Orchidectomy of middle-aged rats decreases liver deiodinase 1 and pituitary deiodinase 2 activity

Branka Šošić-Jurjević, Branko Filipović, Kostja Renko¹, Vladimir Ajdžanović, Milica Manojlović-Stojanoski, Verica Milošević and Josef Köhrle¹

Department of Cytology, Institute for Biological Research ‘Siniša Stanković’, University of Belgrade, Despot Stefan Boulevard 142, 11000 Belgrade, Serbia
¹Institut für Experimentelle Endokrinologie, Charité - Universitätsmedizin Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany
(Correspondence should be addressed to B Šošić-Jurjević; Email: brankasj@ibiss.bg.ac.rs)

Abstract

Endogenous androgens are involved in regulation of thyroid function and metabolism of thyroid hormones. As serum testosterone level progressively declines with age, this regulation may change. We tested how androgen deprivation, achieved by orchidectomy, affects thyroid homeostasis in middle-aged rats. Fifteen-month-old Wistar rats were orchidectomized (Orx) or sham–operated under ketamine anesthesia (15 mg/kg body weight). Five weeks after the surgery, animals were decapitated. Thyroids were used for histomorphometric and ultrastructural examinations and together with livers and pituitaries for real-time quantitative PCR and deiodinase (DIO) activity measurements. Serum testosterone, TSH, T₃-thyroxine (T₃), and cholesterol (Chol) levels were determined. As expected, middle-aged control rats had lower (P<0.05) testosterone and T₄ compared with 3-month-old males. In the Orx middle-aged group, we detected diminished serum testosterone (P<0.05), no change in TSH and T₄ levels, and higher Chol level (P<0.05), in comparison with age-matched controls. Histomorphometric analysis of thyroid tissue revealed decreased relative volume densities of follicles and colloid (P<0.05). Relevant gene expressions and DIO1 enzyme activity were not changed in the thyroids of Orx rats. Liver Dio1 gene expression and DIO1 activity were decreased (P<0.05) in comparison with the control values. Pituitary levels of TSHβ, Dio1, and Dio2 mRNAs did not change, while DIO2 activity decreased (P<0.05). In conclusion, orchidectomy of middle-aged rats affected thyroid structure with no effect on serum T₄ and TSH. However, decreased liver DIO1 and pituitary DIO2 enzyme activities indicate compensatory–adaptive changes in local T₃ production.


Introduction

Homeostasis of thyroid hormone (TH) status is regulated by systemic control of TH release from the thyroid and by local enzymatic activation and inactivation of TH in extrathyroidal tissues that is catalyzed by iodothyronine deiodinase (DIO) enzymes. Endogenous sex steroids, both estrogen and testosterone, play an important yet poorly defined role in regulation of the hypothalamus–pituitary–thyroid (HPT) axis and TH homeostasis.

Clinical evidence demonstrated that female patients have higher incidence of thyroid dysfunction and carcinomas than men (Ron et al. 1987). However, elderly men with thyroid carcinomas have much worse prognosis than women (Morganti et al. 2005). In animal models, rats in particular, sexual dimorphism is more clearly evident than in humans: young adult males have higher serum TSH levels than females (Chen 1984, Banu et al. 2001, Marassi et al. 2007). In addition to effects on hypothalamic–pituitary hormone regulation (Donda et al. 1990, Köhrle et al. 1995, Schomburg & Bauer 1997), both testosterone and estrogen may differently affect thyroid homeostasis in vivo by affecting thyroid follicular epithelium (Pelletier & El-Alfy 2000, Banu et al. 2002), level of TH carrier proteins in circulation (Bisschop et al. 2006), and/or DIO enzyme activities in peripheral organs (Köhrle et al. 1995, Lisbôa et al. 2001).

