Iron Leads to Memory Impairment that is Associated with a Decrease in Acetylcholinesterase Pathways

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Abstract: Increasing evidence indicates that excessive iron in selective regions of the brain may be involved in the etiology of neurodegenerative disorders. Accordingly, increased levels of iron have been described in brain regions of patients in Parkinson's and Alzheimer's diseases. We have characterized neonatal iron loading in rodents as a novel experimental model that mimics the brain iron accumulation observed in patients with neurodegenerative diseases and produces severe cognitive impairment in the adulthood. In the present study we have investigated the involvement of the cholinergic system on iron-induced memory impairment. The effects of a single administration of the acetylcholinesterase (AChE) inhibitor galantamine or the muscarinic receptor agonist oxotremorine on iron-induced memory deficits in rats were examined. Male Wistar rats received vehicle or iron (10.0 mg/kg) orally at postnatal days 12 to 14. At the age of 2-3 months, animals were trained in a novel object recognition task. Iron-treated rats showed long-term impairments in recognition memory. The impairing effect was reversed by systemic administration of galantamine (1 mg/kg) immediately after training. In addition, iron-treated rats that received oxotremorine (0.5 mg/kg) showed enhanced memory retention. Rats given iron showed a decreased AChE activity in the striatum when compared to controls. The results suggest that, at least in part, iron-induced cognitive deficits are related to a dysfunction of cholinergic neural transmission in the brain. These findings might have implications for the development of novel therapeutic strategies aimed at ameliorating cognitive decline associated with neurodegenerative disorders.

Keywords: Acetylcholinesterase, muscarinic cholinergic receptors, neurodegeneration, object recognition memory, rat, striatum.

INTRODUCTION

Iron is a transition metal playing a central role in neural development as an essential component of oxidative metabolism and a co-factor for numerous enzymes [1, 2]. Increasing evidence indicates that iron accumulation in brain areas might be involved in the pathogenesis of neurodegenerative disorders, like Alzheimer's (AD) [3, 4], Parkinson's [5-9], Huntington's (HD) [10] and neurodegeneration with brain iron accumulation (NBIA) [11]. In the human brain, iron content is higher in globus pallidus, substantia nigra and striatum. Patients with neurodegenerative disorders show high brain iron concentrations and increased associated oxidative stress providing evidence for a pivotal role of iron in neurodegeneration [12].

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It is well documented that the neonatal period is critical for the establishment of iron content in the adult brain, and iron uptake by the brain is maximal during the neonatal period [13]. We developed an animal model in which iron administration in the neonatal period, in rodents, promotes midbrain iron accumulation [14] and enhanced vulnerability to toxic injury [15] in adult life. In fact, neonatal iron administration induces impairments in cognitive function in several memory tasks. In previous reports we have demonstrated that this treatment induces selective iron accumulation in brain regions, specifically in the basal ganglia, which was associated with spatial memory deficits in adult mice [16, 17] and memory disruption in an emotionally motivated learning model in rats [14]. In addition, this treatment impairs long-term recognition memory [18], a form of non-spatial and non-conditioned memory that might be altered in non-demented aged individuals as well as in patients with AD [19-22].

The cholinergic pathways are intimately involved in cognitive functions, such as learning and memory, and disruption of this system produces impairments in many

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learning and memory models [23]. The loss of cholinergic pathways is a hallmark of aging and AD, demonstrated by decreased acetylcholinesterase (AChE) activity, which is related to impaired cognitive function [24-26]. In normal aging or AD, modulation of cholinergic stimulus, with AChE inhibitors or cholinergic agonists, have been used aiming to improve the process of learning and memory [27-31].

Although we have extensively reported that neonatal iron treatment is deleterious to cognitive functioning in adult animals, the precise mechanisms involved in iron-induced memory disruption still remain to be investigated. In the present study, aiming to determine the involvement of the cholinergic system in iron-induced memory impairment, we have studied the effects of pharmacological manipulation of cholinergic system on iron-induced recognition memory impairment. Additionally, we evaluated the activity and expression of AChE in brain regions of rats treated neonatally with iron.

METHODS

Animals

Pregnant Wistar rats were obtained from Fundação Estadual de Pesquisa e Produção em Saúde, Porto Alegre, Brazil. After birth, each litter was adjusted within 48 hours to contain eight rat pups. Each pup was maintained together with its respective mother in a plastic cage with sawdust bedding in a room at a temperature of 22 +/- 1^{o} C and a 12 hours light/dark cycle. At the age of 4 weeks the pups were weaned and the males were selected and raised in groups of four rats. At postnatal treatment, the animals were supplied with standardized pellet food and tap water *ad libitum*. All efforts were made in order to minimize the number of animals used and their suffering. All experimental procedures were performed in accordance with the NIH Guide for the care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996), and the Brazilian Society for Neuroscience and Behavior recommendations for animal care. The protocol for this research was approved by the Institutional Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (013/08 - CEUA).

