Sexual dimorphism in thyroid function and type 1 iodothyronine deiodinase activity in pre-pubertal and adult rats

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Abstract

Iodothyronine deiodinase activities are regulated by sex steroids; however, the mechanisms underlying the reported sexual dimorphism are poorly defined. In the present report, we aimed to investigate whether type 1 deiodinase (D1) sexual dimorphism exists early in sexual development by studying pre-pubertal male (Pm) and female (Pf) rats, as well as adult controls (C) and gonadectomized male and females rats. Adult male Wistar rats were studied 21 days after orchietomy (Tex), and adult females were studied 21 days after ovariectomy (Ovx), and after estradiol benzoate (Eb) replacement. Serum total triiodothyronine (T3) was higher in pre-pubertal (P) rats than in the matching adults, with no difference between genders, although in adult males T3 was significantly lower than in females. There were no sex or age differences in serum total T4. Serum TSH in pre-pubertal (P) rats was within the adult female range, and both were significantly lower than in adult males. D1 activity in liver was greater in Pm than in Pf. In adult females, liver D1 activity was lower, while in adult males it was higher than in P rats. The same pattern of D1 activity was found in kidney. In thyroid and pituitary, D1 activity was

similar in Pm, Pf, and adult females, which were all significantly lower than in the adult male. There were no differences in serum T3 and T4 between C and Tex males, but serum TSH was significantly decreased in Tex rats. Hepatic and renal D1 activities were lower in Tex than in C, but no changes were detected in thyroid and pituitary. In Ovx females, T3 was significantly lower than in the C group. Serum T4 was significantly decreased by estradiol replacement therapy in Ovx rats, in both doses used, whereas TSH was unchanged. Eb replacement increased liver and thyroid D1 activity, but in the kidney, only the highest estradiol dose promoted a significant D1 increase. In conclusion, in males, hepatic and renal D1 activity appears to be significantly influenced by gonadal hormones, in contrast to females, in which only exogenous Eb treatment stimulated D1 activity. The comparison between pre-pubertal and adult rats suggests that serum T3 is not the main regulator of D1 activity, and other factors, besides T3 and gonadal hormones, can modulate D1 activity during murine maturation.

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Introduction

Thyroxine (T4), the main secretory product of the thyroid gland, is converted into active (triiodothyronine; T3) or inactive (reverse T3; rT3) metabolites after deiodination of its outer (5') or inner ring (5) respectively. Based on several functional criteria and molecular characterization, three distinct deiodinase enzymes have been identified: type 1 (D1), type 2 (D2), and type 3 (D3). D1 seems to be the only selenodeiodinase that can function as either an outer or inner ring iodothyronine deiodinase, while D2 and D3 are exclusively outer (D2) or inner (D3) deiodinases respectively. D1 is expressed in many tissues, such as liver, kidney, thyroid gland, central nervous system, pituitary, intestine, placenta, and others (St Germain 1994, Bianco *et al.* 2002).

The prevalence of thyroid dysfunction is greater in women than in men. These disorders often appear concomitantly

with changes in endogenous levels of sex steroids, in particular during puberty, pregnancy, and at the time of menopause. Besides this clinical aspect, various experimental studies with rats have established that sex hormones influence thyroid function, but the nature and significance of this influence have yet to be ascertained (Donda *et al.* 1990).

Cheron *et al.* (1980) reported lower serum T3 and higher serum thyroid-stimulating hormone (TSH) in adults than in 21- and 30-day-old pre-pubertal male (Pm) rats, without any significant difference in serum T4. The T4-5'-monodeiodination rates in liver homogenates were lower in 2-, 4-, and 9-day-old rats than adults, reaching the adult value in 21-day-old rats; however, the intrapituitary T4-5'-monodeiodination rate was higher in 2- and 4-day-old rats and fell to the adult values by 30 days of age.

In the adult rat, numerous sex-related differences in thyroid function have been reported, in particular lower plasma T4 and

TSH concentrations associated with a higher plasma T3 level in the female than in the male rats (Rapp & Pyun 1974, Fukuda *et al.* 1975, Greeley *et al.* 1983, Chen 1984, Corrêa da Costa *et al.* 2001, Moreira *et al.* 2005). Some studies demonstrated that liver D1 activity in females is lower than in male rats (Harris *et al.* 1979, Donda *et al.* 1990, Santini *et al.* 1994, Miyashita *et al.* 1995) and pituitary D1 activity in males is lower than in female rats (Donda *et al.* 1990, Köhrle *et al.* 1995).

