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Reversion of age-related recognition memory impairment by iron chelation in rats

Maria Noêmia Martins de Lima [a,b], Caroline Pietá Dias [a,b], Juliana Presti Torres [a,c], Arethuza Dornelles [a,c], Vanessa Athaíde Garcia [a], Felipe Siciliani Scalco [a], Marcelo Rewsaat Guimarães [a], Roberta Cristina Petry [a], Elke Bromberg [a,b], Larissa Constantino [d], Patricia Budni [d], Felipe Dal-Pizzol [d], Nadja Schröder [a,b,c,*]

[a] *Neurobiology and Developmental Biology Laboratory, Faculty of Biosciences, Pontifical Catholic University, 90619-900 Porto Alegre, RS, Brazil*
[b] *Graduate Program in Biomedical Gerontology, Institute for Geriatrics and Gerontology, São Lucas Hospital, Pontifical Catholic University, 90619-900 Porto Alegre, RS, Brazil*
[c] *Graduate Program in Cellular and Molecular Biology, Faculty of Biosciences, Pontifical Catholic University, 90619-900 Porto Alegre, RS, Brazil*
[d] *Physiopathology Laboratory, Department of Medicine, University of Southern Santa Catarina, 88806-000 Criciúma, SC, Brazil*

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Abstract

It is now generally accepted that iron accumulates in the brain during the ageing process. Increasing evidence demonstrate that iron accumulation in selective regions of the brain may generate free radicals, thereby possessing implications for the etiology of neurodegenerative disorders. In a previous study we have reported that aged rats present recognition memory deficits. The aim of the present study was to evaluate the effect of desferoxamine (DFO), an iron chelator agent, on age-induced memory impairment. Aged Wistar rats received intraperitoneal injections of saline or DFO (300 mg/kg) for 2 weeks. The animals were submitted to a novel object recognition task 24 h after the last injection. DFO-treated rats showed normal recognition memory while the saline group showed long-term recognition memory deficits. The results show that DFO is able to reverse age-induced recognition memory deficits. We also demonstrated that DFO reduced the oxidative damage to proteins in cortex and hippocampus. Thus, the present findings provide the first evidence that iron chelators might prevent age-related memory dysfunction.

Keywords: Aging; Recognition memory; Desferoxamine; Neuroprotection; Iron; Oxidative stress; Protein carbonyl; Rat

1. Introduction

It is now generally accepted that iron accumulates in the brain during the ageing process (Polla et al., 2003; Schipper, 2004; Zecca et al., 2004). In humans, it is known that concentrations of non-haem iron increase in the putamen, motor cortex, prefrontal cortex, sensory cortex and thalamus during the first 30–35 years of life (Hallgren and Sourander, 1958; Martin et al., 1998). Recent studies have shown that levels of ferritin, the major iron storage protein, in older individuals were higher than in younger controls in the frontal cortex, caudate nucleus, putamen, substantia nigra and globus pallidus (Connor et al., 1995; Zecca et al., 2001). A study comparing cellular and regional distribution of ferritin and iron between young and aged rats has indicated that in the normal aging brain there is an intracellular accumulation of iron in neurons (Benkovic and Connor, 1993).

Excessive iron content in selective regions of the brain may generate cytotoxic free radical formation, thereby possessing

* Corresponding author at: Department of Physiological Sciences, Faculty of Biosciences, Pontifical Catholic University, Av. Ipiranga, 6681 Prédio 12C, Sala 266, 90619-900 Porto Alegre, RS, Brazil. Tel.: +55 51 33203545; fax: +55 51 33203612.
E-mail address: nadja_s@terra.com.br (N. Schröder).

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implications for the etiology of neurodegenerative disorders (Qian and Shen, 2001; Thomas and Jankovic, 2004). Increased levels of iron have been reported in several neurodegenerative disorders, such as Parkinson's (PD) (Dexter et al., 1994, 1991; Ebaldi et al., 1996; Grifiths et al., 1999; Kienzl et al., 1995; Riederer et al., 1989), Alzheimer's (AD) (Bishop et al., 2002; Lynch et al., 2000; Ong and Farooqui, 2005; Pratico et al., 2002; Quintana et al., 2006) and Huntington's (HD) (Bartzokis and Tishler, 2000; Bartzokis et al., 1999) diseases. Despite years of investigation, it is still not known why iron levels are abnormally high in some regions of the brain in neurodegenerative disorders. Also, it is not clear whether iron accumulation in the brain is an initial event that causes neuronal death or is a consequence of the disease process.

