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Testosterone and estradiol treatments differently affect pituitary-thyroid axis and liver deiodinase 1 activity in orchidectomized middle-aged rats

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ABSTRACT

We previously reported that orchidectomy (Orx) of middle-aged rats (15–16-month-old; MA) slightly affected pituitary-thyroid axis, but decreased liver deiodinase (Dio) type 1 and pituitary Dio2 enzyme activities. At present, we examined the effects of subsequent testosterone-propionate treatment (5 mg/kg; Orx + T), and compared the effects of testosterone with the effects of estradiol-dipropionate (0.06 mg/kg; Orx + E) treatment. Hormones were subcutaneously administered, daily, for three weeks, while Orx and sham-operated (SO) controls received only the vehicle. The applied dose of T did not alter serum TSH, T_4 and T_3 concentrations in Orx- MA, though it increased TSH when administrated to Orx young adults (2.5-month-old; Orx-YA). However, pituitaries of Orx–MA + T rats had higher relative intensity of immunofluorescence (RIF) for TSHβ; in their thyroids we found increased volume and height of follicular epithelium, decreased volume of the colloid and higher RIF for T_4-bound to thyroglobulin (Tg-T_4). Liver Dio1 activity was increased. E-treatment did not affect serum hormone levels, pituitary RIF for TSHβ, or liver Dio1 activity in Orx-MA rats. Thyroids had decreased relative volume and height of follicular epithelium, increased relative volume of the colloid, decreased volume of sodium-iodide symporter-immunopositive epithelium and lower RIF for Tg-T_4. Detected changes were statistically significant. In conclusion, androgenization enhanced pituitary TSHβ RIF, thyroid activation and liver Dio1 enzyme activity in Orx-MA, without elevating serum TSH as in Orx-YA rats. Estrogenization induced pituitary enlargement with no effect on pituitary TSHβ RIF, serum TSH or liver Dio1 activity. E also induced alterations in thyroid histology that indicate mild suppression of its functioning, and contributed to thyroid blood vessel enlargement in Orx-MA rats.

1. Introduction

Serum testosterone (T) levels in men decline progressively with age. This is associated with numerous symptoms and poor health condition, including type-2 diabetes, higher incidence of cardiovascular disease, and increased mortality (Samaras et al., 2013).

Testosterone supplementation therapy (TST) creates a wide range of positive effects on most symptoms of andropause (Isidori et al., 2015). Although there is a potential risk of this treatment (Samaras et al., 2013), use of TST has increased over the past decade (Klotz, 2015). Estrogen (E) plays important role in male physiology. Some negative andropause symptoms are reduced by E-treatment. In men with prostate carcinoma androgen ablation therapy increases the risk of osteoporosis and bone fractures (Lipton et al., 2012). Estrogen therapy can also induce state of androgen deprivation and exert a beneficial effect on bone preservation (Eriksson et al., 1995), but is toxic and induce breast growth in men (Cox and Crawford, 1995). However, parenteral administration of E in men with metastatic prostate cancer was reported to reduce risk of its toxicity (Oh, 2002; Hedlund et al., 2008).

Thyroid cancer is more common in women than in men and it is most common in women during their reproductive years. However, among elderly population, men are at higher risk and the thyroid cancer is more aggressive then in elderly women (Rukhman and Silverberg, 2011). Several aspects of the hypothalamic-pituitary-thyroid axis are sexually dimorphic. This is more obvious in rodents than in humans — male rodents have higher thyrotropin (TSH) values in sera than females (Chen, 1984). Sex steroids differently affect proliferation of follicular

Abbreviations: Dio1, deiodinase type 1 enzyme; Dio2, deiodinase type 2 enzyme;; DAB, diaminobenzidine tetrahydrochloride; E, estradiol; HRP, horseradish peroxidase; IHC, immunohistochemistry; IFC, immunofluorescence; MA, middle-aged adults; NIS, sodium-iodide symporter; PBS, phosphate buffer saline; RER, rough endoplasmic reticulum; RIF, relative intensity of fluorescence; ROI, region of interest; SD, standard deviation; Orx, orchidectomy; Orx-YA, orchidectomized young adult males; Orx-MA, orchidectomized middle-aged males; OrxPBS, phosphate saline buffer; RIA, radioimmunoassay; SO, sham-operated testicle-intact males; TEM, transmission electron microscopy; T, testosterone; TST, testosterone supplementation therapy; Tg, thyroglobulin; Tg-T_4, T_4bound to thyroglobulin; TH, thyroid hormones; T_4, thyroxine; T_3, triiodothyronine; TSH, thyroid-stimulating hormone; TRH, thyrotropin-releasing hormone; YA, young-aged adults;; VEGF, vascular endothelial growth factor.

