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Experimental gestational hypothyroidism evokes hypertension in adult offspring rats

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ABSTRACT

Gestational hypothyroidism is a prevalent disorder in pregnant women. We aimed to investigate the impact of experimental gestational hypothyroidism (EGH) on cardiovascular and autonomic nervous systems (ANS) in the offspring of rats. EGH was induced with methimazole (MMI) 0.02% in drinking water from day 9 of gestation until birth. Sixty day old offspring from MMI-treated dams (OMTD, n = 13) or water-treated dams (OWTD, n = 13) had femoral arteries surgically assessed for the measurements of heart rate (HR), mean (MAP), systolic (SAP) and diastolic arterial pressure (DAP), and spontaneous baroreflex sensitivity (BRS). To investigate the balance of ANS, we established the high (HF) and low frequency (LF) bands of pulse interval (PI) and LF band of SAP spectrum. OMTD had increased MAP (130.2 ± 2.0 vs 108.8 ± 3.0 mm Hg, $p<0.001$), SAP (157.3 ± 2.9 vs 135.7 ± 4.5 mm Hg, $p<0.001$) and DAP (109.7 ± 1.9 vs 88.4 ± 2.6 mm Hg, $p<0.001$) when compared to OWTD, and had lower HR (355.1 ± 8.9 vs 386.8 ± 9.2 bpm, $p<0.05$). After spectral analysis of PI and SAP, only LF band of SAP spectrum was higher (7.2 ± 0.8 vs 4.0 ± 0.6 mm Hg^2, $p<0.01$) in OMTD under spontaneous condition. Despite bradycardia, EGH promotes spontaneous hypertension in 60 day old offspring, probably due to increased sympathetic modulation of vessels, which is suggested by the higher LF of SAP. These findings suggest a critical role of maternal THs in the development of fetal cardiovascular and autonomic nervous systems.

1. Introduction

Thyroid hormones (THs), thyroxin (T4) and 3,5,3′-triiodothyronine (T3), are essential to normal body development, especially to structural and functional formation of the central nervous system (CNS) (Bernal, 2005; Koibuchi et al., 2001; Koromilas et al., 2010), not only during development but also in adulthood (Bauer and Whybrow, 2001; Calzà et al., 1997; Joffe and Sokolov, 1994). High prevalence of hypothyroidism has been reported in pregnant women (Glinoer, 1998; Nambiar et al., 2011; Stagnaro-Green et al., 2011). The role of THs in the development of mammalian brain, heart and vessels has been extensively studied (Chattergoon et al., 2012; Porterfield, 1994). However, it is not clear how thyroid dysfunctions during intra-uterine life can affect the offspring later in life.

THs, synthesized in the maternal thyroid gland, can easily cross the placental barrier. Before the onset of fetal thyroid gland, maternal THs are the only source of these hormones to fetus during a crucial period of neurons, heart and vessel development (Gärtner, 2009; Mogil et al., 2000). An inadequate support of THs from pregnant mothers can temporarily or permanently affect tissue differentiation in the offspring (Fowden and Forhead, 2004). Thus, lower THs plasma levels during intra-uterine life may affect the cardiovascular function, directly or indirectly, by reprogramming the function of both cardiovascular and autonomic nervous systems (Chattergoon et al., 2012). In addition, it may potentially explain, at least in part, the etiology of unknown cardiovascular disorders.

It is known that thyroid dysfunction itself determines changes in cardiovascular functioning. For example, clinical and experimental studies have demonstrated that hypothyroidism decreases cardiac output, induces bradycardia and increases peripheral resistance and arterial blood pressure (Kisso et al., 2008; Ohga et al., 2002; Patel et al., 2011). In the vessels, the THs can directly affect total peripheral vascular resistance. Studies from several authors have shown high prevalence of systolic and diastolic hypertension in hypothyroidism (Biondi et al., 2002; Danzi and Klein, 2002; Dillmann, 2002; Kotsis et al., 2007). Because THs are required to the synthesis of nitric oxide (NO) in peripheral blood vessels, they exert an indirect vasodilation effect (Delp et al., 1995; McAllister et al., 2005; Quesada et al., 2002;

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Vargas et al., 1995). In this context, the hypertension observed in hypothyroid patients and animals has been implicated with low NO production in endothelium (Danzi and Klein, 2003; Endo et al., 1979; Polikar et al., 1993; Saito and Saruta, 1994; Saito et al., 1983; Streeten et al., 1988).