Iodothyronine DIO plays an important role in maintaining systemic TH status, as well as local control of TH action. Three DIO isoenzymes have been identified in mammals (Bianco et al. 2002) so far: type 1 (DIO1), type 2 (DIO2), and type 3 (DIO3). Their expression and activity are highly development and tissue specific and depend on numerous hormonal, environmental, and nutritional factors (Köhrle 2002). DIO1 enzyme activity is mainly regulated by triiodothyronine (T₃; Köhrle 1994), but sex steroids may also be involved in this regulation. Liver DIO1 activity is higher in young male than in female rats (Harris et al. 1979, Donda et al. 1990, Miyashita et al. 1995), while pituitary DIO1 activity is lower in young males than in females (Köhrle et al. 1995, Lisbôa et al. 2001). It seems that sexual dimorphism in
this enzyme expression and activity may change with advancing age, as was reported for DIO1 activity in mouse liver (Schomburg et al. 2007).

Hormone level changes are an important part of aging. Aside from well-documented partial decline in circulating testosterone, aging could not be clearly associated with hypothyroidism in men (Lamberts et al. 1997). However, more recent studies suggest that elderly people and centenarians have higher serum TSH concentrations than younger individuals (Surks & Hollowell 2007, Atzmon et al. 2009). In male rats, aging is characterized by lower serum testosterone and TH levels, along with unchanged serum and pituitary TSH levels (Reymond et al. 1992, Ciza et al. 1995). At the level of hypothalamic–pituitary hormone regulation, aging seems to be associated with mild central hypothyroidism in both genders (Donda et al. 1990, Cizza et al. 1995).

Although numerous experimental studies have been undertaken to determine mechanisms and molecular targets of testosterone impact on thyroid homeostasis, results obtained so far are incomplete. Much more data are currently available for young than for aged animal models. The aim of our study was to determine changes in thyroid homeostasis after testosterone deprivation achieved by bilateral castration of middle-aged rats, which we used as a model of andropause. Besides hormonal, histomorphometric, and ultrastructural assessment of thyroid tissue, we examined changes in Dio gene expressions and activities, as potential molecular targets of testosterone action in the pituitary, thyroid, and liver.

Materials and Methods

Experimental animals

Male Wistar rats were housed in the unit for experimental animals at the Institute for Biological Research ‘Sinisa Stankovic’ in Belgrade, Serbia. They were kept individually under constant laboratory conditions – room temperature (22 ± 2 °C) and lighting (12 h light:12 h darkness).

At the age of 15–16 months, animals were randomly divided into two groups: one was bilaterally orchidectomized (Orx, n = 12) under ketamine anesthesia (15 mg/kg body weight) via scrotal route. The second group (SO; n = 12) was sham-operated (SO), in which testicles were exposed but not removed. Three-month-old young adult rats (C; n = 6) served as control group for SO middle-aged rats and were SO as well. Five weeks after the surgery, all animals were decapitated. Blood was collected from the trunk of both young and middle-aged animals and the sera were stored at −70 °C.

The pituitaries, thyroids, and livers from middle-aged rats were excised and weighed. Organs from six animals were used for histological and ultrastructural examinations, while organs from another group of six animals were used for molecular and enzyme analyses.

The protocols were approved by the Animal Care and Use Committee of our Institute, following recommendations provided in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS no. 123, Appendix A).

Determination of serum hormone and cholesterol levels

Total testosterone level was determined using enzyme immunoassay kit (Adaltis Italia spa, Bologna, Italy). According to the manufacturer, the lower limit of detection was 0.2 ng/ml, and the calibration range was up to 16 ng/ml. The sensitivity was 0.01 ng/ml. The cross-reactivity with DHT was 10%, with androstenedione 0.8%, androsterone 0.0%, and with DHEA-S 0.0%. All samples were assayed in duplicate together in one run, with intra-assay CV of 3–9%.