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epithelium and sodium-iodide symporter (NIS) gene expression in the thyroid tissue (Banu et al., 2002; Stanley et al., 2010). Liver iodothyronine deiodinase type 1 (Dio1) enzyme activity is higher in males (Miyashita et al., 1995). Some of these sex-related differences may change with advancing age (Chen, 1984; da Costa et al., 2001; Schomburg et al., 2007). In rodents, aging is associated with mild central hypothyroidism in both genders (Donda et al., 1987; Cizza et al., 1992).

Orchidectomy (Orx) of young male rats has been reported to decrease concentration of TSH in circulation, while subsequent T treatment increased TSH. However, the stimulatory effect of androgens seems to cease with advancing age (Chen, 1984). When we tested how pituitary-thyroid-periphery signaling responds to Orx in middle-aged rats (MA), liver Dio1 and pituitary Dio2 enzyme activities were decreased. Minor changes in thyroid structure, besides unchanged serum thyroxine (T_4) and TSH in Orx-MA, were detected. Our testicle-intact MA model was characterized by almost 50% and 30% lower serum T and T_4, respectively, without apparent changes in concentration of TSH in comparison to young adult (YA) males (Šošić-Jurjević et al., 2012). Therefore, we hypothesized that the pituitary–thyroid axis of MA adults would be less sensitive to changes in the androgen milieu.

To further test this hypothesis, in the present study we examined if the high dose of testosterone may provoke expected stimulation of pituitary-thyroid axis and increase liver Dio1 activity in Orx-MA rats. Keeping in mind that both T and E treatments are applied in therapy of aging men, and that several points of HPT system regulation are sexually dimorphic, we also aimed to examine potential differences between the effects of androgenization and estrogenization. Effects of both sex steroid treatments on serum concentration of TSH, total T_4 and T_3, which we used as markers of pituitary-thyroid axis functioning, were evaluated in comparison with the age-matched Orx- and sham-operated (SO)- MA controls, as well as with equally-treated Orx young-aged (Orx-YA) animals. Histological and liver Dio1 activity assessments were performed in MA rats. The special attention has been paid to histostructural, ultrastructural, immunohistochemical (IHC) and immunofluorescence (IFC) examination of the thyroid tissue, due to increased risk of thyroid carcinomas (La Vecchia et al., 1999; Stanley et al., 2012; Zhang et al., 2015) development related to sex steroid treatments.

2. Material and methods

2.1. Animals, treatments and organ processing

Wistar rats were housed in the unit for experimental animals at the Institute for Biological Research "Siniša Stanković", Serbia. All animals were fed ad libitum and were maintained at constant light (12 h light/12 h dark) and temperature (21 ± 2 °C) conditions.

At the age of 2 (YA) and 15 (MA) months, rats were orchidectomized (Orx-YA and Orx-MA, respectively) under ketamine anesthesia (15 mg/kg body weight of Ketamidor10%, Richter Pharma, Wels, Austria. Two weeks after the surgery, Orx animals were divided in the following groups: (i) testosterone-treated Orx-YA (Orx-YA + T) and Orx-MA (Orx-MA + T) groups were injected subcutaneously (*s.c.*) with 5 mg/kg b.w. testosterone-propionate (Fluka Chemie AG, Buchs, Switzerland); (ii) estradiol-treated Orx-YA (Orx-YA + E) and Orx-MA (Orx-MA + E) group was injected *s.c.* with 0.06 mg/kg b.w. estradiol-dipropionate (ICN Galenika Pharmaceuticals, Belgrade, Serbia). All substances were administered daily for three weeks. Rats in the control sham-operated (SO) and Orx group were *s.c.* administered the same volume of vehicle (sterile olive oil) according to the same schedule. Each group contained 6 animals.