Experimental hypothyroidism induced by oral administration of methimazole reduces the expression and function of β1 and β2 adrenergic receptors, whereas it increases β3 expression, resulting in negative chrono- and inotropic effects (Arioglu et al., 2010).

Abnormalities in brain development of hypothyroid rats are commonly found in the postnatal period (Patel et al., 2011). Hypothyroidism during rat development implies impairment in synaptic transmission leading to disastrous effects on neurological function which can be permanent (Ahmed et al., 2010).

The state of maternal thyroid during pregnancy and lactation may affect the thyroid status of the pups. Rats that received methimazole during the intrauterine period via the placenta and lactation became hypothyroid (Hasebe et al., 2008). Experimental studies have been developed to assess the long term effects of gestational hypothyroidism (GH) over the offspring's lives. Moreover, there is lack of data of whether GH can make cardiovascular system more vulnerable in the offspring. These same studies have used models of induction of hypothyroidism in the pre-and postnatal (i.e.: from pregnancy to lactation period). Differently, in our study, the induction of maternal hypothyroidism has been carried out during pregnancy.

Therefore, considering that (i) GH is a common hormonal disorder, (ii) THs are crucial for fetal development, and (iii) imbalances occurring during intra-uterine life can result in transient or permanent dysfunctions in the offspring, we aimed to evaluate the impact of GH in the cardiovascular system of the offspring when adult.

2. Materials and methods

2.1. Animals and the induction of gestational hypothyroidism

All animals used were obtained from the Animal Care Facility of the Federal University of Sergipe and maintained under controlled light/dark cycle (12/12 h) and room temperature (23 ± 2 °C). They had free access to standard chow and drinking solution. Female Wistar rats (~200 g) had their estrus cycle followed on daily basis through vaginal smear. Once proestrus phase was detected, adult males (~300 g) were put in female cages for mating overnight. On the next morning, the presence of spermatozoid on vaginal smear confirmed day 0 of gestation (GD). To induce gestational hypothyroidism, dams were given 0.02% methyl mercaptoimidazole (methymazole, MMI, Sigma-Aldrich, Saint Louis, MO, USA) in the drinking water from GD 9 up to delivery day (GD 21–22). MMI given for 10 days to pregnant rats from GD 9 is able to cause hypothyroidism in dams, as shown by the decrease of circulating levels of maternal total T3 and total T4 (Ahmed et al., 2010). Four male and four female offspring rats were maintained per dam. Offspring from MMI-treated dams (OMTD) were compared to the corresponding control offspring (offspring from water-treated dams; OWTD). Newborns were weaned at 21st postnatal day (PND). At 60th PND, all offspring were tested and analyzed together (n = 13 per group).

All procedures are in accordance with the Ethics Committee for Animal Research (CEPA) of the Federal University of Sergipe (Protocol # 02/2011) which operates under the rules of the National Council for the Control of Animal Research (CONCEA) and International Guiding Principles for Biomedical Research Involving Animals. All efforts were made to minimize animal suffering and reduce the number of animals used.

2.2. Surgical procedure

At age 60 days, animals were anesthetized with thiopental sodium (50 mg/kg, i.p.) and were implanted with a polyethylene catheter (PE-10/PE-50, Intramedic, Becton Dickinson and Company, Sparks, MD, USA) into the femoral artery. A single injection of a combination of penicillin (240,000 IU/kg) and streptomycin (100 mg/kg), was used to prevent infection. The catheter was tunneled to the back of the rats and exteriorized on the back neck in the nape, and surgical incision sites were closed by sutures. Twenty-four hours later, the arterial catheter was connected to a pressure transducer (Edwards Lifescience, Irvine, CA, USA) coupled to a preamplifier (BioData, Model BD-01, PB, Brazil). Pulsatile arterial pressure (BP) was recorded for 10 min using an IBM/PC equipped with an analog-to-digital interface (2 kHz; BioData, BD, Brazil). The pulsatile arterial pressure recordings were processed using a computer software (Advanced CODAS/Windaq, Dataq Instruments Inc., Akron, OH, USA) that identifies inflection points on signals and generates beat-by-beat time series with systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), heart rate (HR) and pulse interval (PI) values.