Serum TSH was measured with a commercially available rat TSH RIA kit (Immunodiagnostics Systems GmbH, Frankfurt am Main, Germany). According to the manufacturer, the lower limit of detection was 1–03 ng/ml and the calibration range was up to 30 ng/ml. The sensitivity was 0.33 ng/ml. The cross-reactivity with GH was <0.1%, with PRL <0.1%, with LH <1%, and FSH <4%. All samples were assayed in duplicate together in one run, with an intra-assay CV of 6–1%.

As rat serum has a different composition of TH binding proteins, we adapted human total thyroxine (T4) RIA assay (INEP, Zemun, Serbia) by dissolving the standards in rat TH-free serum (Stringer & Wynford-Thomas 1982). The rat TH-free serum was depleted of endogenous iodothyronines by pretreatment with 8-anilino-1-naphthalenesulfonic acid followed by overnight charcoal adsorption. The calibration range of the novel RIA was from 12.5 to 500 nmol/l, with the lower limit of detection of 6 nmol/l. The intra-assay CV was 4.7–10%. The assay showed good median recoveries of about 108% (range 94.4–115.8%). No significant cross-reaction was observed with T3 or with reverse T3 (rT3).

Total serum cholesterol (Chol) was determined with colorimetric CHOD/PAP assay, according to the manufacturer’s instructions (Serbolab, Kragujevac, Serbia). According to the manufacturer, the limit of detection was 0–156 nmol/l. All samples were assayed in duplicate, with CV of 2%.

Histological, morphometric, and ultrastructural analyses

Thyroid glands were excised together with trachea. One thyroid lobe (n = 6) was randomly chosen and further processed according to routine protocols for light microscopy. It was fixed in Bouin’s fixative for 48 h and dehydrated in increasing concentrations of ethanol and xylene. After embedding in Histowax (Histolab Product Ab, Gothenburg, Sweden), each tissue block was serially sectioned at 5 μm thickness on a rotary microtome (RM 2125RT Leica Microsystems, Wetzlar, Germany). Sections were further stained with hematoxylin and eosin (HE), according to standard procedures. Morphometric analyses of thyroids were carried out on HE sections, exactly as previously reported (Sosić-Jurjević et al. 2010). In brief, four to five transversal
sections from the anterior, central, and posterior parts of each animal's thyroid were analyzed by point-counting method (Weibel 1979), using the M12 multipurpose test grid inserted into the ocular of a Zeiss light microscope (Jena, Germany). Fifty test fields were counted per animal at a total magnification of 40 × 400.

Digital light microscopic images of thyroid tissue were made on a Leica DM RB Photo Microscope with a JVC TK 1280E Video Camera using the Qwin program (Leica) for the acquisition and analysis of the images.

For electron microscopy, sections of the other thyroid lobe (n = 4) were immersed immediately in 4% glutaraldehyde in 0-1 mol/l PBS (pH 7-4) for 24 h and postfixed in 1% OsO4 in the same buffer for 1 h. Tissue samples were dehydrated in a graded alcohol series and embedded in ERL. Ultrathin sections were cut on LKB Ultramicrotome III (type 8802A; Sweden), stained with uranyl acetate and lead citrate, and examined under a MORGAGN 268 (FEI Company, Hillsboro, OR, USA) transmission electron microscope.

Sample collection and preparation of tissue homogenates

Pituitaries, thyroids, and livers were removed, immediately frozen in liquid nitrogen, and stored at −80 °C for further processing. Frozen liver tissues (n = 6) were first pulverized under dry ice (solid CO2) in precooled Teflon containers, using a micro-dismembrator (B. Braun Biotech International GmbH, Melsungen, Germany). Aliquots of the powdered tissues were stored for further processing at −80 °C.

