014; Filipović et al., 2013).

Materials and methods

Animals and experimental design

The experiments involved 24 middle-aged (16-month-old) Wistar rats. They were bred at the Institute for Biological Research "Siniša Stanković", Belgrade, Serbia; housed one *per* cage, and maintained under constant laboratory conditions (22 ± 2 °C, 12–12 h light–dark cycle) with free access to food (standard diet; the chemical composition is given in Table 1) and water. At the age of 15 months, the experimental animals were randomly bilaterally orchidectomized (Orx; $n = 16$ animals) or sham-operated (**SO**; $n = 8$ animals) under Ketamine anaesthesia (15 mg/kg b.w.; Richter Pharma, Wels, Austria). Recovery period was two weeks. Orchidectomized rats were then divided into two groups of eight animals ($n = 8$) each. The first group was subcutaneously treated with testosterone propionate (Fluka Chemie AG, Buchs, Switzerland; **Orx + T**) in a dose of 5 mg/kg b.w. (Filipović et al., 2013) every day except on Sundays, for 3 weeks. The final injected volume was 0.5 ml *per* animal. The second orchidectomized group (**Orx**) and the **SO** group were given the same volume (0.5 ml) of vehicle (sterile olive oil) alone, and served as controls. Body mass in all the groups was weighed before surgery, before treatment and after treatment. All the animals ($n = 24$) were sacrificed by decapitation under ether anesthesia (*ether ad narcosis* Ph. Iug. III., Lek, Ljubljana, Slovenia) 24 h after the last injection. All experimental procedures were in compliance with Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

Table 1
Chemical composition of the rat food used in experiment.

Chemical composition	
Metabolizable energy	11,000 kJ/kg
Protein, not less than	20%
Moisture, not more than	13%
Ash, not more than	10%
Cellulose, not more than	8%
Calcium, not less than	1%
Lysine, not less than	0.90%
Methionine + cystine, not less than	0.75%
Phosphorus, not less than	0.50%
Sodium	0.15–0.25%
Vitamin A, not less than	10,000 IU/kg
Vitamin D3, not less than	1600 IU/kg
Vitamin E, not less than	25 mg/kg
Vitamin B12, not less than	0.02 mg/kg
Zinc, not less than	100 mg/kg
Iron, not less than	100 mg/kg
Manganese, not less than	30 mg/kg
Copper, not less than	20 mg/kg
Iodine, not less than	0.5 mg/kg
Selenium, not less than	0.1 mg/kg
Antioxidant, not less than	100 mg/kg

Light microscopy, histochemistry and immuno-histochemistry

The pituitary and adrenal glands were excised, weighed, fixed in 4% paraformaldehyde for 24 h, dehydrated in a series of increasing concentrations of ethanol, enlightened in xylol and embedded in paraplast. Serial 5-μm thick tissue sections were deparaffinized in xylol and rehydrated in a series of decreasing concentrations of ethanol. For the light microscopy analysis, adrenals were histochemically stained following the Novelli procedure, while ACTH-producing cells were immunolabelled using the peroxidase–antiperoxidase complex (PAP) method of Sternberger et al. (1970).

In brief, Novelli histochemical staining is used for gaining insight into the tissue vascular profile, and beside deparaffinization and rehydration of adrenal sections involved their incubation in hot 1 N HCl (60 °C, 3 min), 1% acid fuchsine (30 s) and 1% light green (30 s), respectively. The mentioned individual incubations were followed by washing in distilled water, and after the last one, dehydration and mounting in DPX were carried out. As the result, purple erythrocytes were clearly visible against the bright green background of the adrenal cortex.

After rehydration of the pituitary sections, the endogenous peroxidase activity was blocked by incubation in 9 mmol (0.3%) hydrogen peroxide solution in methanol for 15 min at 24 °C. Before the application of specific primary antisera, nonspecific background staining was prevented by incubation of the sections with non-immune, *i.e.* normal porcine serum, diluted (1:10) with phosphate buffered saline (PBS) pH 7.4, for 45 min at 24 °C. Pituitary sections

Download English Version:

https://daneshyari.com/en/article/1923515

Download Persian Version:

https://daneshyari.com/article/1923515

Daneshyari.com