2.3. Data analysis

The PI and SAP variability analysis was performed using a custom computer software (CardioSeries v1.2 — http://sites.google.com/site/cardioseries). Beat-by-beat series obtained from pulsatile arterial pressure recordings were converted to data points every 100 ms using cubic spline interpolation (10 Hz). The interpolated series were divided into half-overlapping sequential sets of 512 data points (51.2 s), which were tested for stationarity. It is important to remind that cardiovascular variability analysis through spectral analysis requires at least weakly stationary data series (i.e. mean and covariance stable over time) (Berntson et al., 1997; Porta et al., 2004). Data stationarity can be verified by means of stationarity tests (i.e. better reproducibility of the results among users and laboratories) (Magagnin et al., 2011; Porta et al., 2004), as well as through visual inspection of data series (Dias et al., 2010; Porta et al., 2001; van de Borne et al., 1997). In the current study, a well-experienced researcher in cardiovascular variability analysis visually inspected the segments of interpolated time series (i.e. PI or SAP values) looking for transients that could affect the calculation of the power spectral density (PSD). To confirm that the visual inspection of the time series was properly performed, a *Hanning* window was used to attenuate side effects and all segments had the spectrum computed using a direct fast *Fourier* transform (FFT) algorithm for discrete time series. All segments were visually inspected for abnormal spectra. Lastly, taking together the results from the time series and spectra inspections, nonstationary data were not taken into consideration for PSD calculation. The spectra were integrated in the low frequency band (LF; 0.2–0.75 Hz) and the high frequency band (HF; 0.75–3 Hz), and results are presented in absolute and normalized form, by dividing LF and HF power by the total power minus very low frequency (VLF; <0.2 Hz) power.

The baroreflex sensitivity (BRS) was assessed in the time-domain by means of the Sequence technique, as described by Di Rienzo et al. (1985). A custom computer software (Analyzer v4.4 — http://www.haraldstauss.com) scanned beat-by-beat time series of SAP and PI searching for sequences of at least 4 consecutive beats in which increases in SAP were followed by PI lengthening (up sequence) and decreases in SAP were followed by PI shortening (down sequence), with a linear correlation higher than 0.85. The slope of the linear regression lines between SPB and PI was taken as a measure of BRS, as described by Bertinieri et al. (1985).

2.4. Statistical analysis

Body weight of the offspring was analyzed by repeated measures two-way ANOVA with the testing across time as the repeated dependent variable, and treatment (OMTD and OWTD) as independent factor. Bonferroni test was used for post hoc analyses when required. Student *t* test was used for the analysis of the cardiovascular parameters, the

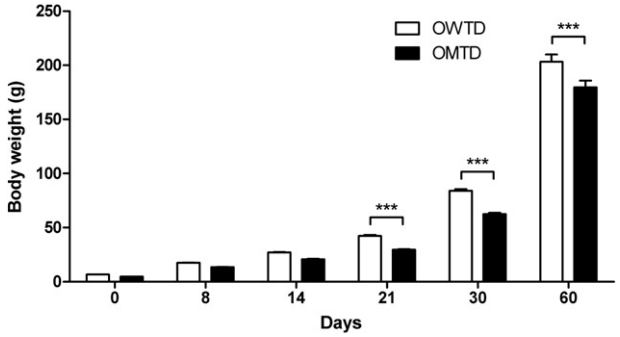

Fig. 1. Effect of experimental gestational hypothyroidism (EGH) on the body weight (BW) of offspring (at 0, 8, 14, 21, 30 and 60 days old). Offspring of water-treated dams (OWTD, n = 26) and offspring of MMI-treated dams (OMTD, n = 26). (***) $p<0.001$. For analysis, we used a two-way ANOVA followed by Bonferroni post hoc test.

total number of segments obtained following segmentation of interpolated series, as well as the percentage of segments used. The threshold of statistical significance was set at $p<0.05$. The results are expressed as mean and standard error of mean.

