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Effects of ageing and pharmacological hypothyroidism on pituitary–thyroid axis of Dutch-Miranda and Wistar rats

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Abstract

To evaluate the ability of the aged rat pituitary to increase TSH secretion in response to major decreases in serum thyroid hormones, hypothyroidism was induced by methimazole in young and old, male and female, Dutch-Miranda and Wistar rats. Before MMI-treatment there were no differences in serum TSH of young and old rats, but serum T_4 was significantly decreased in aged rats from both genders and strains, while serum T_3 was significantly decreased in aged male rats from both strains, and in old Wistar females. MMI treatment significantly decreased serum T_4 and T_3 in all treated animals, and progressively increased serum TSH in both male and female rats, but the increase was significantly smaller in the elder rats. The pituitary TSH content was higher in Wistar than in Dutch-Miranda rats, of both genders, and was not significantly affected by age. MMI treatment decreased the pituitary TSH in both young and old Dutch-Miranda rats, but in the Wistar strain only the old females had a significant decrease. Our results show that the ability of the pituitary thyrotrophs to increase hormonal secretion in response to decreased levels of thyroid hormones is impaired in the old rat, even when the thyroid hormone levels are dramatically reduced.

Keywords: Thyroid hormones; Thyrotropin; Ageing; Hypothyroidism; Rat; Methimazole

1. Introduction

Thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), are important determinants of growth and development, and modulate many metabolic processes including basal metabolic rate and calorigenesis. Thyrotropin (TSH) is the major regulator of the morphologic and functional states of the thyroid gland, stimulating all steps of thyroid hormones biosynthesis. TSH production and secretion are stimulated by the hypothalamic thyrotropin-releasing hormone (TRH) and suppressed by thyroid hormones in a classical negative feedback model. The circulating T_4 is entirely originated by the thyroid gland, while T_3 may also be produced by 5′-monodeiodination of T_4 in thyroid and non-thyroidal tissues (Larsen et al., 2003).

Ageing has been associated with several changes in endocrine function that also affect the hypothalamic–pituitary–thyroid axis. Some of these alterations have been described in humans and in experimental animals. Hypothyroidism is more common in elderly subjects, and physiologic changes due to ageing may also mimic hypothyroidism and other endocrine diseases (Morley, 2001).

In rats ageing has been characterized by unchanged circulating TSH associated with low serum thyroid hormones. We previously reported that low serum T_4 together with normal serum TSH are found in aged Dutch-Miranda rats of both genders, but low serum T_3 was only detected in male rats, suggesting gender-related differences (Corrêa da Costa et al., 2001). Cizza et al. (1992) also reported progressive follicular loss with advancing age in the thyroid of male Fisher rats, with significant decreases in free serum T_4 and T_3, but not in serum TSH. Apparently, the thyroid responsivity to TSH in older rats is decreased due to

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lower TSH binding to thyrocytes, which might explain the decreased thyroid hormone secretion (Reymond et al., 1992). On the other hand, under physiological conditions a decrease in serum thyroid hormones would induce an increased secretion of TSH by the thyrotrophs, which does not occur in the aged rat (Cizza et al., 1992; Corrêa da Costa et al., 2001). These findings suggest that the hypothalamic TRH-secreting neurons and/or the pituitary thyrotrophs have a diminished ability to increase their hormonal secretion in response to a decreased serum T_4 in the aged rat.

The aim of the present study was to evaluate the aged rat pituitary ability to increase TSH secretion in response to major (methimazole-induced) decreases in thyroid hormones, and to compare the residual pituitary TSH content in young and aged rats of both genders and from two different strains.

2. Materials and methods

2.1. Animals

The study was approved by the institutional Committee for the Use of Animals in Research, and the procedures used were in compliance with the International Guiding Principles for Biomedical Research Involving Animals, Council for International Organisations of Medical Sciences (Geneva, Switzerland), and the guiding principles for care and use of animals from American Physiological Society.