Liver D1 activity is significantly decreased in orchiectomized rats and increased by testosterone administration. Besides, pituitary D1 activity is increased in castrated male rats, and testosterone administration does not affect pituitary D1 activity (Lisbôa *et al.* 2001). In contrast to male rats, several reports have shown that ovariectomy or β -estradiol administration does not alter liver D1 activity in female rats (Harris *et al.* 1979, Miyashita *et al.* 1995). However, Lisbôa *et al.* (1997, 2001) reported that ovariectomy produced a decrease in both liver and pituitary D1 activities and that treatment with estradiol was able to return these activities to control levels and to significantly increase thyroid D1.

The respective roles of androgens and estrogens on rat thyroid function are a matter of debate, since the previous studies are controversial. Probably, the inconclusive previous reports are due not only to different rat strains used, but mainly to the different developmental stages that have been studied. Hence, the aim of the present study was to further evaluate the sex-related differences found in thyroid function and D1 activity by comparing pre-pubertal rats, adult control, and gonadectomized rats. In the first phase of this study, we compared pre-pubertal with adult rats to assess the effects of emerging gonadal function on pituitary–thyroid axis regulation. In an attempt to better understand the respective roles of androgens and estrogens, in the second protocol, using only adult rats, we carried out the removal of gonads to evaluate the importance of gonadal hormones on thyroid function.

Materials and Methods

Animals

The study was approved by the Institutional Committee for Use of Animals in Research, and the procedures used were in compliance with the International Guiding Principles for Biomedical Research Involving Animals, the Council for International Organizations of Medical Sciences (Geneva, Switzerland), and the guiding principles for care and use of animals from the American Physiological Society. Pre-pubertal male (80–100 g) and female (70–90 g), and adult male (250–300 g) and female (200–250 g) Wistar rats were kept from birth in a temperature-controlled (22–25 °C) animal room, with a 12 h light:12 h darkness cycle, and pelleted commercial chow (Paulínea, São Paulo, Brazil; iodine content 2 mg/kg) and water were available *ad libitum*.

Experimental groups

Pre-pubertal animals did not suffer any treatment. Adult male animals were divided into two groups: sham-operated (control) and orchiectomized (Tex) rats killed 21 days after the surgery. All adult female rats showed a regular 4–5 day estrous cycle monitored by vaginal cytology collected each morning for 2 consecutive weeks before starting the experiments. Adult female rats were divided into four groups: sham-operated (control), ovariectomized treated with vehicle (Ovx), or treated with 17 β -estradiol benzoate (Eb; Sigma), in a physiological dose of 0.7 μ g/100 g body weight per day (Ovx+0.7), or in a supraphysiological dose of 14 μ g/100 g bw per day (Ovx+14), administered subcutaneously for 21 days. The pre-pubertal and adult animals were killed by decapitation and blood was collected for hormone concentration analyses. Serum was obtained after centrifugation of the blood at 3000 r.p.m. for 20 min and stored at –20 °C. Rat tissues (liver, kidney, thyroid, and pituitary) were dissected out and stored at –70 °C until processing for enzymatic measurements.

RIA for estradiol and testosterone

Serum total estradiol and testosterone were determined by specific Coated-Tube RIA kits (estradiol: DSL-4400, EUA; testosterone: DSL-4100, EUA). All the procedures were carried out following the fabricant recommendations.

RIA for total T3, T4, and TSH

The measurement of serum TSH levels was carried out using a specific RIA for rat TSH obtained from the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK, Bethesda, EUA), and expressed in terms of reference preparations 3 (RP-3).

Serum T3 and T4 were determined by specific Coated-Tube RIA kits (T3: DLS-3100 Active TX, EUA; T4: DLS-3200 Active TX, EUA). Hormone-stripped rat serum was used for the standard curves of total T3, T4, and TSH. All the procedures were carried out following the fabricant recommendations.