3. Results

Body weight was lower in OMTD rats from PND 21 to PND 60 ($p<0.001$) (Fig. 1). In Fig. 2A, it is shown that OMTD rats present lower HR in comparison to OWTD (355.0 ± 8.9 bpm vs 386.8 ± 9.2 bpm, n = 13, $p<0.05$). Contrarily, basal MAP (130.2 ± 2.0 mm Hg vs 108.8 ± 3.0 mm Hg, n = 13, $p<0.001$; Fig. 2B), SAP (157.3 ± 2.9 mm Hg vs 135.7 ± 4.5 mm Hg, n = 13, $p=0.001$; Fig. 2C) and DAP (109.7 ± 1.9 mm Hg vs 88.4 ± 2.6 mm Hg, n = 13, $p<0.001$; Fig. 2D) were higher in OMTD, when compared to OWTD rats.

No differences were observed between OWTD and OMTD rats regarding the total number of segments obtained following segmentation of interpolated series, as well as the percentage of segments used for spectral analysis (Fig. 3A and B).

As shown in Fig. 4, OMTD rats showed no differences in the power of the LF band (normalized units, Fig. 4A) and HF band (normalized and absolute units, Fig. 4B and C) of PI spectrum, as well as in LF/HF (Fig. 4D) and BRS (Fig. 4F), when compared to OWTD rats. However, the power of the LF band of SAP spectrum was found higher in OMTD rats (7.2 ± 0.8 vs 4.0 ± 0.6 mm Hg^2, $p<0.01$; Fig. 4E), when compared to OWTD rats.

4. Discussion and conclusions

The current evidence demonstrates, for the first time, that the deficiency of THs, exclusively during intrauterine life, can permanently affect cardiovascular function later in life. This suggests that hypothyroidism during gestation can change the functioning of one or more organs of this system (i.e. heart, vessels and/or autonomic nervous system).

We found that 60 day old OMTD rats have higher basal MAP, SAP and DAP, beside the lower HR. It has been demonstrated by several groups that low thyroid function promotes changes in cardiac contractility, decreases cardiac output, increases peripheral vascular resistance, decreases chronotropic and inotropic function and exerts cardiac atrophy (Kisso et al., 2008). However, similar to Kisso's study, most of those found in the current literature did not aim to investigate the role of maternal THs in the fetal programming of cardiovascular function. Moreover, very few clinical and experimental studies have focused on the understanding of the impact of gestational hypothyroidism over the life of the offspring. Our findings add new information to the literature, once we show for the first time, the increase in MAP, SAP and DAP, and a reduction in HR in 60 day old OMTD rats. Interestingly, no changes were found in the autonomic control of cardiac function, at least under basal conditions, as demonstrated by spectral analysis of PI. Moreover no change was observed in BRS. These findings suggest that bradycardia observed in OMTD rats might be related to a direct effect of a lack in THs in the heart.

In this context, it has been extensively demonstrated that THs deficiency causes a reduction in the sensitivity and responses to catecholamines as a result of decreased expression of β1 and β2 adrenergic receptors in the heart, as well as increased expression of the β3 adrenergic receptor, which taken together promotes reduction in chronotropic and inotropic function of the heart (Arioglu et al., 2010). It is known that T3 acts directly on cardiomyocytes, thereby altering cardiac function, regardless of its action in peripheral vessels