RNA extraction and quantification

Pituitaries for SO n = 6; for Orx n = 4) were initially homogenized with a TissueLyzer (Qiagen) and thyroids (for SO n = 6; for Orx n = 6) with Ultra Turrax (IKA Analytical, Staufen, Germany), and total RNAs were isolated using Qiagen RNeasy Mini kit (Qiagen). Total RNA from the liver tissue powder (for SO n = 6; for Orx n = 6) was isolated using the Trizol reagent (Invitrogen), according to the manufacturer's protocols. The purity and integrity of the total RNA of all samples were assessed using NanoDrop u.v.-vis spectrophotometer ND-1000 (PEQLAB, Erlangen, Germany). Absorbance ratio at 260/280 was calculated and found to be in the range of 1.8–2.0 for all RNA samples.

cDNA from all tissue homogenates was generated from 500 ng total RNA, using the iScript cDNA synthesis kit (Bio-Rad Laboratories). Transcript concentrations of Dio1 (pituitary, thyroid and liver), Dio2 (pituitary), Spot14 (Thy5) (liver), hypoxanthine guanine phosphoribosyl transferase (Hprt; pituitary, thyroid, and liver), thyroid peroxidase (Tpo; thyroid), and thyroglobulin (Tg; thyroid) were determined by quantitative real-time PCR (qRT-PCR) analysis. In short, primers were chosen with the help of Primer3 (http://www.bioinformatics.nl/cgi-bin/prime3plus/prime3plus.cgi). The primer sequences were

\( \text{Dio}1-e: 5'-\text{T}TTAAGAACAACGTGGACATCAGG-3'; \)
\( \text{Dio}1-r: 5'-\text{GGTTACCTTGTACGACTCTCT-3'}; \)
\( \text{Dio}2-e: 5'-\text{GGCACCTGACCCCAAATCTAG-3'}; \)
\( \text{Dio}2-r: 5'-\text{GCAGACATGCGCTTCTTCTGTG-3'}; \)
\( \text{TSHB}-e: 5'-\text{ATCACAGACTCCCTACAGAAGCAG-3'}; \)
\( \text{TSHB}-r: 5'-\text{GGCAAACGGGGTGGAGAAATAAA-3'}; \)
\( \text{Hprt}-e: 5'-\text{TATGAGACAGACTGAAAGACTTT-3'}; \)
\( \text{Hprt}-r: 5'-\text{CAGCGAGCTGCAAAGAAACTTATA-3'}; \)
\( \text{Tpo}-e: 5'-\text{TGCGATCTGACATTCAGTCAGTTG-3'}; \)
\( \text{Tpo}-r: 5'-\text{ATCTTGTGACCATGCTTCTTG-3'}; \)
\( \text{Tg}-e: 5'-\text{CGGCAATATCTGCAGACAGAT-3'}; \)
\( \text{Tg}-r: 5'-\text{GGCAAGCTTTGGATATATGGA-3'}; \)
\( \text{Tg}-f: 5'-\text{CTTACCCACCT-0-TTGTTACCCTTCCTGAGG-3'}; \)
\( \text{Spot14}-e: 5'-\text{CTTACCCACCT-0-TTGTTACCCTTCCTGAGG-3'}; \)
\( \text{Spot14}-r: 5'-\text{CAATATCTGCAGACAGAT-3'}; \)
\( \text{Spot14}-f: 5'-\text{GGCAAGCTTTGGATATATGGA-3'}; \)

Amplifications were conducted in duplicate with the Absolute qPCR SYBR Green Fluorescein Mix (Thermo Fisher Scientific, Inc., ABgene products, Epsom, UK), on a RT-PCR detection system (iCycler; Bio-Rad Laboratories). The program included the following steps: initial denaturation at 95 °C for 10 min, followed by 40 cycles, each including a denaturation step at 95 °C for 30 s, an annealing step at 60 °C for 45 s, and an elongation step at 72 °C for 30 s. At the end of a final elongation step at 72 °C for 3 min the reaction mix was cooled at 8 °C.

The optimization of PCR and validation of all PCR primer sets used in this study was previously determined (http://edoc.hu-berlin.de/docviews/abstract.php?id=36909).