Female and male Dutch-Miranda and 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 received pelleted commercial chow (Paulínea, São Paulo, Brazil; iodine content 2 mg/kg) and water ad libitum.

2.2. Induction of hypothyroidism and hormone measurements

Hypothyroidism was induced by methimazole (MMI 0.03% in drinking water during 21 days) in male and female, 3–5 (young) and 12–15 (old) months old Dutch-Miranda and Wistar rats. Blood was collected from the jugular vein, under light ether anaesthesia, before the start of MMI (day 0), and weekly thereafter (days 3, 7, 14 and 21). Sera were separated by centrifugation and stored at −20 °C until hormone determination. On the 21st day of MMI treatment, the pituitaries of the MMI-treated and of control animals were rapidly excised, weighed, homogenised in 10 mM phosphate buffered saline, pH 7.6, and centrifuged at $3000 \times g$ for 30 min at 4 °C. The supernatant was collected and stored at −20 °C for measurement of the intra-pituitary TSH content.

Serum and pituitary TSH were measured using a kit supplied by the National Hormone and Peptide Program, NIDDK (Bethesda, MD, USA) and expressed in terms of the preparation (RP-3) provided. Serum T_3 and T_4 were determined by specific Coated-Tube Radioimmunoassay (RIA) kits, using rat hormone-stripped serum for the standard curve. Protein concentration was determined by the method of Bradford (1976).

2.3. Statistical analysis

Hormone measurements are presented as mean ± SEM. Serum TSH analysis was performed on log-transformed values. Two-way analyses of variance complemented by post hoc Newman–Keuls tests or Sheffé's multiple contrasts (Zar, 1996) were used for statistical evaluation of data, using the SuperANOVA program (Abacus Concept, Berkeley, CA, USA).

3. Results

In both strains the aged male or female rats body weights were greater than in the young animals as expected, and the relative pituitary weight in females was about double that found in males (Table 1).

The MMI-induced hypothyroidism increased the absolute and relative thyroid weight in young and old rats of both genders, but the body weight and relative pituitary weight were unaffected. (data not shown). Serum T_3 and T_4 were significantly decreased in the aged female and male Wistar and the male Dutch-Miranda rats. Serum T_4 was also decreased in the aged female Dutch-Miranda rats, but the serum T_3 was not significantly different from that found in young females of the same strain (Table 2).

The MMI treatment significantly decreased serum T_4 and T_3 ($p < 0.01$) in the old and young animals of both genders and strains, as expected. As can be seen in Fig. 1, the serum T_3 values during the first 2 weeks are higher in the females than in the males, but this is due to their higher starting values. In fact, the T_3 disappearance rate is similar in males and females (Fig. 1).

There were no differences in the serum TSH of young and old rats in either strain before the MMI-treatment. Serum TSH increased progressively during the MMI-treatment period, in both male and female rats, but the increase was significantly slighter ($p < 0.01$) in the elder rats of both strains (Fig. 2), reaching no more than ≈60% of the values attained by the young animals, as assessed by the area under the serum TSH curve (Old Dutch-Miranda

Table 1
Pituitary weight (mg/100 g body weight) in young and old rats (mean ± SEM)

	Dutch-Miranda		Wistar	
	Females	Males	Females	Males
Young	6.13 ± 1.08	2.53 ± 0.09	4.60 ± 0.39	1.72 ± 0.37
Old	5.90 ± 0.56	2.47 ± 0.15	5.81 ± 0.52	2.28 ± 0.24

Table 2
Serum T_4 and T_3 in young and old rats (mean ± SEM)

	Dutch-Miranda		Wistar	
	Females	Males	Females	Males
T_3 (ng/dl)				
Young	41.2 ± 4.2	33.9 ± 4.4	41.4 ± 4.2	22.2 ± 1.7
Old	33.9 ± 3.4	18.2 ± 3.9*	29.7 ± 3.1*	17.2 ± 1.7*
T_4 (μg/dl)				
Young	3.53 ± 0.21	3.84 ± 0.16	3.21 ± 0.33	3.84 ± 0.54
Old	2.90 ± 0.17*	2.86 ± 0.22*	2.04 ± 0.27*	1.96 ± 0.13*

*Significantly different from the young ($p < 0.05$ or less).

females 63%, males 57%; old Wistar females 53%, males 56% of the respective young values).