Type 1 iodothyronine deiodinase activity

The type 1 activity was determined using methods previously published (Berry *et al.* 1991), as adapted by A C Bianco and P R Larsen (personal communication). In short, each thyroid, pools of two pituitary glands, and 25 mg liver and kidney were homogenized in 1 ml 0.1 M sodium phosphate buffer containing 1 mM EDTA, 0.25 M sucrose, and 10 mM dithiothreitol, pH 6.9. Homogenates (150 μ g protein for pituitary samples and 30 μ g protein for liver, thyroid, and kidney) were incubated, in duplicate, for 1 h at 37 °C with 1 μ M rT3 (Sigma), freshly purified (Sephadex LH20) tracer [¹²⁵I]rT3 (Perkin–Elmer Life Sciences, Boston, MA, USA), and 10 mM dithiothreitol in 100 mM potassium phosphate buffer, pH 6.9, containing 1 mM EDTA. Total reaction

volume was 300 µl. Blank incubations were carried out in the absence of protein. The reaction was stopped in an ice-bath followed by immediate addition of 200 µl fetal bovine serum (Cultilab, BR) and 100 µl trichloroacetic acid (50%, v/v). After thorough mixing (Vortex), the samples were centrifuged at 8000 g for 3 min, and an aliquot of the supernatant was collected for measurement of ^{125}I liberated during the deiodination reaction. The specific enzyme activity was expressed as picomoles of rT3 deiodinated/min mg protein. Although type 2 deiodinase (D2) can also be present in the thyroid and pituitary glands, in our assay conditions only D1 activity is measured since deiodinase activities were completely blocked in the presence of 100 mM propylthiouracil (PTU), a specific D1 inhibitor (data not shown).

Protein was measured by the Bradford method (Bradford 1976), after incubation of homogenates with NaOH (2.5 M) for 30 min at room temperature.

Statistical analyses

We used at least four animals in each experimental group and the experiments were repeated thrice. Data from total T3, T4, and deiodinase activities are expressed as means $\pm$ S.E.M. and were analyzed by two-way ANOVA using the SuperANOVA program (Abacus Concept, Berkeley, CA, USA), by one-way ANOVA, or by unpaired *t*-test using the Graphpad Prism software (version 4, Graphpad Software, Inc., San Diego, USA). The results of serum TSH, estradiol, and testosterone, which do not have a normal distribution, are expressed as median and minimum–maximum values and were analyzed by non-parametric ANOVA (Kruskal–Wallis test) or by Mann–Whitney test, using the Graphpad Prism software (version 4, Graphpad Software, Inc., San Diego, USA). A value of $P \leq 0.05$ was considered statistically significant.

Results

Pre-pubertal versus adult rats

Serum total T3, T4, and TSH concentrations Serum total T3 did not differ between female and male pre-pubertal rats, but was higher in adult female than in adult male rats. Moreover, serum T3 was significantly higher in pre-pubertal rats than in adult animals. Serum total T4 was not significantly

different among the groups studied. Serum TSH levels were not different between pre-pubertal and female adult rats, but were significantly higher in male adults when compared with the other groups (Table 1).

D1 activity Liver and renal D1 activities were significantly higher in male than in female pre-pubertal rats (liver, male pre-pubertal = 80.1 ± 4.7 and female pre-pubertal = 61.4 ± 2.6 ; renal, male pre-pubertal = 70.7 ± 3.2 and female pre-pubertal = 62.9 ± 1.3 pmoles rT3/min mg protein). The liver and renal D1 activities were markedly higher in male adult rats than in pre-pubertal and female adult animals (liver, male adult = 95.9 ± 3.2 and female adult = 28.2 ± 2.1 ; renal, male adult = 96.8 ± 2.4 and female adult = 41.2 ± 2.8 pmoles rT3/min mg protein). However, liver and renal D1 activities were lower in female adult rats than in pre-pubertal rats (Fig. 1A and B).

In the thyroid and pituitary, D1 activities did not differ between female and male pre-pubertal rats (thyroid, male pre-pubertal = 39.7 ± 2.4 and female pre-pubertal = 40.7 ± 2.9 ; pituitary, male pre-pubertal = 1.40 ± 0.3 and female pre-pubertal = 2.27 ± 0.8 pmoles rT3/min mg protein), but it was significantly higher in male adults than in pre-pubertal and female adult rats (thyroid, male adult = 126.2 ± 6.6 and female adult = 48.3 ± 3.6 ; pituitary, male adult = 3.33 ± 0.3 and female adult = 1.85 ± 0.3 pmoles rT3/min mg protein) (Fig. 1C and D).

Adult male rats

Body and tissue weights Body weight gain was significantly impaired in castrated male (Tex) rats in comparison with control animals (Fig. 2).