Hprt, Gapdh, and 18S rRNA were found to be most stably expressed genes in different rat tissues under different experimental conditions (methimazole or T4 treatment, gonadectomy, and subsequent hormone replacement). Based on these data, in this study, the three genes had the most similar expression level and amplification efficiency with all examined genes in different tissues.

![Figure 1](http://www.endocrinology-journals.org)

**Figure 1** Serum total testosterone (a), TSH (b), total thyroxine (T4; c), and cholesterol (Chol; d) levels of control young adult (C), sham-operated (SO) and orchidectomized (Orx) middle-aged rats; serum testosterone was determined by enzyme immunoassay, while TSH and T4 were determined by RIAs. Statistics: one-way ANOVAs and Bonferroni's multiple range post hoc test. The values are mean ± S.D.; n = 5–7; *P < 0.05 compared with C rats; **P < 0.05 compared with SO rats.
Orx 10.4 Group
homogenization buffer, pH 7, respectively, on ice. The buffer
SO 9.9 per animal) and further analyzed by an unpaired Student’s
modified Bradford assay using IgG as standard (Bio-Rad).

DIO activities in pituitary, thyroid, and liver were measured
samples were assayed in a reaction mixture containing
60 µg (pituitary), 7.5 µg (thyroid), or 20 µg (liver) proteins.
DTT (10 mmol/l) served as cofactor and [125I]rT3 was added
in the absence of 6-6-propyl-2-thio-uracil (PTU). Conditions had been optimized so that deiodination of
the substrate was <15%. For pituitaries and thyroids, the
fraction of iodide release blocked by 1 mmol/l PTU was ascribed to DIO1. However, no thyroid DIO2 activity has
been detected. Before use, [125I]rT3 was purified from free
iodide by chromatography using Sephadex LH-20 (Sigma–Aldrich).

After starting the enzymatic reaction by adding
[125I]rT3-containing substrate mix, the samples were incubated in a water bath at 37 °C. The reaction was stopped
by adding 50 µl BSA-PTU stop solutions and 10%
thiocloroacetic acid for precipitation of protein. After
centrifugation (20 °C, 14 000 g, 5 min), the supernatant was transferred on Dowex-50 WX–2-column (Bio–Rad).

Table 1 Morphometric parameters of the thyroid gland in sham-operated (SO) and orchidectomized (Orx) middle-aged rats. The results were obtained on four to five transversal sections from the anterior, central, and posterior parts of the thyroid, using M42 test system (50 test fields per animal) and further analyzed by an unpaired Student’s t-test. The values are mean ± S.D. (n = 6)

<table>
<thead>
<tr>
<th>Group</th>
<th>τ (µm)</th>
<th>ia = Vae/Vac</th>
<th>Vae (%)</th>
<th>Vai (%)</th>
<th>Vac (%)</th>
<th>Vif (%)</th>
</tr>
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<tbody>
<tr>
<td>SO</td>
<td>9.9 ± 0.3</td>
<td>1.4 ± 0.1</td>
<td>38.0 ± 2.4</td>
<td>32.5 ± 3.9</td>
<td>29.5 ± 2.9</td>
<td>67.6 ± 2.9</td>
</tr>
<tr>
<td>Orx</td>
<td>10.4 ± 0.5</td>
<td>1.5 ± 0.2</td>
<td>37.8 ± 1.4</td>
<td>36.7 ± 1.6*</td>
<td>25.4 ± 1.9*</td>
<td>63.8 ± 0.7*</td>
</tr>
</tbody>
</table>

τ, the height of thyroid follicular cells; ia, index of activation rate; Vae, relative volume densities of epithelium; Vai, interstitium; Vac, colloid; Vif, follicles. *P<0.05 vs SO rats.

Laboratories). Released $^{125}$Iodide was eluted by adding 10% acetic acid. $^{125}$-Iodide samples were measured on a gamma counter (Gamma Master, LKB Wallac, Turku, Finland) and background corrected. The mouse liver homogenate of known protein concentration was used as interassay variability control. After the assay, protein concentration from all samples was again determined by the Bradford assay, and the obtained results were used for calculation of the enzyme activities as described previously (Schomburg et al. 2006).

