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Selegiline protects against recognition memory impairment induced by neonatal iron treatment

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Abstract

Excess of iron in the brain has been implicated in the pathogenesis of several human neurodegenerative diseases, for example Alzheimer's disease and Parkinson's disease. It has been shown that the neonatal period is critical for the establishment of normal iron content in the adult brain. Moreover, it is known that aging alters the cerebral distribution of this metal. We have recently described that neonatal administration of iron severely impaired novel object recognition memory in rats. The aim of the present study was to determine whether selegiline, a monoamine oxidase (MAO) inhibitor known for its neuroprotective properties, could protect rats against cognitive impairment induced by neonatal administration of iron. In the first experiment, male Wistar rats received vehicle (5% sorbitol in water) or iron (10.0 mg/kg) orally from postnatal days 12 to 14 and saline (0.9% NaCl) or selegiline (1.0 or 10.0 mg/kg) intraperitoneally for 21 days, starting 24 h before the first iron dosing. In the second experiment, rats were given either vehicle or iron (10.0 mg/kg) orally from postnatal days 12 to 14 followed by saline or selegiline (1.0 or 10.0 mg/kg) intraperitoneally for 21 days, starting when rats reached adulthood (50th day after birth). Iron-treated rats given selegiline in both doses showed no deficits in recognition memory. Rats receiving iron but no selegiline presented memory deficits. This is the first study reporting the reversion of iron-induced memory impairment, supporting the view that our model can be considered as a useful tool in the search for new drugs with neuroprotective and/or memory enhancing properties.

Keywords: Iron; Recognition memory; Selegiline; Neurodegeneration; Neuroprotection; Oxidative stress; Rat

Introduction

The involvement of iron in several brain metabolic processes and normal development of neurological systems during a critical perinatal period, wherein deficiencies in this metal are associated with disruptions in behavioral performance, has been indicated (Youdim and Yehuda, 2000; Youdim et al., 1991; Ben-Shachar et al., 1986). However, there is also accumulating evidence that excessive deposits of iron in selective regions of the brain may generate cytotoxic free radical formation and cause alterations in iron metabolism, thereby possessing implications for the etiology of neurologic disorders (Zecca et al., 2004; Thomas and Jankovic, 2004; Kaur and Andersen, 2004; Sengstock et al., 1993). Increased levels of iron in selective brain regions have been reported in several neurodegenerative disorders, such as Parkinson's (PD), HuntIngton's (HD), Hallervorden-Spatz and Alzheimer's (AD) diseases, amyotrophic lateral sclerosis as well as in normal brain aging (Jellinger, 1999).

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In some experimental animal models of PD where degeneration of nigrostriatal dopaminergic neurons has been observed, there is evidence for iron-induced oxidative stress as a pathogenic factor (Youdim et al., 2004; Leret et al., 2002). However, these models of PD are generally focused on the motor alterations associated with this disorder.

The effects of iron administration during the neonatal period on cognition have been well documented. Adult mice (Fredriksson et al., 1999, 2000) and rats (Schröder et al., 2001) that received iron during a critical period of development, which corresponds to the period of maximal uptake of iron by the brain, showed spatial memory deficits when tested in the radial arm maze. In addition, this treatment has proven to promote disruption in the inhibitory avoidance task, a type of aversively motivated conditioning in rats (Schröder et al., 2001). We have recently found that iron neonatal treatment impairs long-term recognition memory in adult rats and induces oxidative damage in brain regions implicated in memory formation, thus raising the possibility that iron-induced cognitive deficits are at least partially mediated by oxidative stress (De Lima et al., 2005b).

The monoamino oxidase B (MAO-B) inhibitor selegiline has been long known by its neuroprotective properties. In animal studies, selegiline has proved to be effective against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonism (Cohen et al., 1984). In addition, selegiline pretreatment can protect neurons from other dopaminergic neurotoxins such as MPP+ and β-carbolinium (Matsubara et al., 2001). Selegiline treatment after 6-OHDA exposure in rats reduced dopaminergic sensitivity to apomorphine without a concomitant increase in striatal dopamine content, suggesting that its effects could be related to neurorescue properties (Spooren et al., 1999).

The purpose of the present study was to investigate the possible protective effect of selegiline against iron-induced cognitive deficits. In order to do that, rats neonatally treated with iron or vehicle, were divided in two main groups which received selegiline in two different phases of life: either during the first month of life, starting 24 h before the first iron dosing, or after reaching adulthood. After that, animals were trained and tested in the novel object recognition task, which is based on the spontaneous tendency of rodents to explore a novel object. It has been proposed that this task provides a close analogy with recognition tests that are widely used in humans to test memory and to characterize amnesic syndromes, by providing an accurate index of the overall severity of declarative memory impairment (Dix and Aggleton, 1999; Reed and Squire, 1997).

Methods

Subjects

Pregnant Wistar rats were obtained from Fundação Estadual de Pesquisa e Produção em Saúde, Porto Alegre, RS, Brazil. After birth, each litter was adjusted within 48 h to eight rat pups and to contain offspring of both genders in about equal proportions. Each pup was kept together with its mother in a plastic cage with sawdust bedding in a room temperature of 22 ± 1°C and a 12/12 h light/dark cycle. At the age of 4 weeks, pups were weaned and the males were selected and raised in groups of three to five rats. For postnatal treatments, animals were supplied with standardized pellet food and tap water ad libitum. Behavioral testing started when animals reached the age of 3 months. 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. The protocol for this research was approved by the Institutional Ethics Committee of the Pontificia Universidade Catolica do Rio Grande do Sul (307/03-CEP).

Treatments

Pharmacological treatments are depicted in Fig. 1. The neonatal iron treatment has been described in detail elsewhere (Fredriksson et al., 1999; Schröder et al., 2001). Briefly, 12-day-old rat pups received orally a single daily dose (10.0 ml/kg solution volume) of vehicle (5% sorbitol in water) (control group) or 10.0 mg/kg of body weight of Fe^{2+} (Ferromyn®, AB Hässle, Göteborg, Sweden; iron concentration in the solution was 1.0 mg/ml) via a metallic gastric tube, over 3 days (postnatal days 12–14). In this model, iron is given orally during the period of maximal iron uptake by the brain, so that the model correlates with dietary iron supplementation to infants. We have previously characterized that this treatment protocol induces a selective accumulation of iron in the rat basal ganglia (Schröder et al., 2001). In Experiment I, selegiline (0.0, 1.0 or 10.0 mg/kg of body weight) (Sigma-Aldrich, SP, Brazil) was administered intraperitoneally during 21 days starting 24 h before the first iron dosing. The doses of selegiline, as well as the method of injection and treatment duration, were chosen on the basis of previous studies (Brandeis et al., 1991; Yavich et al., 1993; Stoll et al., 1994; Head et al., 1996; Kiray et al., 2004; Maia et al., 2004; De Lima et al., 2005a; Kiray et al., 2005). In Experiment II, selegiline at the same doses used in Experiment I was administered for 21 days starting when animals were 50-day old. Selegiline was dissolved in saline in a 1.0 ml/kg injection volume.

Novel object recognition

A rectangular open field (45 × 40 × 60 cm) with sawdust covering its floor was used in the novel object recognition task. On the first day, rats were submitted to a habituation session during which they were placed in the empty open field for 5 min. On the following day, rats were given one 5-min training trial in which they were exposed to two identical objects (A1 and A2). All objects were made of