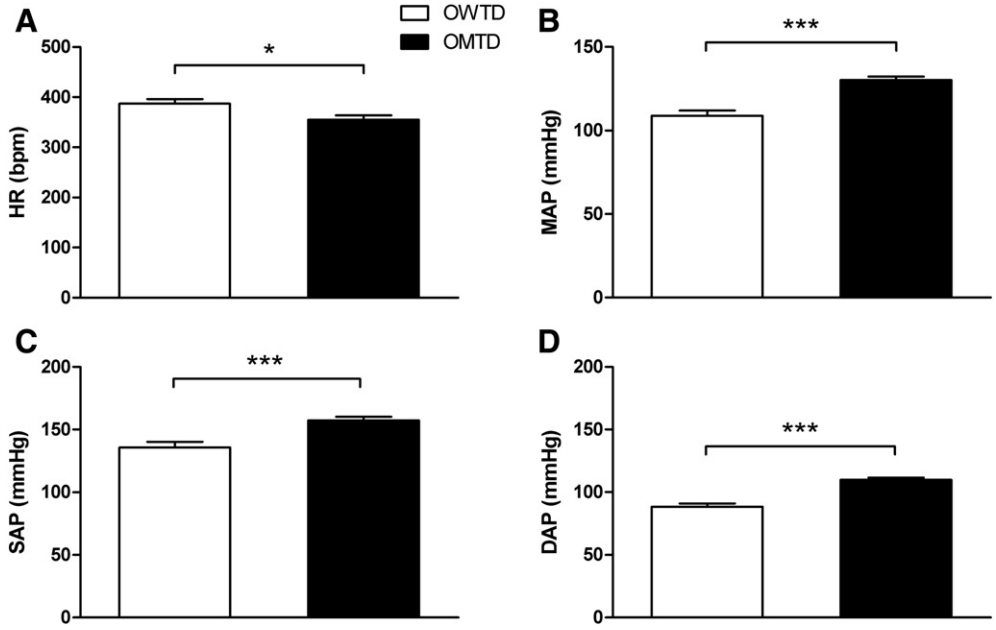

Fig. 2. Effect of experimental gestational hypothyroidism (EGH) on the heart rate (HR, panel A), mean arterial pressure (MAP, panel B), systolic arterial pressure (SAP, panel C), diastolic arterial pressure (DAP, panel D) of 60 day old offspring. Offspring of water-treated dams (OWTD, n = 13) and offspring of MMI-treated dams (OMTD, n = 13). (*) $p<0.05$, (***) $p<0.001$. For analysis, Student *t* test was used.

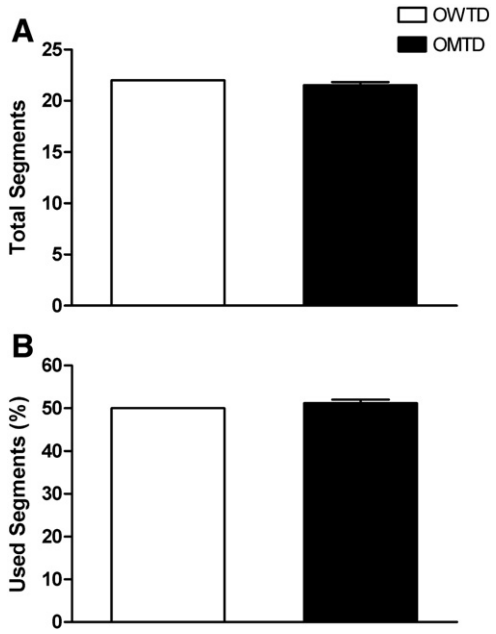

Fig. 3. Total number of segments following segmentation of interpolated series (panel A) and percentage of segments used for spectral analysis through *fast Fourier transform* (panel B) of 60 day old offspring. Offspring of water-treated dams (OWTD, n = 13) and offspring of MMI-treated dams (OMTD, n = 13). For analysis, Student *t* test was used.

(Danzi and Klein, 2011; Morkin, 1993). Simultaneously, T3 also acts to determine vascular relaxation. This effect has been related to the increase in the expression of a critical enzyme for the synthesis of an important vasodilating gaseous factor, nitric oxide (NO) (i.e. nitric oxide synthase; NOS) (Delp et al., 1995; McAllister et al., 2005; Quesada et al., 2002; Vargas et al., 1995). On the other hand, the lack of T3 leads to the increase in peripheral vascular resistance because of the reduction in NOS expression, which may contribute to the genesis of hypertension (Moulakakis et al., 2008).