The animals were decapitated 24 h after the last treatment. Blood was collected from the trunk of each animal (both YA and MA groups) and allowed to clot by leaving it at room temperature in glass tubes (without any coagulant) for at least 30 min. The clot was removed by centrifuging (room temperature; 1000–2000 ×*g*, 15 min). The sera (supernatant) were transferred to eppendorf tubes and, together with liver samples, stored at −70 °C.

The pituitaries and one thyroid lobe only from MA group of animals were fixed in Bouin's solution, dehydrated and embedded in Histowax (Histolab Product Ab, Göteborg, Sweden) for light or confocal microscopy. The other thyroid lobe was further processed for transmission electron microscopy (TEM).

Weight loss/gain for each animal was calculated by subtracting final treatment weight from pretreatment weight (in case of orchidectomy from precastration weight). The relative organ weights are expressed as an absolute organ weight (mg)/final body weight (g) × 100.

All animal procedures were in agreement with the EEC Directive (86/609/EEC) Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes, and approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

2.2. Novelli (acid fuchsin-light green) histochemical staining

Novelli histochemical staining (Novelli, 1959) was used for gaining insight into the thyroid tissue vascular profile. In brief, deparaffinized and rehydrated thyroid sections were incubated in hot 1 N HCl (60 °C, 3 min), followed by staining in 1% acid fuchsin (Fluka Chemie AG, Buchs, Switzerland; 30 s) and 1% light green (Sigma-Aldrich, St. Louis, MO, USA; 3 min), respectively. In between, the slides were washed in distilled water, and after the last one, dehydration and mounting in DPX (Sigma-Aldrich, Barcelona, Spain) were carried out. As the result, acid fuchsin-stained purple erythrocytes within the blood vessels and capillary network were clearly visible at the green background of the thyroid tissue.

2.3. Immunohistochemistry

Procedures for IHC and IFC staining were performed as previously described (Miler et al., 2014; Šošić-Jurjević et al., 2014).

Pituitary thyrotrophs or lactotrophs were identified by incubation with polyclonal rabbit anti–rat TSHβ or prolactin (donation from Dr. A. F. Parlow, National Institute of Health, Bethesda, MD, USA; 1:500) or monoclonal mouse anti-human prolactin (Prl; Abcam, Cambridge, UK; 1:200) antibodies overnight at 4 °C.

For double immune-detection, DAKO EnVision Doublestain System was applied (Dako North America, Inc. Carpinteria, CA, USA) according to the manufacturer's instruction.

For IHC and IFC characterization of thyroid tissue, the following primary antisera were applied overnight at 4 °C: the rabbit antisera directed against rat NIS (Acris antibodies GmbH, Herford,Germany; 1:1200); the rabbit antisera directed against human vascular endothelial growth factor (VEGF; Abcam, Cambridge, UK; 1:100); the rabbit antisera directed against human thyroglobulin (Tg; Dakopatts, Glostrup, Denmark; 1:500); and the mouse antisera directed against human T_4 bound to thyroglobulin (Tg-T_4 monoclonal antibody; QED Bioscience, San Diego, CA, USA; 1:300).

For immunodetection of NIS and Tg, swine anti-rabbit IgG- horseradish peroxidase (HRP; Dakopatts, Glostrup, Denmark; 1:100) was applied as a secondary antiserum for 1 h. For VEGF, Vectastain ABC Kit (Vector Laboratories, Burlingame, USA) was applied according to procedure suggested by the manufacturer.

Visualizations were performed using diaminobenzidine tetrahydrochloride (DAB; Dakopatts, Glostrup, Denmark) or fast red (Sigma-Aldrich, Barcelona, Spain) chromogen substrates at concentrations suggested by the manufacturer.

The sections were counter-stained with hematoxylin and mounted in DPX medium (Sigma-Aldrich, Barcelona, Spain). Digital images were made on a DM RB Photomicroscope with a DFC 320 CCD Camera (Leica, Germany).