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Research report

Testing the correlation between experimentally-induced hypothyroidism during pregnancy and autistic-like symptoms in the rat offspring

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HIGHLIGHTS

- We tested the association between maternal hypothyroidism and autism in the offspring.
- We used an experimental model based on methimazole (MMI) administration to rat dams.
- MMI-exposed rats had no deficits in communication, social interaction and anxiety.
- MMI-exposed rats showed increased novelty-directed exploratory behaviors.

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ABSTRACT

Thyroid hormones are important for the development of the central nervous system. Since the fetal thyroid gland is not functioning until mid-gestation, transport of maternal thyroid hormones across the placenta is essential during the early phases of gestation. Maternal thyroid deficiency has been associated with a higher incidence of neurodevelopmental disorders in the newborns. The relationship between maternal hypothyroidism and the onset of autism spectrum disorders (ASD) in the offspring, however, is still debated. To address this issue, we used a validated animal model of prenatal hypothyroidism based on the administration of the thyroid peroxidase inhibitor methimazole (MMI, 0.02 g/100 ml in tap water) to rat dams from gestational day 9 up to delivery. The offspring was tested in behavioral tasks during infancy (PNDs 5, 9, 13) and adolescence (PND 35-40) to capture some of the core and associated symptoms of ASD. MMI-exposed pups were able to vocalize as controls when separated from the nest, and showed intact social discrimination abilities in the homing behavior test. At adolescence, the offspring from both sexes did not show an anxious-phenotype in the elevated plus maze and showed intact object recognition. However, MMI-exposed male rats showed increased novelty-directed exploratory behaviors: they solicited their partner to play more and showed more interest for novel rather than familiar objects compared to control rats. Our results show that prenatal MMI-induced hypothyroidism does not cause in the rat offspring behaviors that resemble core and associated ASD symptoms, like deficits in communication and social interaction and anxiety.

1. Introduction

Thyroid hormones play a fundamental role in the development of the central nervous system, since they are involved in many processes underlying brain development and maturation [1]: neuronal and glial cell differentiation and proliferation, axonal and dendritic growth, synapse formation, cell migration and myelination [2,3].

Optimal thyroid functioning is required during pregnancy. Thus, hyperthyroidism during pregnancy is associated with severe maternal tachycardia, thyromegaly and exophthalmia, along with several adverse effects on pregnancy outcomes, such as miscar-

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riage, stillbirth, preterm delivery, intrauterine growth retardation, preeclampsia [4]. Compared to hyperthyroidism, hypothyroidism is more common during pregnancy, with 0.3–0.5% of pregnant women showing overt hypothyroidism and 2–2.5% subclinical hypothyroidism [4,5].