The pituitary TSH content was systematically higher in Wistar than in Dutch-Miranda rats, independent of gender, and was not significantly affected by age, even though there was a slight increase in the old male Dutch-Miranda rats, and a decrease in the old female Wistar rats. The MMI treatment decreased the pituitary TSH in both young and old Dutch-Miranda rats, but in the Wistar strain only the old females had an equally significant decrease (Fig. 3).

4. Discussion

The present findings of decreased serum thyroid hormones in old Dutch-Miranda and Wistar rats of both genders confirm previous reports (Klug and Adelman, 1979; Donda and Lemarchand-Béraud, 1989; Cizza et al., 1992; Reymond et al., 1992; Corrêa da Costa et al., 2001; Greeley et al., 1983), and indicate that these changes are not strain-related, although there may be differences between the genders. The lower T_3 and T_4 serum levels in elder rats can be attributed to a progressive loss of follicles, as reported by Cizza et al. (1992) in the thyroid gland of aged male rats, as well as to a diminished thyroid response to TSH, probably due to a decrease in the number of TSH receptors (Reymond et al., 1992). However, since we did not measure TSH bioactivity, one cannot discard the possibility that the 'normal' serum TSH of old animals could be less bioactive, as found in situations with absent or diminished TRH effects reviewed by Yamada et al. (2003). Anyway, Weiss and Refetoff (2000) found that thyroid hormone levels decrease with age in patients with resistance to thyroid hormone and in unaffected individuals from the same families, and Weiss et al. (2002) reported a significant inverse correlation between age and serum thyroid hormone levels in wild as well as in transgenic TRα and β knock-out mice. These findings indicate an intrinsic thyroid failure since the age-related decrease in serum thyroid hormones occurs even in the presence of serum levels of TSH as high as those found in patients with resistance to thyroid hormone and TR knock-out mice.

A few reports of increased TSH in ageing rats have been presented (Pekary et al., 1983; Cónsole et al., 1995).