**Statistical analysis**

The statistical analyses were performed using GraphPad Prism v.5.01 for Windows (GraphPad Software, San Diego, CA, USA). The data are expressed as mean ± S.D., except for qRT-PCR results, which represent mean ± S.E.M. of the fold changes compared with basal levels of Hprt as reference gene.

One-way ANOVA was used to compare results on serum hormone and Chol levels, followed by the post hoc Bonferroni’s multiple range tests to detect differences between groups. The differences in morphometric results, DIO activities and organ weights between groups were evaluated by the unpaired Student’s $t$-test. The paired $t$-test was used to analyze the changes in body weight between SO and Orx groups. qRT-PCR data were calculated and evaluated using the Relative Expression Software Tool-Multiple Condition Solver (REST-MCS, version 2), as described (Pfaffl 2001). The minimum level of significance was set at $P<0.05$.

**Results**

**Body and organ weights**

The mean body weight of SO animals before the experiment was 675 ± 72 g and did not significantly change by the end of the experiment (691 ± 59 g). However, in the Orx group, significant body weight loss was revealed (by 11%, $P<0.05$). For the SO group, the mean pituitary, thyroid, and liver weights were 15 ± 1.9 mg, 33 ± 3 mg, and 17.4 ± 1.9 g respectively. No significant changes in organ weights were detected in the Orx group (the mean pituitary, thyroid, and liver weights were 16.3 ± 2.9 mg, 28.6 ± 3.5 mg, and 15.4 ± 2.1 g respectively).

**Serum hormone and Chol levels**

Middle-aged rats had 50 and 34% ($P<0.05$) lower serum total testosterone and T$_4$ respectively in comparison to young male adults. No age-related changes in serum TSH level were detected (Fig. 1).

**Figure 3** Thyroid follicular cells (FC) from sham-operated (a and b) and orchidectomized (c and d) middle-aged rat. BC, red blood cells; BV, blood vessel; CC, thyroid C cell; N, nucleus; RER, rough endoplasmic reticulum; LY, lysosomes; DB, dense bodies; C, colloid; AM, apical membrane; CR, crystalline inclusions. Transmission electron microscopy.
Surgical removal of both testicles in middle-aged rats resulted in reduced total serum testosterone by 90% \((P<0.05)\) compared with values obtained for age-matched SO controls. No change in serum concentration of TSH and total T4 was detected. Serum total Chol was 25% higher \((P<0.05)\) compared with the SO rats (Fig. 1).

Thyroid gland: histomorphometric and ultrastructural findings

Thyroid parenchyma of control SO middle-aged male rat consists of follicles of different sizes. Still, follicles composed of cuboidal follicular epithelium and moderate portion of dense luminal colloid were predominant (Fig. 2a). In Orx rat, thyroid follicles displayed a higher variation in size, and follicles of smaller size appeared more frequent in comparison with the SO rats (Fig. 2b). Further morphometric analyses demonstrated decreased relative volume of the follicles and colloid, by 15 and 17\% \((P<0.05)\) respectively, accompanied by a 12\% \((P<0.05)\) increase in relative volume density of interstitium in Orx rats (Table 1).

Ultrastructurally, thyrocytes of middle-aged SO rats had a cuboidal shape and polar organization of organelles – oval nuclei were at the base of the cells, followed by well-developed rough endoplasmic reticulum (RER), lysosomes, dense bodies, and microvilli projected into the colloid at the apical surface (Fig. 3a). However, quite a few thyrocytes were characterized by dilated and disoriented RER, without losing their cuboidal shape. Some of these contained geometrically shaped (rhomboid or rectangle) crystalline inclusions, encircled by proliferated and dilated RER. (Fig. 3a and b). In thyroids of Orx rats, similar ultrastructural organization of thyrocytes was observed, though no crystalline inclusions were detected (Fig. 3c and d).

