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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).

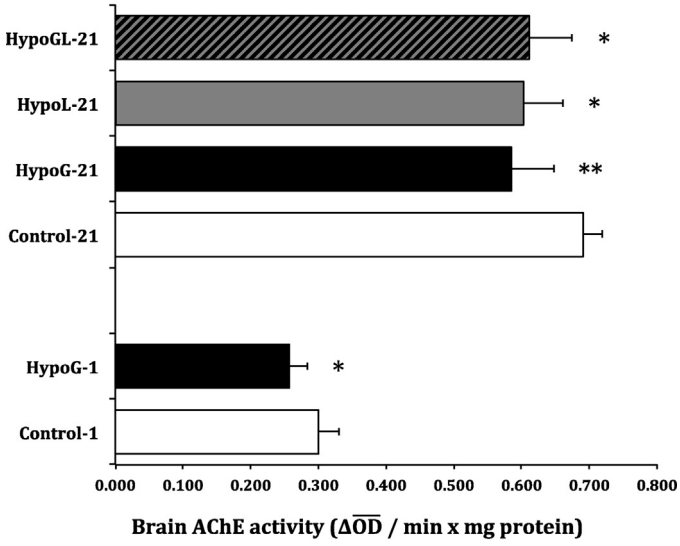

Fig. 1. Effects of gestational and/or lactational exposure to experimental PTU-induced hypothyroidism on offspring rat brain AChE activity. Note: for more details, see Section 2. Each value indicates the mean ± SD of five or six independent experiments. The average value of each experiment was obtained 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

Table 1
Effects of gestational and/or lactational exposure to experimental PTU-induced hypothyroidism on offspring rat body and brain weight.

Group	Body weight	Brain weight	Brain to body weight ratio	*n*
Control-1	6.8 ± 0.3	0.274 ± 0.027	0.040 ± 0.004	6
HypoG-1	5.4 ± 0.3 (−21% *vs* Control-1)***	0.237 ± 0.012 (−14% *vs* Control-1)*	0.044 ± 0.004 (+10% *vs* Control-1)	6
Control-21	39.0 ± 1.3	1.430 ± 0.035	0.037 ± 0.001	6
HypoG-21	42.2 ± 8.4 (+8% *vs* Control-21)	1.422 ± 0.039 (−1% *vs* Control-21)	0.035 ± 0.008 (−5% *vs* Control-21)	6
HypoL-21	31.7 ± 3.8 (−19% *vs* Control-21)**	1.301 ± 0.043 (−9% *vs* Control-21)***	0.042 ± 0.007 (+14% *vs* Control-21)	6
HypoGL-21	24.5 ± 5.2 (−37% *vs* Control-21)***	1.257 ± 0.108 (−12% *vs* Control-21)**	0.053 ± 0.007 (+43% *vs* Control-21)***	6

Note: For more details, see Section 2.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

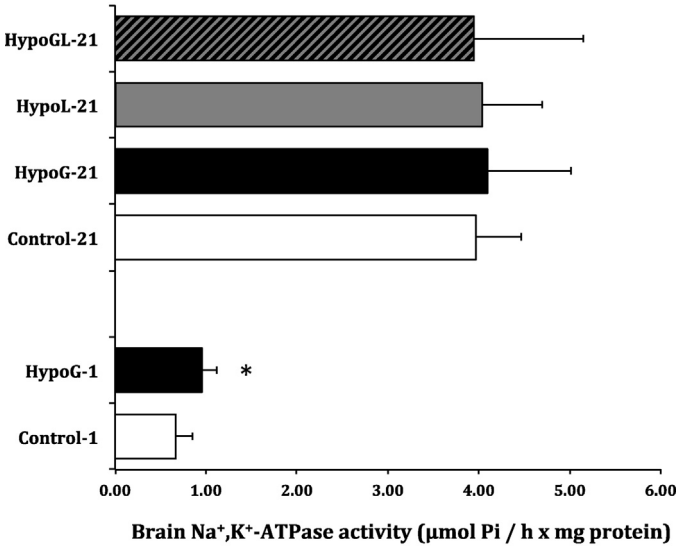

Fig. 2. Effects of gestational and/or lactational exposure to experimental PTU-induced hypothyroidism on offspring rat brain Na^+,K^+-ATPase activity. Note: for more details, see Section 2. Each value indicates the mean ± SD of five or six independent experiments. The average value of each experiment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

