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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