Expression of genes relevant to the HPT axis in the pituitary, thyroid, and liver

In the pituitary, gene expressions of Dio1 and Dio2 and TSH-specific β subunit were examined. Upon quantification, no significant difference in mRNA levels of these genes was detected between SO and Orx groups (Fig. 4). In the thyroid, mRNA levels of Dio1, Dio2, Tg, and TPO were not significantly different between the Orx middle-aged group and SO rats (Fig. 5).

In the liver of Orx group, Dio1 expression was 2.7-fold lower \((P<0.05)\) than the value obtained for the SO group (Fig. 6a). Expression of the Spot14 gene did not significantly differ (Fig. 6b).

DIO activities in the pituitary, thyroid, and liver

As shown in Fig. 7 orchidectomy of middle-aged male rats induced a significant decrease in pituitary Dio2 and liver Dio1 activities, by 27 and 33\% respectively \((P<0.05)\). Pituitary and thyroid Dio1 activities were unchanged in comparison to the SO controls.

Discussion

This study demonstrates that castration of middle-aged rats induces small changes in thyroid structure, namely reduction in relative volume of follicles, accompanied by the corresponding changes of some components of the HPT axis, such as reduced activities of DIO1 in the liver and DIO2 in the pituitary. Serum concentrations of TSH and TH remained unchanged.

In this experiment, orchidectomy was applied as a classical approach for studying the effects of endogenous androgens on hormonal homeostasis. As expected, orchidectomy diminished serum testosterone levels (by 90\%) in our model of andropause. A small but significant decrease in body weight was detected in the Orx group, which may be due to a skeletal muscles atrophy (Rincon et al. 1996, Axell et al. 2006) and/or decreased food intake (Gentry & Wade 1976) induced by lack of androgens. Serum testosterone level in control middle-aged males was less than half that in young adults. Other researchers also demonstrated decreases in blood testosterone concentration with advancing age (Chen et al. 1994, 1996). Age-related partial decline of serum testosterone in men is associated with decline in sexual function, muscle function, bone density, and other physiological parameters, popularly termed andropause (Harman et al. 2001, Matsumoto 2002).

In addition to the decline in serum testosterone, in our middle-aged rats, T4 levels were significantly decreased in comparison with young adult males. Thyroid tissue of SO rats was characterized by the presence of inactive follicles, distented with dense colloid. Rao-Rupanagudi et al. (1992) and other researchers (Cizza et al. 1992, Mariotti et al. 1995) also reported similar histological changes and progressive loss of follicular tissue with advancing age in the thyroid of male Sprague–Dawley or Fisher rats respectively with significant decreases in free serum TH levels.
At the ultrastructural level, both in SO and in Orx middle-aged rats, besides numerous follicular cells with typical organization of cell organelles, quite a few thyrocytes were characterized by dilated and distended RER cisternae. Similar ultrastructural changes were previously reported in thyrocytes of aged mouse thyroid (Tamura & Fujita 1981, Nève & Rondeaux 1991). Tamura & Fujita (1981) described such ultrastructural changes in thyrocytes of ‘active’ follicles as degenerative. Both groups related them to follicular cell hyperactivity explained with compensation for lack of TH production in inactive, so-called ‘cold’ mice follicles. Further quantitative ultrastructural studies with rats at different life stages are needed to understand the observed features of thyrocytes under our experimental conditions.

In the thyroids of Orx middle-aged rats, no significant changes were detected in expression of genes involved in TH synthesis (Tg and Tpo genes) and deiodination (Dio1 and Dio2 genes). Similar to results of our previous study (Sosić-Jurjević et al. 2010), morphometric analyses of thyroid sections revealed changes in thyroid structure, namely reduction in relative volume density of follicles and colloid. Taking all obtained results together, we suppose that these histomorphometric changes reflect increased colloid reabsorption in Orx middle-aged rats.