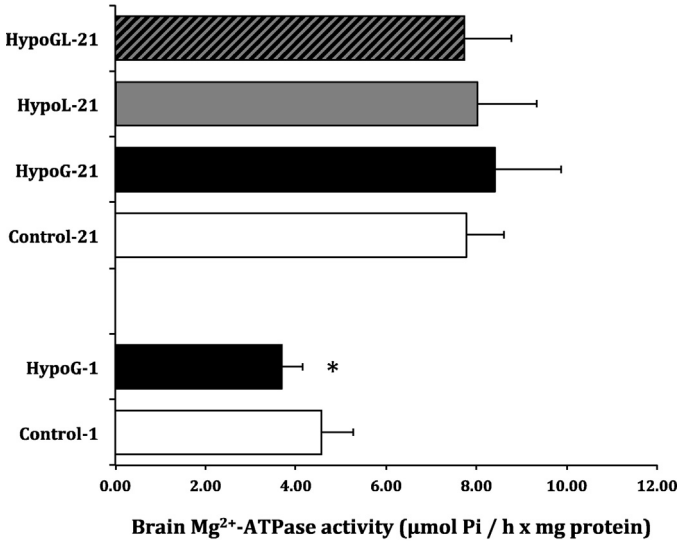

Fig. 3. Effects of gestational and/or lactational exposure to experimental PTU-induced hypothyroidism on offspring rat brain Mg^{2+}-ATPase activity. Note: for more details, see Section 2. Each value indicates the mean ± SD of five or six independent experiments. The average value of each experiment was obtained from three evaluations in the homogenized rat brain of newborn (1-day-old) or 21-day-old rat offspring. * $P < 0.05$ (*vs* Control-1).

activity (−15%, $P < 0.01$; −13%, $P < 0.05$; −11%, $P < 0.05$, respectively, when compared to Control-21; Fig. 1).

On the other hand, Na^+,K^+-ATPase activity was only found to be increased in the brain of PTU-exposed newborn rats (+43%, $P < 0.05$; Fig. 2); none of the 21-day-old offspring groups demonstrated a statistically significant difference in their mean brain Na^+,K^+-ATPase activity as compared to their respective 21-day-old control group (Control-21; Fig. 2). Brain Mg^{2+}-ATPase was found to be inhibited in the PTU-exposed newborn offspring rats (−19%, $P < 0.05$), but did not demonstrate any significantly differentiating trend among the examined 21-day-old rat groups when compared to the respective age-matched control (Fig. 3).

4. Discussion

The maternal administration of PTU is known to block TH biosynthesis and peripheral T4 deiodination to T3, making it an excellent means for the experimental induction of hypothyroidism to both foetuses and suckling pups (as PTU crosses the blood–placenta barrier and passes into the milk) (Rami et al., 1989; Zoeller and Crofton, 2005). Maternal PTU administration is known to lower the rat pups' circulating serum TH levels in a dose-dependent way, even if this is only applied throughout lactation (Goldey et al., 1995). Our study introduces the use of PTU for the induction of maternal hypothyroidism during gestation, lactation or combined gestation and lactation, as a three-way assessment approach to the neurodevelopmental effects of this type of experimentally-simulated hypothyroidism on 21-day-old offspring Wistar rats. This approach is designed so as: (i) to simulate the clinical entities of congenital hypothyroidism (exposure to PTU during lactation) and of maternal hypothyroidism[2] of varying timeframe/severity (exposure to PTU during gestation or gestation and lactation), (ii) to assess the different patterns of defects that hypothyroidism could exert on the studied neurochemical parameters, as well as to evaluate the similarities that these patterns bear with other established experimental approaches. The extended (during gestation and lactation) exposure timeframe is, according to our opinion, of particular clinical interest: given the fact that a significant part of the rat neurogenesis is taking place postpartum (Howdeshell, 2002), this approach is closer to human maternal hypothyroidism-induced neurotoxicity.