The absolute and relative thyroid and pituitary weights were not statistically different between control and Tex groups (thyroid: control = 16.27 ± 0.65 mg or 46.76 ± 1.79 µg/g bw, $n = 30$; Tex = 15.37 ± 0.66 mg or 45.71 ± 1.93 µg/g bw, $n = 19$ and pituitary: control = 9.53 ± 0.27 mg or 27.61 ± 1.02 µg/g bw, $n = 30$; Tex = 10.26 ± 0.34 mg or 30.95 ± 1.46 µg/g bw, $n = 19$).

Serum total testosterone, T3, T4, and TSH concentrations

Serum testosterone and TSH were significantly decreased in the Tex group despite the fact that serum T3 and T4 were unchanged (Table 2).

Table 1 Serum total T3, T4, and TSH concentrations in pre-pubertal and adult, female and male rats. Data are shown as mean $\pm$ S.E.M. TSH is presented as median (minimum–maximum); n = total number of rats.

	Pre-pubertal rats		Adult rats	
	Female	Male	Female	Male
T3 (ng/dl)	105.3 ± 4.69^a ($n = 17$)	105.1 ± 3.52^a ($n = 17$)	74.02 ± 6.25^b ($n = 21$)	50.82 ± 5.58^c ($n = 27$)
T4 (µg/dl)	1.62 ± 0.14^a ($n = 17$)	$1.95 \pm 0.17^{a,b}$ ($n = 17$)	$2.15 \pm 0.16^{a,b}$ ($n = 22$)	2.21 ± 0.22^b ($n = 25$)
TSH (ng/ml)	0.97 (0.81–1.30) ^a ($n = 17$)	1.11 (0.74–1.92) ^a ($n = 17$)	1.05 (0.76–1.90) ^a ($n = 23$)	1.37 (0.81–3.11) ^b ($n = 24$)

Different letters indicate statistically significant differences for: T3, $P < 0.001$; T4, $P < 0.05$; TSH, $P < 0.001$ b vs pre-pubertal female and $P < 0.05$ b vs other groups.

Figure 1 Type 1 iodothyronine deiodinase activity in liver (A), kidney (B), thyroid (C), and pituitary (D) from pre-pubertal and adult, female and male rats. Data are shown as mean $\pm$ S.E.M.; n =total number of rats. Different letters indicate statistically significant differences for: liver, $P<0.001$; kidney, $P<0.05$ a versus b and $P<0.001$ a versus other groups; thyroid, $P<0.001$; pituitary, $P<0.01$.

D1 activity Hepatic D1 activity was significantly decreased in the Tex group (control = 92.6 ± 3.45 , Tex = 63.7 ± 3.40 pmoles rT3/min.mg protein; Fig. 3A) as was the renal D1 activity (control = 94.3 ± 2.56 , Tex = 84.1 ± 4.69 pmoles rT3/min.mg protein; Fig. 3B). However, thyroidal D1 activity was unchanged in the castrated group (control = 127.5 ± 6.23 , Tex = 126.9 ± 9.36 pmoles rT3/min.mg protein; Fig. 3C), regardless of diminished serum TSH. In the pituitary, D1 activity was also unchanged by castration (control = 2.81 ± 0.35 , Tex = 2.53 ± 0.52 pmoles rT3/min.mg protein; Fig. 3D).

Adult female rats

Body and tissue weights Body weight gain was significantly higher in ovariectomized (Ovx) rats during the second and third weeks after castration in comparison with control animals, as previously described in the literature (Kimura *et al.* 2002). Estradiol replacement therapy was able to circumvent the higher body weight gain caused by

ovariectomy. In fact, body weight gain in OvX rats treated with $0.7 \mu\text{g}/100 \text{ g bw}$ estradiol benzoate (Ovx + 0.7) was lower than in the OvX and control groups. When OvX rats

Figure 2 Body weight gain in adult sham-operated (control) and castrated (Tex) male rats. Data are shown as means $\pm$ S.E.M.; n =total number of rats. * $P<0.05$, † $P<0.01$.

Table 2 Effects of orchiectomy on serum total testosterone, T3, T4, and TSH concentrations. Data are shown as mean $\pm$ S.E.M., testosterone and TSH are presented as median (minimum–maximum); n =total number of rats.

