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Alterations in the memory of rat offspring exposed to low levels of fluoride during gestation and lactation: Involvement of the α7 nicotinic receptor and oxidative stress

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ABSTRACT

Daily exposure to fluoride (F) depends mainly on the intake of this element with drinking water. When administered during gestation and lactation, F has been associated with cognitive deficits in the offspring. However, the mechanisms underlying the neurotoxicity of F remain obscure. In the current study, we investigated the effects of oral exposure to low levels of F during the gestational and lactation periods, on the memory of adult female rat offspring. We also considered a possible underlying neurotoxic mechanism. Our results showed that this exposure reduced step-down latency in the inhibitory avoidance task, and decreased both mRNA expression of the α7 nicotinic receptor (nAChR) and catalase activity in hippocampus.

Our data indicates that low F concentrations administrated during gestation and lactation decrease the memory of 90-day-old female offspring. This suggests that the mechanism might be connected with an α7 nAChR deficit in the hippocampus, induced by oxidative stress.

1. Introduction

The most important factor contributing to fluoride (F) exposure is its content in drinking water [1,2]. In the World Health Organization (WHO) guideline [3] the permissible limit of F in drinking water is 1.5 mg/l. Beneficial effects of F are achieved with low concentrations (0.8–1.2 mg/l) in drinking water and by mixing it with dental paste (1000 ppm and above) [1,4]. However, among the 25 countries that have naturally occurring high F concentrations (> 1.5 mg/l) in groundwater, such as China, India, México and Argentina [5], more than 200 million people suffer from endemic fluorosis [6]. In Argentina, in particular in some areas of the Chaco-Pampean plain, shallow groundwater with very high F concentrations (11.5 mg/l) has been found, which may lead to a potential risk of fluorosis [5].

F exists in drinking water in an ionic form and, following ingestion, rapidly passes through the intestinal mucosa where it interferes with metabolic pathways of living systems [7]. F is a cumulative poison [2]. On average, only 50% of the F ingested by our body each day is excreted through the kidneys while the remaining accumulates in tissues [8,9]. In the organisms of infants and children, about 80–90 % of the absorbed F is accumulated [2]. F is biologically active even at very low concentrations (equal to the 1 ppm in fluoridated drinking water) [10]. F can cross the placenta barrier and diffuse into cord blood [11]. In addition, the significant high F in breast milk indicates the accessibility of fluoride for infants [12,13]. Young individuals are less resistant to the toxic influence of F due to the fact that their defensive mechanisms are not fully developed and the permeability of their blood-brain barrier is higher than among adults [14]. In recent years, scientists have focused on the toxic influence of this element on the nervous system [15]. Epidemiological studies have found that the levels of mental work capacity and the Intelligence Quotient (IQ) are lower for children in the areas with endemic fluorosis as compared to reference areas [16–19]. F accumulation over a period of time has been shown to cause significant neurological damage and neuro-degenerative disorders in animals

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[20,21]. Supported by numerous animal experimental studies, the hippocampus has been postulated to be one of the neurotoxic target sites [22–24]. Prolonged exposure to F in the prenatal and postnatal stages of development has a toxic influence on the metabolism of neurons and glia which results in disorders in memory and in learning processes [25–27]. However, the exact mechanisms by which F decreases cognitive and learning abilities and causes memory loss are not clear.

Neuronal nicotinic receptors (nAChRs) are a class of neurotransmitter-gated ion channels present throughout the central nervous system (CNS). nAChRs are involved in cognitive functions, such as learning, memory and attention and executive function, both in humans and in animals [28–31]. Neuronal nAChRs can be homomeric, composed of an α-type subunit, such as α7, or heteromeric, which includes α and non-α subunits. To date, nine different α (α2-α10) and three β (β2-β4) subunits have been cloned in CNS. α7, α4β2, and α3β4 nAChRs are present in the hippocampus [32]. The α7 nAChR has received considerable attention as a consequence of its high expression in the hippocampus and in the neocortex, its ability to form homo-oligomeric receptors, its involvement in several types of learning and memory-related behavior, and its neuroprotective effect [30,33,34]. The key role of the hippocampus in the formation of many forms of memory, including inhibitory avoidance and maze tasks, has been well-documented [35]. Importantly, the α7 nAChR deficit might be connected with functional disorders of the brain and the decreased IQ influenced by fluorosis [36]. Therefore, it is interesting to understand the mechanisms by which the fluorosis produced a decrease in this nAChR.