Neonatal Iron Treatment

The neonatal iron treatment has been described in detail elsewhere [14, 18, 32-34]. Briefly, thirty eight 12-day-old rat pups received a single oral daily dose (10.0 ml/kg solution volume) of vehicle (5% sorbitol in water) (control group), and thirty nine 12-day-old rat pups received a single oral daily dose (10.0 ml/kg solution volume) of 10.0 mg/kg of body weight of Fe^{2+} (Ferromyn ®, AB Hässle, Göterborg, Sweden; iron concentration in the solution was 1.0 mg/ml) via a metallic gastric tube, for 3 days (postnatal days 12-14). Experimental design is depicted in Fig. (**1**).

Cholinergic Drugs Administration

At the age of 2-3 months, 29 vehicle-treated and 30 iron-treated rats were trained in a novel object recognition task (see below). Immediately after the training trial, both groups were divided into three experimental groups receiving a single intraperitoneal injection of saline (vehicle), or Galantamine (Reminyl ®, Janssen-Cilag), a selective AChE inhibitor, at dose of 1.0 mg/kg or Oxotremorine (Sigma-Aldrich, SP, Brazil), a muscarinic receptor agonist, at dose of 0.5 mg/kg. The doses were chosen on the basis of previous studies [29, 35-37] and pilot experiments in our laboratory. Drug solutions were prepared freshly before each experiment.

Novel Object Recognition

The novel object recognition task was chosen based on previous studies performed in our laboratory indicating that this type of memory is consistently affected by iron neonatal treatment [18, 32-34] and aging [22, 38]. Novel object recognition has been used as an experimental tool for the characterization of cognitive alterations associated with aging, genetic manipulations, and neurotoxic treatments in rodents, and it has been proposed that this task may depend on the hippocampus [39, 40], as well as other brain areas including the striatum [41, 42].

A rectangular open field (45 x 40 x 60 cm) with sawdust covering its floor was used in the novel object recognition task. On the first day, adult rats were submitted to a habituation session during which they were placed in an empty open field for 5 min. On the following day, rats were given a training trial in which they were left to explore two identical objects (objects A1 and A2) until they had accumulated 30 s of total object exploration time or for a maximum of 20 min as described in previous studies [34, 38, 43].

All objects were made of plastic Duplo Lego Toys, and had a height of about 10 cm. Objects presented similar textures, colors and sizes, but distinctive shapes. The objects were positioned in two adjacent corners, 9 cm from the walls. Between trials, the objects were washed with a 10% ethanol solution. In the testing trial (24 hours after the training session), rats were allowed to explore the open field for 5 min in the presence of the two objects: the familiar object A and a novel object B. These were placed in the same locations as in the training session. In retention test trials, the novel object was placed in 50% trials in the right side and 50% in the left side of the open field. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered as exploration. A recognition index calculated for each animal was expressed by the ratio $T_B/ (T_A + T_B)$ (T_A = time spent exploring the familiar object A; T_B = time spent exploring the novel object B) [32, 33, 34, 43].

AChE Activity

AChE activity was determined using the method of Ellman, 1961 [44]. At the age of 2-3 months-old, five vehicle-treated and five iron-treated rats were euthanized by decapitation. Cortex, hippocampus and striatum were dissected and the tissue was homogenized in 5 volumes of buffered solution containing 320 mM Sucrose, 5.0 mM Hepes and 0.1 mM EDTA, pH 7.5, in a motor driven Teflon glass homogenizer. The protein concentrations used for enzymatic analysis were 0.4, 0.5-0.8, and 0.6-0.8 mg/ml, from striatum, hippocampus and cortex, respectively. Homogenates were incuba-

ted in a solution composed by DTNB and potassium phosphate buffer (DTNB final concentration 1 mM, pH 7.5) at the proportion of 1:4. The preincubation time was 2 min at 25° C and the enzyme reaction was initiated by addition of 8.0 mM acetylthiocholine (ASCh). Substrate hydrolysis was monitored by the formation of thiolate dianion by DTNB at 412 nm for 2 min (30s intervals). AChE activity was expressed as micromole of thiocholine released per hour per milligram of protein. Controls to determine non enzymatic hydrolysis of ASCh were performed by incubation of ASCh, DTNB and potassium phosphate at the same proportion and concentration described, without addition of homogenates [45].