	Testosterone (ng/ml)	T3 (ng/dl)	T4 (μ g/dl)	TSH (ng/ml)
Control	3.92 (1.13–15.59) (n =15)	54.76 $\pm$ 5.62 (n =30)	2.18 $\pm$ 0.20 (n =28)	1.45 (0.81–3.35) (n =26)
Tex	<0.1 [†] (n =17)	56.53 $\pm$ 6.04 (n =19)	1.99 $\pm$ 0.19 (n =16)	1.19 (0.91–1.98)* (n =19)

* P <0.05, [†] P <0.0001.

were treated with 14 μ g/100 g bw estradiol benzoate (Ovx + 14), the body weight gain was significantly decreased during the 3 weeks of evaluation in comparison with Ovnx and control rats (Fig. 4).

The absolute thyroid weight was not statistically different among control, Ovnx, Ovnx+0.7, and Ovnx+14 groups (control = 13.70 $\pm$ 0.34 mg, n =27; Ovnx = 13.53 $\pm$ 0.60 mg, n =15; Ovnx+0.7 = 13.13 $\pm$ 0.73 mg, n =16; Ovnx+14 = 14.38 $\pm$ 0.57 mg, n =16). On the other hand, the absolute pituitary weight was statistically increased in Ovnx+0.7 and Ovnx+14 rats in comparison with the two other groups (control = 10.41 $\pm$ 0.52 mg, n =27; Ovnx = 9.73 $\pm$ 0.49 mg, n =15; Ovnx+0.7 = 16.38 $\pm$ 0.69 mg, n =16; Ovnx+14 = 32.13 $\pm$ 2.86 mg, n =16), as previously described (Scheithauer

et al. 1990). Pituitaries from rats treated with the higher estradiol dose (Ovx + 14) were even heavier than in Ovnx+0.7.

Serum total estradiol, T3, T4, and TSH concentrations Serum estradiol was decreased by ovariectomy, and this decrease was reversed by estradiol 0.7 μ g/100 g bw, although these changes do not reach significance. In the rats that received the supraphysiological dose of estradiol (14 μ g/100 g bw), serum estradiol was significantly higher than in control and Ovnx groups. Serum total T3 was lower in the Ovnx group than in control rats and both doses of estradiol normalized serum T3. Serum total T4 was not significantly changed by ovariectomy, but estradiol benzoate administration (Ovx + 0.7 and Ovnx + 14) significantly decreased

Figure 3 Type 1 iodothyronine deiodinase activity in liver (A), kidney (B), thyroid (C), and pituitary (D) of male sham-operated (control) and castrated (Tx) rats killed 3 weeks after castration. Data are shown as means $\pm$ S.E.M.; n =total number of rats. * P <0.05, [†] P <0.001.

Figure 4 Body weight gain in adult female sham-operated (control) and ovariectomized rats treated with vehicle (Ovx) or with 0.7 (Ovx+0.7) or 14 (Ovx+14) µg/100 g bw per day estradiol benzoate, subcutaneously for 21 days. Data are shown as means ± S.E.M.; n=total number of rats. *P<0.05, †P<0.01, ‡P<0.001 vs control.

serum T4 in comparison with the control group. Serum TSH levels did not differ among these groups (Table 3).

D1 activity Liver, renal, thyroid, and pituitary D1 activities were not significantly modified by ovariectomy. However, liver D1 activity was higher in OvX+0.7 and OvX+14 than in control rats (control=30.3±2.05, OvX=30.5±2.04, OvX+0.7=37.0±1.92, OvX+14=36.7±2.79 pmoles rT3/min·mg protein; Fig. 5A). Renal D1 activity (Fig. 5B) was significantly increased in the ovariectomized rats treated with the highest estradiol benzoate dose (Ovx+14) in comparison with control and OvX animals (control=43.3±2.73, OvX=42.5±2.73, OvX+0.7=51.0±2.11, OvX+14=53.2±4.32 pmoles rT3/min·mg protein). The thyroid D1 activity was significantly higher in OvX+0.7 and OvX+14 than in controls and OvX rats (control=56.9±5.11, OvX=57.4±5.35, OvX+0.7=84.2±10.54, OvX+14=79.4±6.91 pmoles rT3/min·mg protein; Fig. 5C). In the pituitary, D1 enzyme activity did not change (control=1.62±0.25, OvX=1.54±0.29, OvX+0.7=2.14±0.57, OvX+14=1.67±0.26 pmoles rT3/min·mg protein; Fig. 5D).