Several factors like genetic mutations, infections, nutrients and environmental contaminants can affect thyroid function during gestation and early postnatal development. The most common cause of maternal and fetal thyroid dysfunction is iodine deficiency from inadequate alimentary habits [6]: it has been estimated that nearly 2 billion individuals globally have an insufficient iodine intake that can result in inadequate thyroid hormone production [7]. The fetal thyroid gland is not functioning until mid-gestation, being active at 16–20 weeks post-conception in humans and at gestational day 17.5 in rats [1,8]. However, even before the onset of fetal thyroid function, the fetal cerebral cortex is capable of generating T3 from maternal T4 by local deiodination, and significant levels of T3 and thyroid hormone receptors (TRs) can be found in the fetal brain [1,9]. Thus, active transport of maternal thyroid hormones across the placenta has to occur during the early phases of gestation and it is essential for the development of the mammalian central nervous system [1]. Conversely, thyroid hormone deficiency, present at the time of birth through low maternal iodine levels, is a major contributing factor to congenital hypothyroidism in newborns [10]. Maternal thyroid hormone deficiency has adverse consequences on pregnancy outcome and offspring neurodevelopment. Overt hypothyroidism is associated with increase in prevalence of abortion, anemia, pregnancy-induced hypertension, preeclampsia, placental abruption, postpartum hemorrhage, premature birth, low birth weight, intrauterine fetal death and neonatal respiratory distress, associated with long term effect on the cognitive function of the offspring [4]. Recent evidence suggests that even more moderate forms of maternal thyroid dysfunction, particularly during early gestation, may have a long-lasting influence on child development, impairing the offspring's cognitive and motor development and increasing the risk of neurodevelopmental disorders [1]. In particular, thyroid hormone deficiencies during brain development, either due to a genetic deficiency of the thyroid receptor interacting protein, which codes for a transcriptional regulator associated with nuclear TRs [11], or due to maternal hypothyroidism, have been associated with an increased risk of autism spectrum disorders (ASD) [6,12,13]. However, other studies found no association between neonatal T4 levels and ASD [14], or reported an inverse correlation between mid-pregnancy thyroid stimulating hormone levels and ASD risk [15]. Thus, it is still unclear whether maternal hypothyroidism is a risk factor for ASD and can therefore become a target for effective public health risk reduction efforts. The aim of this study was to test this possibility at the preclinical level, by investigating whether maternal hypothyroidism induces in rats behavioral features that resemble some of the core and associated symptoms of ASD. To this aim, we used a validated animal model of prenatal hypothyroidism based on methimazole (MMI) administration to rat dams. In Europe and many other countries, MMI is used to treat Graves' disease, the most common cause of hyperthyroidism in pregnancy [16]. MMI affects thyroid hormone synthesis and deiodinase activity by inhibiting thyroid peroxidase and it has been extensively used to model maternal hypothyroidism in laboratory animals [17–19]. Since ASD patients typically show poor communication and social interaction [20], deficits in social play [21], increased interests for objects and intact object recognition [22], we tested the offspring born from MMI-exposed and control (CTRL) dams in the following behavioral tests: 1. the pup isolation-induced ultrasonic vocalizations (USVs) test, that provides quantitative and qualitative measures of the USVs emitted by rodent pups when isolated from the nest, which play an essential communicative role in mother-offspring interaction [23,24]; 2. the homing behavior test, that provides an early measure of social discrimination, since it allows to detect the pups' cognitive, sensory and motor ability necessary to discriminate between a neutral odor and their own nest odor [23,25]; 3. the social interaction test at adolescence, to detect social behaviors both related and unrelated to play [23,26]; 4. the object recognition test, a two trial cognitive paradigm that assesses object recognition memory in rodents [27]. Last, since anxiety is a frequent symptom displayed by autistic patients [28], we also tested whether MMI prenatal exposure affected the behavior of the offspring in the elevated plus-maze test, the most common behavioral paradigm used to assess anxiety-like behaviors in rodents.

2. Materials and methods

2.1. Animals and treatments

Primiparous female Wistar rats (Charles River, France), weighing 250 ± 15 g, were mated overnight. The morning when spermatozoa were found was designated as gestational day 0 (GD0). Pregnant rats were singly housed in Macrolon cages (40 × 26 × 20 cm), under controlled conditions (temperature 20–21 °C, 55–65% relative humidity and 12/12 h light cycle with lights on at 07:00 a.m.). Food and water were available *ad libitum*. Gestational hypothyroidism was induced by adding 20 mg of MMI to tap water (100 ml) [17–19], from gestational day (GD) 9 until the day of the birth [18]. Indeed, it has previously been shown that MMI, given for 10 days to pregnant rats from GD9, is able to cause hypothyroidism in the dams by inducing a decrease of circulating maternal total T3 and T4 levels [19,29]. CTRL females had access to the same volume of tap water. Fluid consumption was recorded daily throughout gestation to ensure that both CTRL and MMI-exposed dams had a similar water intake. The mean dose of MMI consumed daily by each pregnant dam was 12 mg. On PND 1, the litters were culled to eight animals (4 males and 4 females). On PND 21, rats were weaned and housed in group of three with same litter and same sex partners. Experiments were carried out on the male and female offspring during infancy (PNDs 5, 9 and 13) and adolescence (PND 35-40). The total number of litters used for the behavioral experiments was 8 CTRL- and 9 MMI-exposed dams. For the USV and homing behavior tests, we used two male and two female pups per litter; for the behavioral tests performed during adolescence, one male and one female rat per litter from different litters per treatment group were used in each experiment.

All the experiments were approved by the Italian Ministry of Health (Rome, Italy) and performed in agreement with the guidelines released by the Italian Ministry of Health (D.L. 26/14) and the European Community Directive 2010/63/EU.

2.2. Measurements of thyroid hormones serum levels

Thyroid status was evaluated through the determination of total T3 and T4 serum levels in pregnant rats. For the measurement of thyroid hormones, at GD21, pregnant rats (n = 6 animals/group) were decapitated and trunk blood was collected in plastic tubes. Serum samples were obtained after clotting and were stored at −80 °C. Total T3 and T4 levels in serum were measured by competitive enzyme-linked immunosorbent assay (ELISA) complete kit according to the manufacturer's instructions sheet (Interteck, Katal, MG, Brazil). The final hormone concentration was calculated based on a standard curve constructed for each assay using recombinant hormone standards. All hormone assays were conducted at the same time using supplies from the same assay.