Oxidative stress is caused by a disturbance in the balance between the synthesis of reactive oxygen species and the activity of anti-oxidative enzymes [37]. The products and inescapable by-products of oxidative metabolism can damage macromolecules like nucleic acids, proteins, and lipids [12]. The CNS is especially sensitive to free radical oxidative damage as it contains high levels of iron, easily oxidisable fatty acids, low antioxidant defense system, and it uses large amounts of oxygen [38]. Moreover the heterogeneity of the developing nervous system, with different cell types and function, makes it more vulnerable to environmental contaminants than the adult nervous system [39]. A certain amount of oxidative damage takes place even under normal conditions; however, the rate of this damage increases during fluorosis, as the efficiency of antioxidative and repair mechanisms decreases leading to oxidative stress in neurons and glia [25,37]. Several previous studies revealed that F induces excessive production of oxygen free radicals and caused a decrease in biological activities of some antioxidant enzymes like catalase (CAT) and glutathione peroxidase (GPx). It also produces lipid peroxidation. As an indicator of the level of lipid peroxidation products malondialdehyde (MDA) is assay as thiobarbituric acid-reactive substance (TBARS) [12,13,20,40–44].

Literature is limited regarding the effects of the exposure to low F doses during gestation and lactation on the CNS of the offspring. It is hypothesized that F exposure during gestation and lactation could lead to structural alterations in the neuronal circuit which may later manifest as functional deficits. Since it has been shown previously that female offspring have a greater sensitivity to F effects, compared to male offspring, in neuroconductural studies [45], only the female offspring, of mothers exposed to low F concentrations during the gestation and lactation, were studied. Thus, the purpose of the present work was to study the effect on memory and the underlying effects of the exposure to low levels of F during gestation and lactation of adult female rat offspring. To this end, Wistar rats were exposed to low F concentrations (5 and 10 mg/l) during gestation and lactation. Short-term memory (STM) and long-term memory (LTM) were evaluated by step-down inhibitory avoidance test; the expression level of α7 nAChR mRNA in the hippocampus was determined by real-time PCR; and the antioxidant enzyme activities and lipid peroxidation levels were measured both in the whole brain and in the hippocampus of female adult offspring. To evaluate damage inflicted by oxidative stress, antioxidant enzymes, such as CAT and GPx, along with lipid peroxidation products, such as MDA, were studied as potential biomarkers.

2. Materials and methods

2.1. Materials

Sodium fluoride (NaF) was purchased from Anedra (San Fernando, Argentina).

2.2. Animals

Male and nulliparous female Wistar rats (90–120 days old) were obtained from colonies maintained under specific pathogen-free conditions from our breeding center of the Universidad Nacional del Sur, Bahía Blanca, Argentina. They were maintained under constant temperature (22° ± 1 °C) and under humidity (50–60%) conditions in a 12L:12D cycle (lights on at 7:00 a.m.) and with standard rodent pellet diet and filtered tap water *ad libitum*. In the evening of the proestrus day, they were housed overnight with the male rats. The presence of spermatozoa in the vaginal smears was registered as an index of pregnancy and it was referred to as gestational day 0 (GD 0). Pregnant females were housed individually in cages and were randomly assigned to one of the three following groups: control group (n = 10; filtered tap water), F treated group with 5 mg/l in filtered tap water (n = 10) and F treated group with 10 mg/l in filtered tap water (n = 10), equivalent to doses of 0.6 and 1.2 mg/kg, respectively. Drinking water was changed daily. Dams received the treatment from GD 0 to weaning on postnatal day (PND) 21. Maternal weight gain, food intake and drink consumption were recorded as described before [45]. All pups were weighed and gestation length, litter size and body weight of pups on different PNDs were analyzed as described in Bartos et al., [45]. On PND 21 the female pups of each dam were weaned and housed together according to treatment until PND 90. One female from each litter was randomly selected for the behavioral test, other female from each litter for the α7-AChR expression and another two for the rest of neurochemical determinations in whole brain and in the hippocampus. For memory test we used n = 9–10 per group, and for neurochemical measures we used n = 5 per group. The female offspring that were not utilized in these tests and the full litter of male offspring, were either used for other experiments of our laboratory or were euthanized by using a CO_2 chamber by qualified personnel of our breeding center of Universidad Nacional del Sur.

2.3. Step-down inhibitory avoidance task

Adult female offspring were trained in a step-down inhibitory avoidance paradigm during which stepping-down from a platform presented in a given context was associated with a foot shock, resulting in an increase in the step-down latency. The inhibitory avoidance apparatus was a box with a floor consisting of parallel nonrusting steel bars. A 2.5 cm high platform was placed on the left end of the box. Latency of the rats to step down placing the four paws on the grid was measured. Twenty-four hours prior to training, we conducted a habituation of rats to the new environment, which consisted of placing the rat on the platform and leaving freely explore for 180 s. In the training session, the animals were gently placed on the platform and they received a 0.6 mA, 2 s scrambled foot shock immediately after they stepped down placing their four paws on the grid. Test sessions were carried out 90 min (STM) and 24 h (LTM) after training. They were exactly like the training session, except that the foot shock was omitted. A 180 s ceiling was imposed on test session latency measurements. In the test sessions, step-down latency was used as measure of memory retention [46].

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