Our study has demonstrated a statistically significant decrease in brain AChE activity due to all patterns of maternal exposure to PTU-induced hypothyroidism (Fig. 1). This finding seems to be very encouraging, as it is in agreement with the majority of the available literature and highlights a major neurochemical pathway through

[2] Note: maternal hypothyroidism due to iodine-deficiency, autoimmune thyroiditis (mainly of the Hashimoto's type), drugs, previous thyroidectomy, TH-disrupting chemicals, *etc.*

which hypothyroidism could affect the developing brain cholinergic system; a major determinant of optimal neurodevelopment and higher brain function. Ahmed et al. (2010) have followed a timeframe of study similar to ours and have reported the effects of methimazole (MMI)-induced maternal hypothyroidism on crucial offspring rat brain enzyme activities, 21 days after birth. In their extremely interesting study, hypothyroidism significantly inhibits AChE in the cerebrum, the cerebellum and the medulla oblongata of 21-day-old hypothyroid rats (as compared to the respective age-matched controls) (Ahmed et al., 2010). In a more recent report, Koohestani et al. (2012) also examined the effect of PTU-induced developmental hypothyroidism, but in a way that resembles our protocol's focus on PTU-induced hypothyroidism during lactation only; in fact, the authors have supplied Sprague–Dawley rats with PTU (0.1% in the drinking water from birth till lactation day 21) and have reported a significantly inhibited spinal cord AChE activity in the PTU-exposed offspring rats (as compared to controls) (Koohestani et al., 2012).

In agreement with the above, congenital hypothyroidism that was induced by the addition of 0.05% PTU in the drinking water from gestation day 9 and continually up to lactation day 15 was recently also reported to cause a significant reduction in the hippocampal AChE activity of immature Wistar rats (when compared to respective euthyroid ones) (Cattani et al., 2013).

In a much older attempt, neonatal radiothyroidectomy was induced by a single injection of 200 μCi of ^{131}I on the day of birth in newborn Sprague–Dawley rats, resulting in a failure to significantly alter the activity of brain AChE (although somewhat lower values were noted in 30-day-old hypothyroid rats) (Hrdina et al., 1975). Following an almost similar approach, Geel and Timiras (1967) had even earlier demonstrated a significant AChE inhibition in the cerebral cortex and the hypothalamus of hypothyroid 22-day-old Long–Evans rats (as compared to the respective age-matched controls' regions); in their case, radiothyroidectomy was performed by the administration of 100 μCi of ^{131}I to newborn pups born to mothers fed a low iodine diet (Geel and Timiras, 1967).

In a totally different approach to neonatal hypothyroidism, Virgili et al. (1991) have administered MMI subcutaneously (20 mg/kg dissolved in saline) to individual Wistar rat pups from postnatal day 1 to postnatal day 27; this method is argued to be superior to that of ours and others (of maternal PTU-induced hypothyroidism) in that: (i) it does not make the mother itself hypothyroid, (ii) it does not cause unwanted side effects due to overlowering of maternal TH levels, as well as (iii) it has the advantage of allowing the comparison between animals belonging to the same litter (Virgili et al., 1991). Although their results have shown a significant decrease of both AChE and choline-acetyltransferase (ChAT) activities in the prefrontal cortex and the striatum of hypothyroid rats (as compared to controls), and seem to be in agreement with those of ours (to the extent that such a correlation can be considered based on our whole brain findings following a maternal PTU-induced hypothyroidism during lactation; Fig. 1), one should argue that the technical difficulty and the handling stress caused on pups might not make the Virgili et al. (1991) approach a first choice.

Interestingly, adult-onset 4-week PTU-induced hypothyroidism has been reported to cause a significantly increased AChE activity in Sprague–Dawley rat cerebral cortex synaptosomal preparations (Salvati et al., 1994). However, adult-onset hypothyroidism induced by partial thyroidectomy coupled with PTU-administration (in the drinking water) has been reported to cause a significant reduction of AChE activity in the cerebellum, the medulla oblongata and the subcortex, but not in the cortex and the midbrain of Sprague–Dawley rats (as compared to euthyroid control ones) (Ahmed et al., 1993).