Protein concentration was measured by the Commassie blue method using bovine serum albumin as standard [46].

Analysis of Gene Expression by Semi-Quantitative RT-PCR

Analysis of AChE expression in the striatum was carried out by a semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay. Striatum was isolated from four vehicle-treated and four iron-treated rats for total RNA extraction with TRIzol reagent (Invitrogen) in accordance with the manufacturer instructions. The cDNA species were synthesized with SuperScript First-Strand Synthesis System (Invitrogen) for RT-PCR from 2 μg of total RNA and oligo (dT) primer in accordance with the suppliers. RT reactions were performed for 50 min at 50°C. cDNA (1μl) was used as a template for PCR with the specific primer for AChE. β-actin-PCR was performed as a control for cDNA synthesis. PCR reactions were performed (total volume of 25 μl) using a concentration of 0.2 μM of each primer indicated below and 200 μM and 1 U Taq Platinum DNA Polymerase in the supplied reaction buffer. Conditions for AChE PCR were as follows: initial 2 min denaturation step at 94°C, 1 min at 94°C, 1 min annealing step at 55°C, 1 min extension step at 72°C for 32 cycles and a final 10 min extension at 72°C. Conditions for β-actin PCR were as follows: initial 1 min denaturation step at 94°C, 1 min at 94°C, 1 min annealing step at 58,5°C, 1 min extension step at 72°C for 34 cycles and a final 7 min extension at 72°C. The amplification products were: AChE 785 bp; β-actin 210 bp. Fragment length of PCR reactions was confirmed with Low DNA