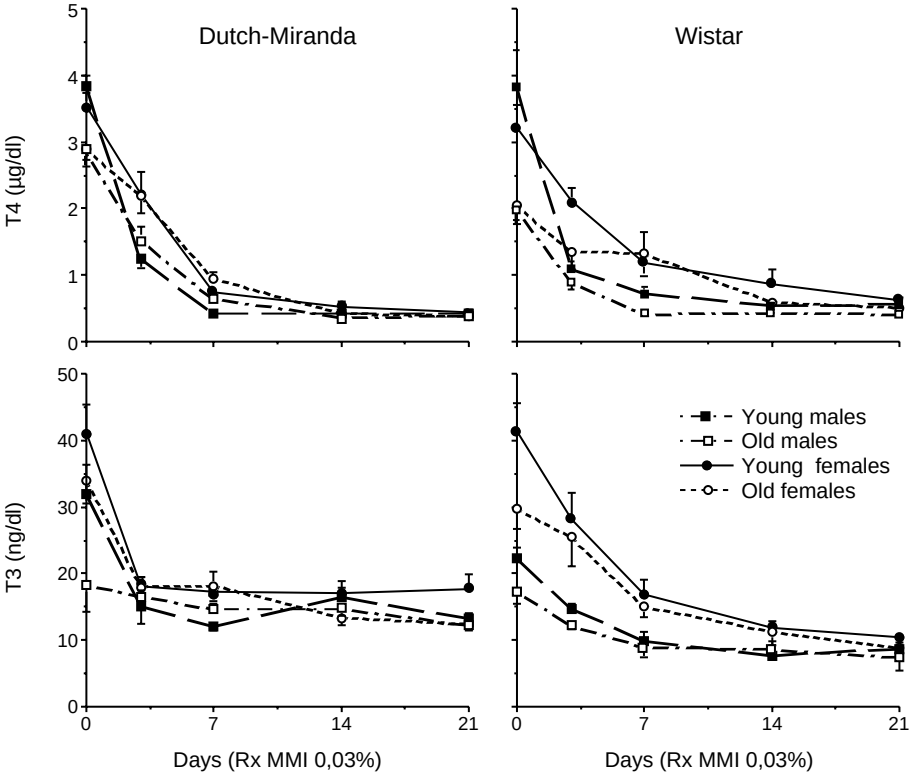

Fig. 1. Serum T_4 and T_3 before and during iatrogenic hypothyroidism induction in young and old, male and female, Dutch-Miranda and Wistar rats. Results are presented as mean ± SEM, $n \geq 10$.

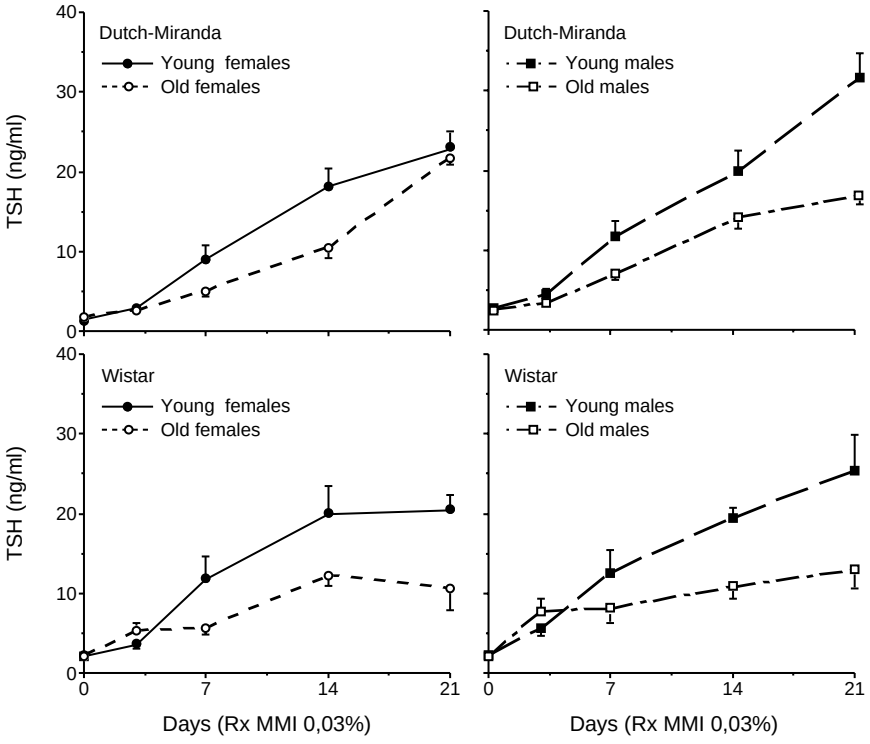

Fig. 2. Serum TSH before and during iatrogenic hypothyroidism induction in young (full symbols) and old (empty symbols), male and female, Dutch-Miranda and Wistar rats. Results are presented as mean ± SEM, $n \geq 10$.