Discussion

There is not much information about sex-related differences in thyroid function of pre-pubertal rats. In the present study, we found no statistically significant differences in serum total T3, T4, and TSH between male and female pre-pubertal rats,

but liver and renal D1 activities were significantly higher in male than in female animals. There are evident pre-pubertal surges in estradiol (Meijs-Roelofs *et al.* 1973) and testosterone (Dohler & Wuttke 1975) during the first 3 postnatal weeks. Banu *et al.* (2002) reported that both testosterone (in males) and estradiol (in females) regulate TSH receptor levels in the immature rat thyroid, suggesting that postnatal elevation of sex steroids might have a physiological relevance to the TSH-induced mitogenic activity in thyrocytes. The postnatal surge in sex steroids might play a relevant role with respect to sex-related differences in D1 activity in pre-pubertal rats. Early testosterone surge might play an important role in the regulation of liver D1 activity in immature rats, and future experiments in this area might enlighten this issue.

During the early postnatal period (pre-puberty), serum testosterone and estradiol are markedly lower than in the adult phase (Banu & Aruldas 2002). Comparing thyroid function in pre-pubertal and adult rats from both genders, we found no differences in total serum T4, but serum total T3 was higher in pre-pubertal than in adult rats, and serum TSH was higher in male adults than in pre-pubertal and female rats. These results are in accordance with the previous reports (Cheron *et al.* 1980, Banu & Aruldas 2002) that showed serum TSH to be higher in male adults than in 30-day-old rats, without differences in serum T4.

In accordance with the previous reports (Donda *et al.* 1987, Corrêa da Costa *et al.* 2001, Moreira *et al.* 2005), we found higher serum T3 in female than in male adult rats. T4 sulfation decreases the outer ring deiodination of T4 and increases its inner ring deiodination, generating rT3. Hepatic sulfotransferase activities, responsible for T4 sulfation, are higher in male than in female adult rats (Gong *et al.* 1992, Santini *et al.* 1994, Visser *et al.* 1998), which could contribute to the decreased serum T3 levels in males.

In addition to higher serum T3, we detected lower serum TSH in female than in male adult rats, which also agrees with the previous studies (Rapp & Pyun 1974, Fukuda *et al.* 1975, Farbota *et al.* 1987, Santini *et al.* 1994). In relation to serum T4, we did not find sex-related differences, as reported by Harris *et al.* (1979), Christianson *et al.* (1981) and Santini *et al.* (1994), but this data disagree with those from Rapp & Pyun (1974) and Fukuda *et al.* (1975), who observed higher serum T4 in male than in female adult rats.

Cheron *et al.* (1980) reported that liver D1 activities in 2-, 4-, and 9-day-old rats were lower than the adult values,

Table 3 Effects of ovariectomy and treatment of ovariectomized rats with different doses of estrogen on serum total estradiol, T3, T4, and TSH concentrations. Data are shown as means ± S.E.M., estradiol and TSH are presented as median (minimum–maximum); n=total number of rats.

	Estradiol (pg/ml)	T3 (ng/dl)	T4 (µg/dl)	TSH (ng/ml)
Control	25.1 (15.3–56.1) (n=10)	79.16±5.81 (n=25)	2.16±0.15 (n=24)	1.11 (0.67–3.03) (n=27)
Ovx	16.2 (9.74–28.9) (n=10)	60.78±7.73* (n=14)	2.03±0.18 (n=14)	1.17 (0.81–2.95) (n=15)
Ovx+0.7	37.6 (19.1–79.8) (n=10)	76.06±6.51 (n=16)	1.69±0.10* (n=14)	1.27 (0.92–1.88) (n=16)
Ovx+14	585 (262–3166) [†] # (n=10)	74.86±5.45 (n=16)	1.70±0.15* (n=14)	1.25 (0.82–2.25) (n=16)

*P<0.05, †P<0.01 vs control; #, P<0.001 vs OVX

Figure 5 Type 1 iodothyronine deiodinase activity in liver (A), kidney (B), thyroid (C), and pituitary (D) of female sham-operated (control) and ovariectomized rats treated with vehicle (Ovx) or with 0.7 (Ovx+0.7) or 14 (Ovx+14) $\mu\text{g}/100\text{ g bw}$ per day estradiol benzoate, subcutaneously for 21 days. Data are shown as means $\pm$ S.E.M.; n =total number of rats. Different letters indicate statistically significant differences for: liver, $P<0.05$; kidney, $P<0.05$; thyroid, $P<0.05$.

