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Effects of experimentally-induced maternal hypothyroidism on crucial offspring rat brain enzyme activities

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ABSTRACT

Hypothyroidism is known to exert significant structural and functional changes to the developing central nervous system, and can lead to the establishment of serious mental retardation and neurological problems. The aim of the present study was to shed more light on the effects of gestational and/or lactational maternal exposure to propylthiouracil-induced experimental hypothyroidism on crucial brain enzyme activities of Wistar rat offspring, at two time-points of their lives: at birth (day-1) and at 21 days of age (end of lactation). Under all studied experimental conditions, offspring brain acetylcholinesterase (AChE) activity was found to be significantly decreased due to maternal hypothyroidism, in contrast to the two studied adenosinetriphosphatase (Na^+,K^+-ATPase and Mg^{2+}-ATPase) activities that were only found to be significantly altered right after birth (increased and decreased, respectively, following an exposure to gestational maternal hypothyroidism) and were restored to control levels by the end of lactation. As our findings regarding the pattern of effects that maternal hypothyroidism has on the above-mentioned crucial offspring brain enzyme activities are compared to those reported in the literature, several differences are revealed that could be attributed to both the mode of the experimental simulation approach followed as well as to the time-frames examined. These findings could provide the basis for a debate on the need of a more consistent experimental approach to hypothyroidism during neurodevelopment as well as for a further evaluation of the herein presented and discussed neurochemical (and, ultimately, neurodevelopmental) effects of experimentally-induced maternal hypothyroidism, in a brain region-specific manner.

1. Introduction

Thyroid hormones (THs), thyroxine (T4) and 3,5,3'-triiodothyronine (T3), are known to exert a broad spectrum of effects on the central nervous system (CNS), during both development and adulthood (Bernal, 2007; Brabant et al., 2011; Calzà et al., 1997; Jahagirdar and McNay, 2012). Within the developing CNS, THs modulate a significant number of factors involved in neuronal migration, growth, differentiation and signalling (Williams, 2008), and are reported to play an important role in the maturation of the synaptic plasma membrane during neurodevelopment (Lindholm, 1984).

Hypothyroidism during neurodevelopment may cause extended structural and functional alterations to certain crucial CNS regions (Koromilas et al., 2010) that can even lead to irreversible mental retardation and neurological deficits (Abduljabbar and Afifi, 2012; Morreale de Escobar, 2003). Experimental simulation of hypothyroidism during neurodevelopment can be achieved through multiple *in vivo* models (Argumedo et al., 2012); among these, the maternal administration of propylthiouracil (PTU) in the drinking water during rodent gestation and/or lactation has been the most popular and can be considered as amongst the easiest to perform.

During the last decade, we have provided a number of reports on the effects of PTU-induced adult-onset hypothyroidism on crucial neurochemical parameters such as the activity of acetylcholinesterase (AChE) and of two major adenosinetriphosphatases

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(ATPases; namely, Na^+,K^+-ATPase and Mg^{2+}-ATPase) in major rat CNS regions (Carageorgiou et al., 2005, 2007a,b). In continuum to these reports, the aim of the present study was (i) to shed more light on the effects of gestational and/or lactational maternal exposure to PTU-induced hypothyroidism on the above-mentioned crucial brain enzyme activities of Wistar rat offspring, at two time-points of their lives: at birth (day-1) and at 21 days of age (end of lactation), as well as (ii) to evaluate the suitability and reliability of these methodological approaches to developmental hypothyroidism (since a wealth of reports already exists on the effects of experimentally-induced hypothyroidism on the herein studied offspring neurochemical parameters, allowing for a critical and comparative technical interpretation).

2. Materials and methods

2.1. Animals

Twenty albino Wistar adult female rats (2 months old) were purchased by the National Center for Scientific Research "Demokritos" (Agia Paraskevi, Athens, Greece) and were housed two in a cage, at a constant room temperature (22 ± 1 °C) under a 12-h light:12-h dark (light 08:00–20:00 h) cycle. Food and water were provided *ad libitum*. Animals were cared for in accordance with the principles for the care, use and protection of experimental animals as set by the EEC Council Directive 86/609/EEC (EEC Council, 1986) and aligned according to the Recommendation 2007/526/EU. Permission for the conduction of the herein described experiments was granted by the local authorities (K/242; 22-01-2010).

2.2. Mating and exposure to PTU during gestation and/or lactation

Ten albino Wistar adult male rats were used for mating purposes only; each male was placed with two females in each cage, in order for mating to be achieved. Following that (as assessed through the examination for the presence of an ejaculatory plug in the vagina), males were removed and female rats were equally divided into four groups: (a) Control (receiving tap water during both gestation and lactation, $n = 6$), (b) HypoG (receiving 0.05% (w/v) of PTU in the drinking water during gestation, $n = 6$), (c) HypoL (receiving 0.05% (w/v) of PTU in the drinking water during lactation, $n = 4$), and (d) HypoGL (receiving 0.05% (w/v) of PTU in the drinking water during gestation and lactation, $n = 4$). A number of newborn offspring ($n = 6$ from each of the Control and HypoG groups) were weighted and sacrificed by decapitation at day 1, providing brain samples for the Control-1 and the HypoG-1 (exposure to PTU during gestation) subgroups[1]. At the end of the lactation period, the 21-day-old rat offspring were weighted, sacrificed by decapitation and their brains were rapidly removed ($n = 6$ from each group), providing brain samples for the Control-21, HypoG-21, HypoL-21 and HypoGL-21 subgroups. All offspring groups consisted of rats of both sexes, as previous data of ours have shown that no significant sex-dependent differences exist amongst 21-day-old rats with regards to their herein studied brain homogenate enzyme activities (Liapi et al., 2007).