However, we and other authors have found that serum TSH levels in young and old rats are not different despite the fact that old rats have lower thyroid hormone serum levels than young animals (Klug and Adelman, 1979; Donda and Lemarchand-Béraud, 1989; Cizza et al., 1992; Reymond et al., 1992; Corrêa da Costa et al., 2001). This was again confirmed in the present study, and suggests some kind of impairment in the hypothalamic–pituitary response to a diminished feedback by the thyroid hormones. Donda et al. (1989) found an increase in the pituitary iodothyronine-5′-deiodinase activity in old male Wistar rats, and proposed that the increased local T_3 generation could explain the inadequately normal serum TSH even when serum T_3 and T_4 levels were significantly lower than in young animals. However, we were unable to confirm their findings, and have previously found a significant decrease in pituitary T_4-5′-deiodinase activity in Dutch-Miranda rats, both female (Corrêa da Costa and Rosenthal, 1996) and male (Corrêa da Costa and Rosenthal, unpublished results). Lewis et al. (1991) also proposed that the association of normal serum TSH and low thyroid hormones seen in some elderly subjects could be due to an apparent resetting of the thyroid hormone feedback regulation of TSH secretion. Our present results show that a major decrease in serum T_3 and T_4, as induced be the MMI treatment, is indeed able to produce a significant response of serum TSH, even in the elder animals. However, the TSH increment was significantly lower in the old animals, irrespective of gender or strain.

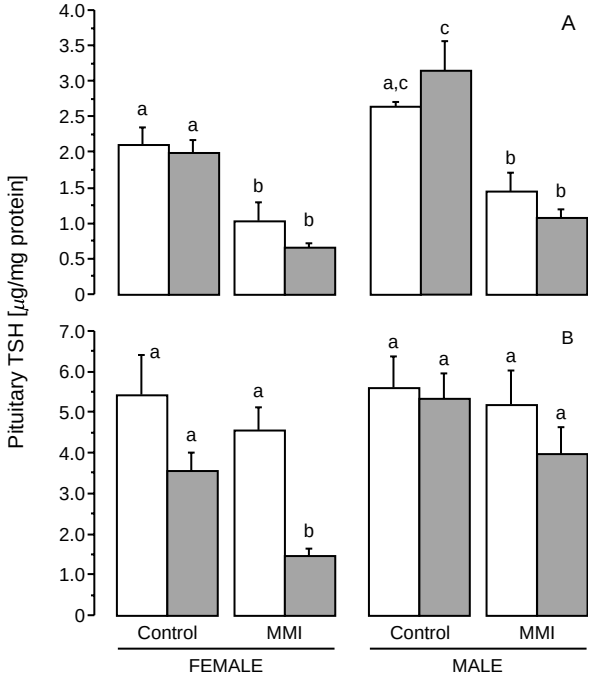

Fig. 3. Effect of iatrogenic hypothyroidism induced by MMI 0.03% in drinking water for 21 days on pituitary TSH in young (clear collumns) and old (shaded collumns) Dutch-Miranda (A) and Wistar (B) rats. Results are presented as mean ± SEM, $n \geq 10$. Same letters indicate not statistically different means.

This agrees with Cizza et al. (1992) report of a progressive age-dependent loss of the in vivo TSH response to exogenous TRH, as well as of TSH-β subunit mRNA levels and TSH content in male Fisher 344/N rats, indicating that the aging hypothalamic TRH-secreting neurons and/or the pituitary thyrotrophs in this rat strain also has a diminished ability to increase hormonal secretion in response to lower levels of thyroid hormones.

The difference between the serum TSH response to iatrogenic hypothyroidism in young and old rats does not seem to correlate with a decreased pituitary TSH content in the elder animals, with the exception of the Wistar females. Nevertheless, it induced a depletion of pituitary TSH in the Dutch-Miranda rats, both young and old, suggesting that rats of this strain are less able than the Wistar rats to maintain/replenish the pituitary TSH pool when subjected to an important secretory stimulus, and that this is not related to aging. Donda et al. (1989) refer that the pituitary TSH concentration was slightly, but not significantly, reduced in aged Wistar males. Our data, do show a slight, not significant, decrement in the pituitary TSH concentration of the old male Wistar rats, but only after their pituitary is challenged by markedly decreased thyroid hormone levels. In the untreated old female Wistar rats, the pituitary TSH content is already decreased an alteration that becomes more noticeable and significant after MMI treatment. Thus, it would appear that although there is no difference in the serum TSH response to induced hypothyroidism between old rats of both strains, the thyrotrophs in Wistar rats are more affected by aging than in Dutch-Miranda.

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