reaching the adult value in 21-day-old rats and that the intrapituitary T4-5'-monodeiodination rate was higher in 2- and 4-day-old rats and fell to the adult value by 30 days of age. However, the present study shows liver, renal, thyroid, and pituitary D1 activities to be lower in the male pre-pubertal rats than in the adult rats. Serum T3 may be decreased in adult males due to their higher hepatic D1, which has recently been shown to also participate in the clearance of serum T3 (Schneider *et al.* 2006). The decreased serum T3 might contribute to the increased serum TSH, which in turn can lead to increased thyroid D1 activity, as previously demonstrated (Köhrle 1999). In fact, increased thyroid D1 might counteract the increased T3 clearance and sustain serum T3 to a certain extent.

Although thyroid hormone-induced increases in D1 activity and/or mRNA levels are well documented in rats (Bianco *et al.* 2002), we found higher D1 activity in male

adult than in pre-pubertal rats, despite lower serum T3. This suggests that directly or indirectly the increase in testicular hormonal activity could act as a potent stimulus of D1 activity. In the female groups, liver and renal D1 activities were higher in pre-pubertal than in adult rats, without significant differences in thyroid and pituitary activities. In fact, after sexual maturation, liver and renal D1 activities seem to be down-regulated in females. Since estrogen seems to positively modulate D1 activity and ovariectomy does not promote any significant change in female hepatic and renal D1, it is tempting to speculate that other factors, besides gonadal hormones, could be modulating D1 activity during murine maturation in the female.

The liver, renal, thyroid, and pituitary D1 activities were significantly lower in female than in male adult rats, corroborating part of earlier reports (Harris *et al.* 1979, Donda *et al.* 1990, Santini *et al.* 1994, Miyashita *et al.* 1995)

that demonstrate decreased liver D1 activity in females, without differences in kidney. However, this data differ from a previous report of lower hepatic D1 activity in males and no sex-related differences in thyroid D1 activity (Corrêa da Costa *et al.* 2001). Donda *et al.* (1990) showed lower pituitary D1 activity in male than in female adult rats, which was not observed in the present study. These controversial results may be related to different rat strains and/or different deiodinase activity determination methodologies.

Thyroid hormones are the main known regulators of liver and renal D1 activity (Köhrle 1999, Koenig 2005). We did not find differences in serum T4 between female and male adult rats, but serum T3 was lower in males than in females, regardless of the higher hepatic and renal D1 activities in males, so this would not be the explanation for sex-related differences. Moreover, Miyashita *et al.* (1995) demonstrated that, in thyroidectomized adult rats, liver D1 activity was lower in females than in males, suggesting that sex-related differences in liver D1 activity are independent of thyroid status and could be due to the presence of testosterone in male rats. Maia *et al.* (2003) suggested that, in human, the skeletal muscle D2 activity could be responsible for a major part of the peripheral T4 to T3 deiodination. It is doubtful whether the same can be proposed for the rat, since previous evaluations were unable to detect D2 activity in the rat skeletal muscle (St Germain *et al.* 2005).

Body weight was significantly decreased in orchietomized rats when compared with the control group. This finding agrees with the results found by Ke *et al.* (2001) and Moreau *et al.* (2001). The absolute and relative weights of the thyroid and pituitary were not changed in castrated male rats. This data disagree with Banu *et al.* (2001), who observed that orchietomy decreased the absolute and relative thyroid weights of 60-day-old rats that were gonadectomized at day 10 *post partum*, and of 160-day-old rats gonadectomized at day 120 *post partum*. This discordance is probably due to differences in the ages of the animals used and the period of castration. Therefore, data obtained in the present study show that androgens are important for body weight gain but do not affect thyroid and pituitary weights, at least during a period of 3 weeks after castration.

The present study corroborates the finding that serum total T3 and T4 are unaltered in orchietomized animals, in agreement with Christianson *et al.* (1981). Christianson *et al.* (1981) reported that serum TSH concentration in adult male rats is androgen mediated. Our data of orchietomy-induced decrease in serum TSH confirm this finding. Farbota *et al.* (1987) and Banu *et al.* (2001) found similar alterations and reported a stimulatory effect of testosterone on TSH secretion in both male and female rats.