In their highly sophisticated studies, Kundu et al. (2006, 2007) have proved that PTU-induced adult-onset peripheral hypothyroidism provokes time-dependent effects on synaptosomal total T3 content, as well as on neuronal AChE and Na^+,K^+-ATPase activities; in fact, they have provided evidence that by injecting adult Sprague–Dawley rats with PTU (intraperitoneally, 2 mg/100 g) on a daily basis for 2 or 20 days, PTU-treated rats demonstrate an increase in synaptosomal membrane Na^+,K^+-ATPase activity and a decrease in AChE activity at both studied time-points (Kundu et al., 2006, 2007). These findings were matched with an increase of synaptosomal total T3 content at day 2 of the undertaken PTU treatment, followed by an expected decrease of this content at day 20; a finding indicating that: (i) during adult-onset experimentally-induced hypothyroidism of this type, the brain seems to maintain TH levels for some time, and (ii) that the TH-induced regulation of crucial brain enzymes such as those of AChE and Na^+,K^+-ATPase is a phenomenon of important complexity (Kundu et al., 2006, 2007). Although these findings match, to an extent, with ours, one should keep in mind that (i) we should avoid any correlation of findings generated at different ages (the neurodevelopmental dynamics of the effects of altered THs do not resemble those performed during adulthood), as well as (ii) the suggested by Meyer and Cooper (1981) inverse relationship between the activities of AChE and Na^+,K^+-ATPase is a phenomenon that does not have a catholic application either in adult-onset (Carageorgiou et al., 2007a,b) or in development-associated hypothyroidism (Figs. 1 and 2).

A major finding of our study is that none of the three studied approaches to developmental hypothyroidism has managed to cause any significant effect on 21-day-old brain Na^+,K^+-ATPase and Mg^{2+}-ATPase activities (Figs. 2 and 3). Continuous gestational and lactational MMI-induced maternal hypothyroidism is reported to cause significant inhibition of both cerebral Na^+,K^+-ATPase and Mg^{2+}-ATPase in the 21-day-old offspring rats (as compared to their age-matched controls) (Ahmed et al., 2010), while on the other hand, radiothyroidectomized newborn Wistar rats have demonstrated an extremely inhibited synaptosomal Na^+,K^+-ATPase activity at postnatal days 30–35 (as compared to controls), accompanied by an unaltered synaptosomal Mg^{2+}-ATPase activity (Billimoria et al., 2006; Katyare et al., 2006).

In an older study, maternal intragastric administration of PTU (50 mg/day) during lactation failed to cause any significant change in the offspring Porton rat forebrain Na^+,K^+-ATPase and Mg^{2+}-ATPase activities at all studied time-points (11-, 22- and 30-days of age) (Atterwill et al., 1985). However, the same experiment has demonstrated a decreased cerebellar Na^+,K^+-ATPase activity due to lactational hypothyroidism at the 22- and 30-day-old offspring rats (Atterwill et al., 1985).

Chaudhury et al. (1996) have reported that maternal forced feeding of PTU (50 mg/day) from gestation day 16 till various postnatal days (1, 5, 10, 15 and 20) resulted in a decreased expression of Na^+,K^+-ATPase mRNA α isoforms in the offspring rat brain. Moreover, congenital hypothyroidism through MMI addition to the drinking water (0.04%) at day 14 of gestation and thereafter has been reported to cause a significant reduction of the ouabain binding sites (an indicator of Na^+,K^+-ATPase abundance) of the 2-week-old offspring Sprague–Dawley rat cerebral cortex and cerebellum (Nomura et al., 1990). Potthoff and Dietzel (1997) have suggested that increases of the various Na^+,K^+-ATPase subunits' expression known to be upregulated by T3 in postnatal neurons could be elicited by an enhanced Na^+ influx through voltage-activated Na^+ currents; a function that could hold the answer to the hypothyroidism-associated neurobehavioural symptomatology. However, the brain does not only consist of neurons; in fact, glial Na^+,K^+-ATPase is a dominant contributor to the activity measured in our experiment as well as in those of many others.