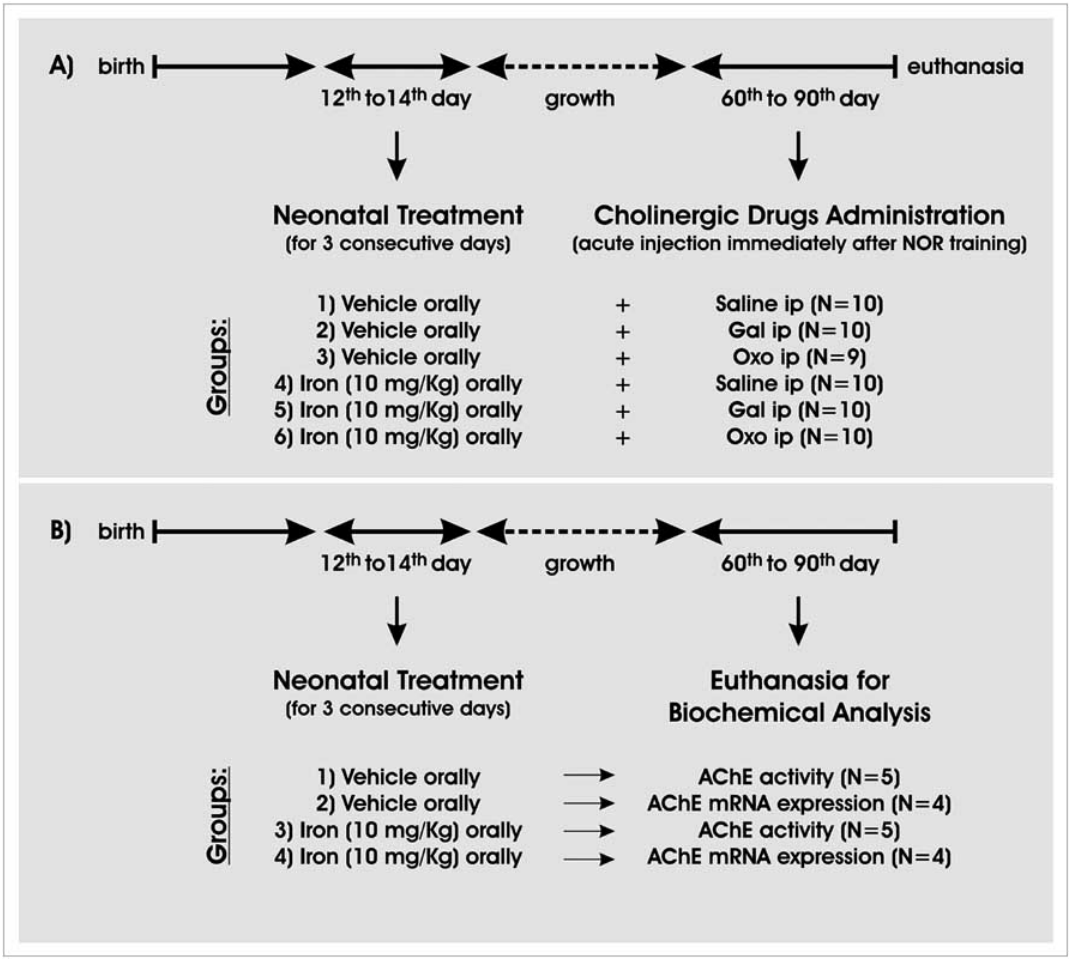

Fig. (1). Experimental design. (**A**) Male rat pups received vehicle (5% sorbitol in water) or iron (10 mg/kg) at post-natal days 12-14. At the age of 2-3 months, 29 vehicle-treated and 30 iron-treated rats were trained in the object recognition task (ORT). Immediately after the training trial, both groups were divided into three experimental groups receiving a single intraperitoneal injection of saline (Sal), or Galantamine (Gal 1.0 mg/kg), or Oxotremorine (Oxo, 0.5 mg/kg). Long-term memory (LTM) retention was assessed 24 hours after the training session. (**B**) AChE activity was determined in the cortex, hippocampus and striatum of five vehicle-treated and five iron-treated rats. Analysis of AChE mRNA expression in striata isolated from four vehicle-treated and four iron-treated rats was carried out by a semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay.

Mass Ladder (Invitrogen), USA) and β-actin was carried out as an internal standard. PCR products were submitted to electrophoresis using a 1% agarose gel and the relative abundance of mRNA *versus* β-actin was determined by densitometry using freeware ImageJ 1.37 for Windows [47].

The following set of primers were used: for AChE: forward 5'- GAC TGC CTT TAT CTT AAT GTG -3'; and reverse 5'- CGG CTG ATG AGA GAT TCA TTG -3'; for β-actin: forward 5'- TAT GCC AAC ACA GTG CTG TCT GG -3'; and reverse 5'- TAC TCC TGC TTC CTG ATC CAC AT -3' [45].

Statistical Analysis

Data for object recognition are expressed as median (interquartile ranges). Comparisons among groups were performed with a Kruskal-Wallis analysis of variance followed by Mann-Whitney *U* tests when necessary. Data for biochemical analysis are expressed as mean ± standard error of the mean (S.E.M.) and were analyzed using Student's test. *p* values of less than 0.05 were considered to indicate statistical significance.

RESULTS

Effects of Galantamine and Oxotremorine on Iron-induced Object Recognition Memory Deficits

In order to exclude the possibility that the effects of iron or cholinergic drugs would alter general behavioral parameters that could interfere with the memory acquisition process, we used a training protocol in which rats had to accumulate 30 seconds of total exploration of both objects. Statistical comparison of latency to reach the criterion of 30 seconds exploring both objects during the object recognition training session was used as an index of motor and exploratory activity. Kruskal-Wallis analysis of variance revealed no significant differences in the latency to reach criterion among groups (H = 5.39, df = 5, p = 0.370), as shown in Table **1**.

Table 1. Latency to Reach Criterion of Time Exploring both Objects in the Object Recognition Training (in Seconds, Data Expressed as Median [Interquartile Range])

Group	*N*	Latency to reach criterion
Veh-Sal	10	172.5 [133.2/217.5]
Veh-Gal	10	191.0 [167.0/221.5]
Veh-Oxo	9	195.0 [144.0/226.5]
Fe-Sal	10	158.0 [146.7/185.5]
Fe-Gal	10	193.0 [183.0/214.0]
Fe-Oxo	10	191.0 [168.2/242.0]

The effects of the AChE inhibitor, galantamine, and the muscarinic agonist, oxotremorine, on object recognition memory in rats treated neonatally with iron are shown in Fig. (**2**). Statistical comparison of recognition indexes in long-term retention test using Kruskall-Wallis analyses of variance indicated a significant difference among groups (H = 25.96, df = 5, $p < 0.0001$).

Rats treated neonatally with iron that received saline in the adulthood showed significantly lower recognition indexes in long-term retention test than the control group Veh-Sal ($p < 0.0001$), indicating that iron given in the neonatal period induces severe recognition memory impairment (Fig. **2**).