Orchietomy and its associated decrease of serum testosterone resulted in a decrease of hepatic and renal D1 activities, without changes in thyroid or pituitary D1 activity. A decrease in hepatic D1 activity in orchietomized rats has been reported previously (Harris *et al.* 1979, Miyashita *et al.* 1995, Lisbôa *et al.* 2001). Since D1 activity is dependent on

thyroid hormone levels, the decreased liver and kidney D1 in castrated male adult rats that have normal plasma T3 and T4 indicates a direct stimulatory action of androgens on D1 activity, at least in liver and kidney, as do Miyashita *et al.* (1995) findings of increased D1 mRNA levels in cultured rat hepatocytes exposed to testosterone. Recent findings in our laboratory also confirm the stimulatory effect of androgens on hepatic and renal D1 activity (Fortunato *et al.* 2006).

In rats, a great proportion of T4 is deiodinated in the thyroid generating approximately 40–50% of circulating T3 (Bianco *et al.* 2002). Thus, despite their lower serum TSH, the unchanged thyroidal D1 activity and decreased hepatic D1 could explain the normal serum T3 levels in orchietomized rats. Previously, castration has been shown to induce an increment in male pituitary D1 activity (Lisbôa *et al.* 2001). However, these data do not agree with our finding of unchanged pituitary D1 activity, but different methodologies were used in these two studies.

As far as we know, this is the first report showing that although androgens can stimulate hepatic and kidney deiodinase, its lack does not affect thyroid D1 activity, at least after a 3-week castration period.

In female adult rats, body weight gain was significantly increased by ovariectomy. Moreover, in ovariectomized rats treated with estradiol, the body weight gain was less than in the control animals. This result corroborates data of Kimura *et al.* (2002) and Meli *et al.* (2004) that suggest a lipolytic effect of estrogen. Estrogens seem to have a central action, inhibiting the neuropeptide Y and consequently food intake (Meli *et al.* 2004). Furlanetto *et al.* (1999) reported increased proliferation of FRTL-5 cells after their exposure to estradiol. However, our *in vivo* study did not reveal changes in thyroid weight among the adult female groups. These data obtained 21 days after ovariectomy disagree with Banu *et al.* (2001), who observed that ovariectomy decreased the absolute thyroid weights of 60-day-old rats that were castrated at day 10 *post partum*, and of 160-day-old rats castrated at day 120 *post partum*. The discordance may be due to the differences in the ages of the animals and the period of castration between Banu *et al.* and our observations. A dose-dependent increase of pituitary weight was found in the ovariectomized estradiol-treated rats; this increase is to be expected considering the hypertrophic effect of estrogen on lactotropic cells.

In this context, it should be noted that no significant changes in serum TSH was detected among the female groups. Chen & Walfish (1978) and Lisbôa *et al.* (1997) have reported that estradiol treatment of castrated females promotes an increase in serum TSH, but neither we nor Christianson *et al.* (1981) found a similar increase in serum TSH.

Our results confirm that serum total T4 concentration is not altered by ovariectomy (Christianson *et al.* 1981, Lisbôa *et al.* 1997), but there is a small significant decrease in serum T3, also reported by Lima *et al.* (2006). The hepatic, renal, thyroid, and pituitary D1 activities were unchanged in ovariectomized rats. After treatment of Ovx rats with estradiol, serum T4 diminished (as in earlier reports of

Chen & Walfish 1978 and Harris *et al.* 1979), but serum T3 returned to control values. This correlates with the increase in hepatic and renal D1 activity seemingly induced by the estradiol treatment. Thyroid D1 also was increased by estrogen treatment and might contribute to the increase in T3/T4 serum ratio. The pituitary D1 activity was unaffected by estradiol treatment. Corroborating our data, Miyashita *et al.* (1995) also did not find any changes in hepatic D1 activity and mRNA in castrated female rats, and Harris *et al.* (1979) found an increase in hepatic D1 activity of ovariectomized rats treated with estradiol. The results of Lisbôa *et al.* (1997, 2001) on liver D1 activity in ovariectomized rats are discordant, but they agree concerning the stimulatory effect of estradiol replacement on hepatic and thyroid D1.

In summary, in adult males, gonadal hormones seem to exert a significant positive influence on hepatic (and renal) D1 activity. In contrast, a decrease in gonadal hormones does not appear to affect adult female hepatic, kidney, or thyroid D1 activity, although they can be stimulated by exogenous estradiol treatment. The comparison between pre-pubertal and adult rats suggests that T3 is not the only or main stimulus of D1 activity and that other factors besides thyroid and gonadal hormones, can modulate D1 activity during murine maturation.

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