2.3. Tissue preparation

The brain tissue was weighted and then homogenized in 10 vol. ice-cold (0–4 °C) medium containing 50 mM Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl), pH 7.4 and 300 mM sucrose, using an ice-chilled glass homogenizing vessel at 900 rpm (4–5 strokes). Then, the homogenate was centrifuged at 1000 × *g* for 10 min to remove nuclei and debris (Tsakiris, 2001). In the resulting supernatant, the protein content was determined according to the method of Lowry et al. (1951) and then the enzyme activities were measured.

2.4. Determination of brain AChE activity

The activity of AChE was determined by following the hydrolysis of acetylthiocholine according to the method of Ellman et al. (1961), as described by Tsakiris (2001). The incubation mixture (1 ml) contained 50 mM Tris–HCl, pH 8, 240 mM sucrose and 120 mM NaCl. The protein concentration of the incubation mixture was 80–100 μg/ml. The reaction was initiated after addition of 0.03 ml of 5,5'-dithionitrobenzoic acid (DTNB) and 0.05 ml of acetylthiocholine iodide, which was used as substrate. The final concentration of DTNB and substrate were 0.125 and 0.5 mM, respectively. The reaction was followed spectrophotometrically by the increase of absorbance ($\Delta\overline{OD}$) at 412 nm.

[1] Note: gestational exposure to PTU resulted into a lower number of pups being born to each dam (~7 pups/dam compared to the untreated dams that delivered on average 11 pups each).

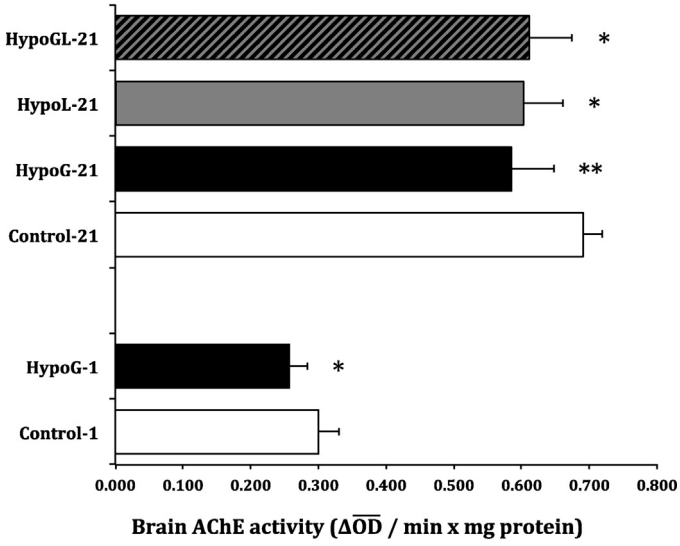

Fig. 1. Effects of gestational and/or lactational exposure to experimental PTU-induced hypothyroidism on offspring rat brain AChE activity. Note: for more details, see Section 2. Each value indicates the mean ± SD of five or six independent experiments. The average value of each experiment was obtained from three evaluations in the homogenized rat brain of newborn (1-day-old) or 21-day-old rat offspring. $^{**}P < 0.01$ (*vs* Control-21); $^{*}P < 0.05$ (*vs* respective Control subgroup).

2.5. Determination of Na^+,K^+-ATPase and Mg^{2+}-ATPase activities

(Na^+,K^+)-ATPase activity was calculated from the difference between total ATPase activity (Na^+,K^+,Mg^{2+}-dependent ATPase) and Mg^{2+}-dependent ATPase activity. Total ATPase activity was assayed in an incubation medium consisting of 50 mM Tris–HCl, pH 7.4, 120 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 240 mM sucrose, 1 mM ethylenediamine tetra-acetic acid K_2-salt (K^+-EDTA), 3 mM disodium ATP and 80–100 μg protein of the homogenate in a final volume of 1 ml. Ouabain (1 mM) was added in order to determine the activity of Mg^{2+}-ATPase. The reaction was started by adding ATP and stopped after an incubation period of 20 min by addition of 2 ml mixture of 1% lubrol and 1% ammonium molybdate in 0.9 M H_2SO_4 (Bowler and Tirri, 1974; Tsakiris, 2001). The yellow colour which developed was read at 390 nm.

2.6. Chemicals

All chemicals used in this study were of analytical grade and/or of the highest purity available and were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.7. Statistical analysis

The data were analyzed using one-way ANOVA followed by Bonferroni correction (where applicable). All analyses were performed by SPSS for Windows Software. Values of $P < 0.05$ were considered statistically significant.

3. Results

Table 1 provides an overview of the changes observed in the body and brain weight of the offspring rats following gestational and/or lactational maternal PTU-induced hypothyroidism. Significant restriction of both body and brain weight gain is caused by PTU-administration even during lactation alone, while gestational exposure to PTU causes a reversible by PTU-free lactation growth retardation (Table 1). Interestingly, the brain to body weight ratio is significantly increased in the offspring rats exposed to gestational and lactational maternal PTU-induced hypothyroidism (as compared to that of age-matched controls (+43%, $P < 0.001$; Table 1).

Newborn rats exposed to PTU during gestation demonstrated a statistically significant decrease in their brain AChE activity (−14%, $P < 0.05$; Fig. 1). This brain AChE inhibition has been a constant finding under all experimental conditions studied: gestational, lactational and the combined gestational and lactational experimentally-induced maternal hypothyroidism resulted in a statistically significant decrease in the offspring rat brain AChE