Besides no changes were found in the parameters that infer the autonomic balance of the heart (i.e. HF and LF band of PI spectrum, BRS and LF/HF), at least in spontaneous conditions, the autonomic control of peripheral vessels seems to be affected by EGH. In the current study, in either absolute or normalized units no differences were observed in the power of the HF band of PI spectrum between OWTD and OMTD rats. The normalization of spectral indexes could make the interpretation of results simpler (Burr, 2007), since data will be represented as percentage (i.e. 0–100%) instead of values spread over a wide range scale (e.g. total power 3466 ms^2, LF power 1170 ms^2, HF power 975 ms^2; values obtained from normal subjects; Task Force, 1996). In addition, the representation of data in normalized units makes it easier to compare results obtained in different studies and/or laboratories (Burr, 2007).

It is noteworthy to mention that, in certain situations, if the power of frequency bands of a spectrum is represented in absolute or normalized units, disagreeing interpretation of the results may arise. Montano et al. (1994) evaluated the HRV (i.e. spectral analysis) of

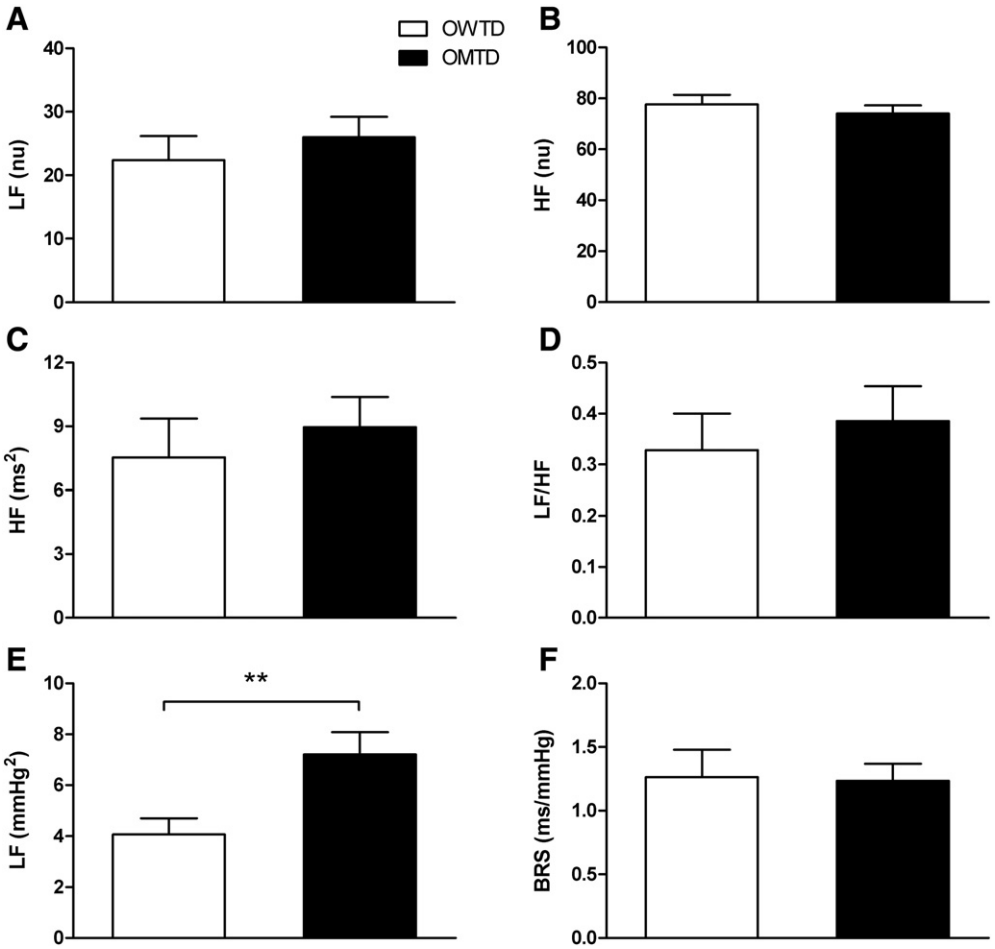

Fig. 4. Effect of experimental gestational hypothyroidism (EGH) on the pulse interval (PI) variability, systolic arterial pressure (SAP) variability and baroreflex sensitivity (SBR) of 60 day old offspring. Power of the low and high frequency bands (LF, panel A; HF, panels B and C), and LF/HF ratio (panel D) of PI spectrum. Power of the LF band (panel E) of SAP spectrum. BRS (panel F). Offspring of water-treated dams (OWTD, n = 13) and offspring of MMI-treated dams (OMTD, n = 13). (**) $p<0.01$. For analysis, Student *t* test was used.

healthy subjects during graded (i.e. 0–90°) orthostatic *tilt*. A correlation between LF power and *tilt* incline was observed only when LF power was expressed in normalized units. On the other hand, a correlation between HF power and *tilt* incline in both, absolute and normalized units was observed. It becomes clear that data should be properly presented and discussed taking into account peculiarities of different projects.

As shown in literature (Cerutti et al., 1991; Furlan et al., 1990; Malliani et al., 1991; Rubini et al., 1993), higher power of the LF band of SAP spectrum strongly indicates an increase in sympathetic modulation of the vessels. Thus, because we found that in OMTD rats, we can suggest that the hypertension observed in these animals may be, at least in part, due to an increased sympathetic modulation to peripheral vessels. Moreover, it has been demonstrated that higher norepinephrine plasma levels in hypothyroid patients are related to increased sympathetic tone (Cacciatori et al., 2000). In addition, it is conceived that hypertension itself is implicated with the reduction in parasympathetic tone (Lombardi et al., 1996; Task Force, 1996; Cacciatori et al., 2000), what was not observed in the present study.