A recent study involving human subjects was the first to correlate iron content, as measured by quantitative magnetic resonance (MR) imaging, and cognitive impairments in elderly participants. Accordingly, R2 an MR imaging parameter affected by changes in brain iron concentration and water content, was different in elderly participants with mild to severe levels of cognitive impairment compared with healthy controls (House et al., 2006), suggesting that iron misregulation might play a role in the decline in cognitive function observed in aged individuals.

The use of animal models has greatly increased our understanding of the iron regulatory mechanisms and the pathogenesis of neurodegenerative disorders related to iron deposition in the brain (Anderson and Powell, 2000; Grabill et al., 2003; Zhang et al., 2005a). In previous reports we have demonstrated that iron supplementation in the neonatal period induces a selective iron accumulation in brain regions, especially in the basal ganglia, which was associated with memory impairments in adult mice (Fredriksson et al., 1999, 2000) and rats (Schröder et al., 2001). In addition, iron supplementation in this period induces lipid peroxidation and protein carbonylation in *substantia nigra* (Dal-Pizzol et al., 2001). Moreover, it was shown that iron load in the early stages of life induces recognition memory impairment possibly by inducing oxidative damage in the brain (De Lima et al., 2005a).

Desferoxamine (DFO) is a metal chelator agent with antioxidants properties. Recently, with the observation that several neurodegenerative diseases involve iron accumulation in the central nervous system, DFO and other metal chelating agents became also investigated as a possible therapeutic agent for this class of pathologies (Crapper et al., 1991; Finefrock et al., 2003).

However, there is little information in the literature about the possible cognitive effects of iron chelation therapy in normal aged subjects or in patients with age-related neurodegenerative disorders. Thus, the purpose of the present study was to evaluate the effect of DFO on age-related recognition memory deficits. In order to do that, we submitted aged male Wistar rats (24 months old) treated subchronically with DFO to a novel object recognition task. Additionally, parameters of oxidative stress in cerebral regions related to memory formation were evaluated.

Recognition memory can be tested in rodents using object recognition tasks that are based on spontaneous activity and the natural preference that rats display to explore a novel object more than a familiar one when the animal remembers previous exposure to familiar object. Advantages associated with this class of measure include the fact that performance does not depend on the retention of a rule, and is not based on usual positive or negative reinforcers, such as food deprivation or application of an electric shock (Bertaina-Anglade et al., 2006; Dix and Aggleton, 1999; Ennanceur and Delacour, 1988; Mumby, 2001). Moreover, these tasks might depend both on the hippocampus and the nigrostriatal dopaminergic pathway (De Lima et al., 2006; Moses et al., 2005; Mumby et al., 2002; Schröder et al., 2003; Wais et al., 2006), brain regions that are severely affected in neurodegenerative disorders in which iron is overloaded.

2. Methods

2.1. Animals

Male Wistar rats were obtained from the State Foundation for Health Science Research (FEPPS-RS, Porto Alegre, Brazil). Animals were kept 3 to a cage on a 12-h light/dark cycle with food and water available *ad libitum*. All behavioral experiments took place between 9:00 and 17:00. All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care and were approved by the Ethics Committee of the Pontifical Catholic University (CEP-996/04).

2.2. Drugs and pharmacological procedures

Aged animals (23 months old) received intraperitoneal (ip) injections of saline (NaCl 0.9%) or desferoxamine mesylate (Desferal, Novartis, SP, Brazil), 300 mg/kg in a 1.0 ml/kg injection volume dissolved in saline three times per week for 2 weeks. The dose of DFO was chosen on the basis of previous studies (Freret et al., 2006; Lan and Jiang, 1997) and pilot experiments performed in our laboratory. Dinitrophenylhydrazine and trichloroacetic acid were purchased from Sigma, St. Louis, MO, USA.

2.3. Novel object recognition memory

Twenty-four hours after open field exploration (see below), animals were trained and tested in a novel object recognition task as previously described (De Lima et al., 2006, 2005a,b,c; Schröder et al., 2003). Training in the object recognition task took place in the same arena used for the open field, except that the arena floor was covered with saw-