Apart from the hormone synthesis and secretion by the thyroid gland, deiodination pathways in liver and kidney are the main contributors to TH metabolism, turnover, and homeostasis (Schweizer et al. 2008). High levels of Dio1 activity were found in these peripheral organs, both in rats and in humans (Visser 1996). Under our experimental conditions, a decrease in liver Dio1 expression, accompanied by a corresponding decrease in enzyme activity, was detected in Orx middle-aged rats, in line with other authors who demonstrated the same for Orx prepubertal and adult rats (Miyashita et al. 1995, Lisbôa et al. 2001, Marassi et al. 2007).

As no change in serum TH hormones was detected in Orx middle-aged males, this effect may be attributed to testosterone deprivation. In accordance with this, Miyashita et al. (1995) demonstrated that liver DIO1 activity in young adult males was higher than in females, both in euthyroid and thyroïdectomized animals.

Differential effects of sex hormones on Dio1 expression in liver and kidney were reported to change with advancing age. Thus, sexual dimorphic expression of liver Dio1 that was detected in young mice vanished at the age of 1 year, while sex-specific differences in renal Dio1 mRNA expression sustained with increasing age (Schomburg et al. 2007). Contrary to this, our results in middle-aged rats demonstrate preserved responsiveness of liver Dio1 to testosterone deprivation, while Dio1 expression in the rat kidney was not affected by castration (results not shown). Species-specific differences in age-related changes of Dio1 expression profile in the peripheral organs may indicate species-specific changes in metabolism and degradation of TH in aged animals. Further studies and comparisons of different animal species at different life stages are needed to determine the factors contributing to these changes.

Our results clearly demonstrate that liver DIO1 significantly decreased in Orx middle-aged rats. Decreased local production of T3 in the liver could act locally on expression of hepatic T3-responsive genes. However, we observed no change in expression of T3-regulated Spot14 gene in Orx middle-aged rats. The responsiveness of this gene to T3 seems to be decreased with advancing age (Mooradian et al. 1991). An increase in serum Chol level was detected in Orx middle-aged males, and we cannot rule out that a local decrease in T3 level might contribute to this change. Further examinations of expression profiles of other hepatic T3-responsive genes involved in regulation of Chol metabolism are needed to evaluate the significance of Orx-induced decline in DIO1 enzyme activity to decline in hepatic T3.

We further investigated whether deprivation of endogenous androgens affects pituitary TSH synthesis and secretion and whether DIO1 and DIO2 enzymes play a role in this regulation. No significant change in TSHβ gene expression and no change in serum TSH level were detected in Orx rats in comparison to the age-matched controls. However,
decreased DIO2 activity detected under our experimental conditions suggests some adaptation or compensation of local T3 production to altered thyroid or androgen status sensed by pituitary.

It was previously documented that the negative feedback control of TSH production by the anterior pituitary involves DIO enzymes, which are distinctly regulated by THs: DIO1 activity is upregulated by T3 (Köhrle 2002), while DIO2 is upregulated by low T4 (Christoffolete et al. 2006). As no change in serum T4 was detected in this experiment, decreased DIO2 activity detected under our experimental conditions was in part compensated by T3 from the serum. Further examinations at different time points during adulthood or after prolonged Orx are needed to distinguish between these two hypotheses. In addition, androgen-modulated paracrine regulation between different pituitary cell types, which express distinct levels of DIO enzymes and/or TH transporters, might influence the fine tuning of anterior pituitary feedback regulation of the HPT axis with increasing age (Baur et al. 2002, Denef 2008, Liao et al. 2011).

In conclusion, our results demonstrate that orchidectomy of rats at middle age induced changes in thyroid structure with no effect on serum T4 and TSH. However, decreased activities of DIO1 in the liver and DIO2 in the pituitary indicate tissue compensation or adaptation through changes in local production of T3.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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