Besides the hypertension seen in OMTD rats, BRS was not changed in these animals, at least in basal conditions. However, other investigators (Irigoyen and Krieger, 1998; Mancia et al., 1997) have reported hypertension as a cause for the reduction in the BRS. Based on these findings, we expected to observe a reduction in BRS in OMTD rats. Interestingly, besides the normal BRS, OMTD rats were unable to restore MAP to control values, suggesting that blood pressure was reprogrammed to a new set point in these animals.

However, despite all the evidences presented in the literature in favor of our initial hypothesis, this was not confirmed with the model used in this work. In this regard, we may take into account the fact that in those reports, the hypothyroid animals were the subject of the studies, whereas in ours, the hypothyroid animals are the mothers, instead of the offspring, which are our subjects of investigation. Moreover, no challenges were applied to the offspring, and, because of it, some potential differences may not be detected.

We also observed that body weight was lower in OMTD rats from PND 21 until PND 60. These data corroborate findings obtained in offspring of mothers that received propyl-thiouracil (PTU), another recognized anti-thyroid drug. The authors found that PTU impairs an adequate body development in offspring, as noted by their lower body weight (Behzadi and Ganji, 2005; Rohani et al., 2009; Sawin et al., 1998). The fact that low body weight has only been observed after PND 21, corroborates with Rohani et al. (2009), which showed reduced body weight of pups from hypothyroid mothers only after the second week of life.

In conclusion, we demonstrated for the first time that experimental hypothyroidism, exclusively during gestation, increases MAP, SAP, DAP and reduces HR in the offspring under basal conditions. Interestingly, in the same conditions, higher power of the LF band of SAP spectrum was observed in OMTD rats, suggesting a higher sympathetic modulation of blood vessels. No changes were found in the drive for the autonomic control of cardiac function. Further studies are necessary in order to elucidate the mechanisms by which hypothyroidism during gestation is able to interfere with cardiovascular function. Moreover, the apparently normal functioning of the autonomic control of the heart, as observed in this study, may be investigated under challenging conditions.

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