Recently, doxorubicin-induced maternal hypothyroidism has been reported to be associated with decreased cerebral and cerebellar Na^+,K^+-ATPase and Mg^{2+}-ATPase activities in 15- and 20-day-old offspring Wistar rats (Ahmed and Incerpi, 2013); however, one should keep in mind that this cannot be considered as an experimental model of congenital or developmental hypothyroidism, since the tested doxorubicin treatment is considered as sufficient to increase the frequency of cellular damage in mammalian systems (Ahmed and Incerpi, 2013), suggesting that this might be more likely linked to a failure of the ATPase system.

An important study is that of Kawada et al. (1988), suggesting that PTU-induced maternal hypothyroidism during lactation followed by surgical thyroidectomy of the offspring could produce a transient decrease of Na^+,K^+-ATPase activity (that can later be regained during adulthood). On the other hand, inherited primary hypothyroid (hyt/hyt) mice are reported to bear significantly lower Na^+,K^+-ATPase activities in tissue homogenates of their cerebral cortex, brain stem and cerebellum (as compared to respective homogenates of corresponding regions from euthyroid littermates) (Li and Chow, 1994).

Beyond neurodevelopment, adult-onset MMI-induced hypothyroidism is known to result in decreased Na^+,K^+-ATPase activity in the cortex, the amygdale and the hippocampus, but not in the cerebellum of male Wistar rats (Pacheco-Rosado et al., 2005). However, Sarkar and Ray (1993) have provided a report on PTU-induced stimulation of synaptosomal Na^+,K^+-ATPase activity in the adult rat cerebral cortex; a phenomenon suggested to be reversed by T3 administration. Our previous findings on adult-onset PTU-induced hypothyroidism have also indicated an increase in both Na^+,K^+-ATPase and Mg^{2+}-ATPase activities of Wistar rat brains (Carageorgiou et al., 2005). On the contrary, by using a more complicated mode of adult-onset hypothyroidism (involving a 20-day-long feeding of a low iodine test diet, 0.05% perchlorate addition in the drinking water and the subcutaneous injection of a 25 mg pellet of PTU), Horowitz et al. (1990) have found no significant changes in Na^+,K^+-ATPase activity or subunits' regulation in the examined hypothyroid male Sprague–Dawley rat brains. Schmitt and McDonough (1986) had earlier used the same protocol of hypothyroidism-induction on pregnant Sprague–Dawley rats (at day 14 of gestation and onwards) and have reported a decreased brain Na^+,K^+-ATPase activity in the 15-day-old offspring, accompanied by a decrease of the abundance of both the α and the $\alpha+$ isoforms of Na^+,K^+-ATPase. What is more interesting is that the same authors have later suggested that the developing brain Na^+,K^+-ATPase α and the $\alpha+$ isoforms are sensitive to THs by as late as postnatal day 15, and that this period of TH-responsiveness is over by postnatal day 22 (Schmitt and McDonough, 1988).

In conclusion, our study has revealed that under all the examined experimental conditions, offspring rat brain AChE activity was found to be significantly decreased due to maternal hypothyroidism, in contrast to the two studied adenosinetriphosphatase activities that were only found to be significantly altered right after birth (following an exposure to gestational maternal hypothyroidism) and were restored to control levels by the end of lactation. Our findings regarding the pattern of effects that maternal hypothyroidism has on these crucial offspring rat brain enzyme activities reveal a wide spectrum of differences with those found in the literature, that could be attributed to both the mode of the experimental simulation approach followed as well as to the time-frames examined. We believe that our study could provide the basis for a debate on the need of a more consistent experimental approach to hypothyroidism during neurodevelopment (either as a result of maternal hypothyroidism or of congenital hypothyroidism) 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. However, it should be noted that despite the fact that PTU is a widely used means of experimentally-induced hypothyroidism, its mode of action during neurodevelopment is not well characterized (Zoeller and Crofton, 2005), and its effect on the offspring developing brain can be lasting even at dose-schemes of undetectable maternal thyroid status changes (Chakraborty et al., 2012).

Conflict of interest

None declared.

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