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Sodium selenite supplementation during pregnancy and lactation promotes anxiolysis and improves mnemonic performance in wistar rats' offspring

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ABSTRACT

Selenium is a micronutrient which is part of selenoprotein molecules and participates in a vast number of physiological roles and, among them, we have fetal and neonatal development. Therefore, the aim of this study was to evaluate possible behavioral changes in offspring of female rats supplemented during pregnancy and lactation with sodium selenite. To address that, we treated two groups of female rats by saline or sodium selenite at a dose of 1 mg/kg through oral route and performed neurochemical and behavioral tests. In the offspring, the thyroid profile and hippocampal neurochemistry were evaluated. Behavioral tests were performed in pups both during childhood and adulthood. We found out that selenium (Se) supplementation increased serum levels of triiodothyronine (25%, $p < 0.001$) and thyroxine (18%, $p < 0.05$) and promoted a tryptophan hydroxylase 2 (TPH 2) expression decrease (17%, $p < 0.01$) and tyrosine hydroxylase (TH) expression increase (202%, $p < 0.01$) in the hippocampus. The cholinesterase activity was decreased (28%, $p < 0.01$) in Se supplemented rats, suggesting a neurochemical modulation in the hippocampal activity. During childhood, the Se-supplemented offspring had a reduction in anxiety-like behavior both in elevated plus maze test and in light–dark box test. In adulthood, Se-treated pups had an increase in the locomotor activity (36%, $p < 0.05$) and in rearing episodes (77%, $p < 0.001$) in the open field test, while in the elevated plus maze test they also exhibited an increase in the time spent in the open arms (243%, $p < 0.01$). For the object recognition test, Se-treated offspring showed increase in the absolute (230.16%, $p < 0.05$) and relative index discrimination (234%, $p < 0.05$). These results demonstrate that maternal supplementation by sodium selenite promoted psychobiological changes both during childhood and adulthood. Therefore, the behavioral profile observed possibly can be explained by neurochemical changes induced by thyroid hormones during the critical period of the central nervous system ontogeny.

1. Introduction

The prevalence of some diseases in adulthood presents a significant correlation with homeostatic disturbances during the fetal stage (Barker, 1990). Several environmental factors contribute to an adverse intrauterine environment during this critical phase, promoting structural and functional changes in the fetus, which could subsequently lead to long-term disease. This process is known as fetal programming (Godfrey & Barker, 2001).

One of the main environmental factors that affect the embryogenesis of different physiological systems is maternal nutrition (Chmurzynska, 2010). Based on this assumption, it is noteworthy that in pregnant and lactating women, plasma concentrations of selenium (Se) are reduced, given that there is an increased demand of this nutrient by the fetus and neonate, making necessary its continuous offering (Smith and Picciano, 1986). Moreover, it has been shown that Se participates in the regulation of fetal and neonatal development (Ewan, 1976), and, importantly, its deficiency has been associated with miscarriages (Barrington et al., 1996) and premature births in women (Dobrzynski

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et al., 1998). Accordingly, the recommended levels for adequate Se supply range from 30 to 85 μg/day for adults (Rayman, 2008). These doses are based on the use of Se needed to maximize selenoenzyme glutathione peroxidase (GPx3) plasmatic activity (Duffield et al., 1999). In general, supplements can provide an additional 10–200 μg Se/day (Fairweather-Tait et al., 2011).

It is known that the beneficial effects of Se to human health are associated to its presence within 25 selenoproteins in the form of the amino acid selenocysteine (Papp et al., 2007; Papp et al., 2010). These proteins are involved in several functions such as: antioxidants or oxidoreductases enzymes, including glutathione peroxidases (GPxs) and thioredoxin reductases (TrxR); metabolism of thyroid hormones by deiodinases (DIOs); transport and delivery of Se to peripheral tissues by selenoprotein P (Sel P); protein folding and endoplasmic reticulum stress inhibition by Sep. 15, Sel M, Sel N, and Sel S. However, some selenoproteins have functions not elucidated so far (Steinbrenner and Sies, 2013).

In this context, in the past few years, many epidemiological studies have demonstrated the involvement of Se deficiency in the genesis and progression of chronic diseases such as metabolic, cardiovascular and neurological diseases (Navarro-Alarcon and López-Martınez, 2000). Furthermore, recent studies suggested a strong association between Se levels and neuronal disorders, particularly in situations of redox imbalance (Chen and Berry, 2003). In fact, humans with Se deficiency are more likely to develop mood disorders, cognitive impairment, anxiety, depression and hostility. However, the increased supply of this element in the diet may reduce and stabilize these disorders (Benton and Cook, 1991; Hawkes and Hornbostel, 1996; Finley and Penland, 1998). Additionally, there is also a correlation between Se status, increased oxidative stress and neural diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy and multiple sclerosis, although this relation is not fully understood (Chen and Berry, 2003; Schweizer et al., 2004; Ashrafi et al., 2007; Shukla et al., 1977).

The reasons for the effect of Se on mood are unclear. But, one of the most plausible hypotheses is related to thyroid hormones (THs) (Sher, 2001). As these hormones play an important role in the regulation of mood and they are involved to some extent in the psychopathology of mood disorders (Esposito et al., 1997; Sher, 2000), the Se effects could be mediated by the action of the deiodinases.

Thus, based on the aspects presented above, we believed Se could be playing a crucial role on cognition and behavioral changes in mood and, therefore, our aim here was to evaluate whether Se supplementation during the critical periods of pregnancy and lactation was able to change behavioral parameters in rats' offspring both during childhood and adulthood. Notwithstanding, once cognitive and mood changes were confirmed, we investigated which neuroendocrine and neurochemical changes were involved in this process.

2. Material and methods

2.1. Subjects

Wistar rats with 60 days of age (~220 g) from Federal Rural University of Rio de Janeiro animal facilities were used in this protocol. After acclimatization period of 15 days, the rats were housed in plastic cages (35 cm × 50 cm × 20 cm) and were mated with a ratio of two females to one male. Day 1 of pregnancy was determined by the presence of spermatozoa in the vaginal smear. Following confirmation that mating had occurred, females were randomly divided into two groups (n = 12): control and Se-supplemented group. The control was treated with 0.9% saline, while the treated group received sodium selenite at a dose of 1 mg/kg. This dose was chosen in accordance with data contained in the literature (Jacobs and Forst, 1981; Boylan et al., 1990). The gavage administration was used for both groups to regulate the solutions ingested by the animals.

After birth, the male rats were removed and the obtained offspring were standardized in maximum of 8 pups (4 males and 4 females) per female. The offspring was weaned at postnatal day (PND) 21 and they were kept in cages, in a group of five rats per cage. On the same day, female pups were euthanized and the serum was collected to assess T3, T4 and TSH levels, whereas the hippocampus was dissected from the whole brain under cold plate and kept at −70 °C to evaluate the TPH2 and TH gene expression or cholinesterase activity. On the other hand, the male pups were submitted to the behavioral analysis on PND 23 or PND 70 as summarized in the Fig. 1.

All animals were housed at controlled temperature (21 ± 2 °C) with daily exposure to a 12 h light–dark cycle and free access to water and standard rodent chow. This investigation was carried out according to Animal Use Ethics Committee (CEUA)/Institute of Biology (IB)/UFRRJ in consonance with Brazilian Animal Welfare legislation and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Serum hormone measurements

Serum total T_4, T_3 and TSH from PND 21 female pups were determined by electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Manheim, Germany) using blood collected from trunk. The tests sensitivities and maximum detection values were: 0.01 μUI/mL and 100 μUI/mL for TSH; 0.42 mg/dL and 24.86 mg/dL for T_4 and 19.5 mg/dL and 651 mg/dL for T_3. Interassay coefficients of variation were, respectively: 7.2% for TSH; 4.2% for T_4 and 9% for T_3.

2.3. RNA analysis

Total RNA was extracted using a standard method (TRIzol reagent; Invitrogen, Carlsbad, CA, USA). The RT-PCR analyses were carried out from 1 μg of total RNA extracted from hippocampus of PND 21 female pups using the Superscript III kit (Invitrogen).

Real-time RT-PCR analyses were performed in a fluorescent temperature cycler (Applied Biosystems 7500; Life Technologies Co., Carlsbad, CA, USA) according to the recommendations of the manufacturer. Briefly, after initial incubation at 50 °C for 2 min and 95 °C for 10 min, reactions were cycled 40 times using the following parameters for all genes studied: 95 °C for 15 s, 60 °C for 30 s and 72 °C for 45 s. SYBR Green (Applied Biosystems, Foster City, CA, USA) fluorescence was detected at the end of each cycle to monitor the amount of PCR product formed during that cycle. Primers used for the amplification of cDNAs of interest were synthesized by Integrated DNA Technologies Inc. The forward and reverse primers sequences were, respectively: 5′-TTCC CACTGGCTGAAAAGGT-3′ and 5′-CGCAGCCGCAAATGC-3′ for *36β4*; 5′-TGAGAACCCCAAATCCTGCA-3′ and 5′-CCCAGCCAACAGACCTAACTG A-3′ for tryptophan hydroxylase 2 (*Tph2*); 5′-CCCCACCTGGAG TATTTT GTG-3′ and 5′-ATCACGGGCGGACAGTAGACC-3′ for tyrosine hydroxylase (*Th*).

We determined relative mRNA levels ($2^{-\Delta\Delta Ct}$) by comparing the PCR cycle threshold (Ct) between groups, after correcting for the internal control 36β4 (Schmittgen and Livak, 2008). Assays were repeated two or three times and the data were merged after normalization.

2.4. Cholinesterase activity assay

The determination of cholinesterase activity in the hippocampus was performed using the Ellman method. This method is based on the hydrolysis of acetylcholine or other choline esters by cholinesterase present in neurons, which occurs in the formation of two products: acetic acid and thiocholine (Ellman et al., 1961).

In order to perform this protocol, animals with 21 days of both experimental groups were euthanized by decapitation. For determination of enzyme activity, the hippocampus was properly weighed and homogenized in phosphate buffered saline (PBS) at a concentration of