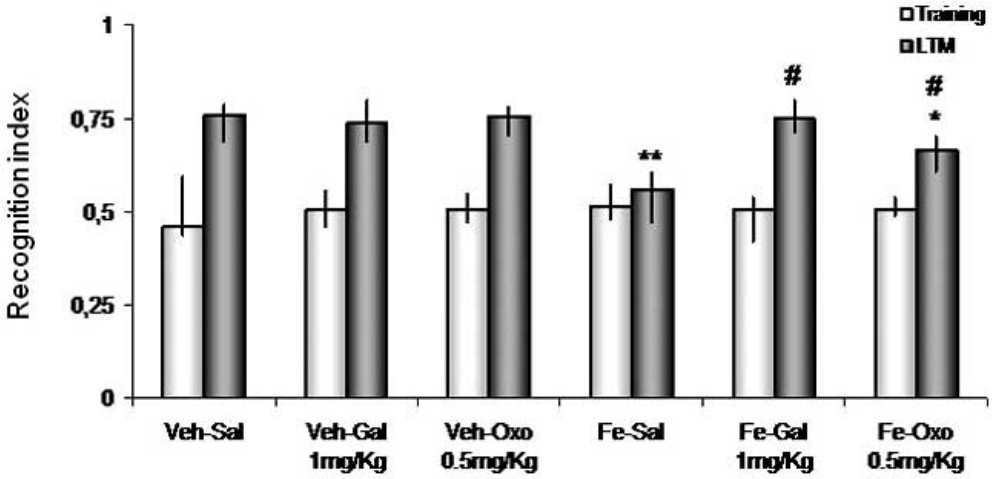

Fig. (2). Effects of galantamine and oxotremorine on iron-induced recognition memory deficits. At the age of 2-3 months, 29 vehicle-treated (Veh) and 30 iron-treated (Fe) rats were trained in a novel object recognition task (see below). Immediately after the object recognition training trial, both groups were divided into three experimental groups receiving a single intraperitoneal injection of saline (Sal), or Galantamine (Gal, 1.0 mg/kg) or Oxotremorine (Oxo, 0.5 mg/kg). Long-term memory (LTM) retention test was performed 24 h after training. The proportion of the total exploration time that the animal spent investigating the novel object was the "Recognition Index" expressed by the ratio TN/(TF+TN), TF = time spent exploring the familiar object and TN = time spent exploring the novel object. Data expressed as median [interquartile ranges]. (Veh-Sal, N = 10; Veh-Gal, N = 10; Veh-Oxo, N = 9; Fe-Sal, N = 10; Fe-Gal, N = 10; Fe-Oxo, N = 10). Differences between Veh-Sal *vs* other groups are indicated as: ** p <0.001 and * p <0.05; differences between Fe-Sal *vs* Fe-Gal or Fe-Oxo are indicated as: # p <0.05, according to Mann-Whitney *U* tests.

Iron-treated rats that received galantamine at the dose of 1.0 mg/kg showed normal recognition memory, as their recognition index did not differ significantly from the control group ($p = 0.705$), and were significantly higher than the Fe-Sal group's index ($p < 0.0001$), indicating that inhibition of AChE in the adulthood was able to completely reverse neonatal iron-induced recognition memory deficits (Fig. **2**).

Animals treated with oxotremorine at the dose of 0.5 mg/kg showed a significantly higher performance on object recognition task than animals that were given Fe-Sal ($p = 0.013$). However, oxotremorine at this dose was not able to completely reverse iron-induced memory impairment, since the performance of iron-treated rats that received oxotremorine was statistically different from the control group Veh-Sal ($p = 0.016$). The results indicate that the selective muscarinic agonist ameliorated recognition memory deficits induced by iron.

The recognition indexes of groups treated with galantamine or oxotremorine treated neonatally with vehicle did not differ significantly from the control group Veh-Sal (Fig. **2**). Indeed, the treatment with cholinergic drugs was not able to improve or ameliorate recognition memory in normal rats.

AChE Activity and Expression in Brain Regions of Rats Treated with Iron in the Neonatal Period

AChE activities in brain regions are shown in Fig. (**3**). Statistical analysis indicated that iron neonatal treatment has not affected AChE activity neither in cortex nor in hippocampus of rats. However, AChE activity was decreased in the striatum of rats treated neonatally with iron in comparison to the control group ($p = 0.007$).

Semi-quantitative RT-PCR experiments were conducted in order to verify if the inhibitory effect induced by iron treatment on striatal AChE activity could be a consequence of transcriptional control. No differences on AChE mRNA levels were found between controls and the iron treatment group (means of AChE/β-actin mRNA ratios from four independent experiments were 0.70 ± 0.02 and 0.72 ± 0.05, for rats receiving vehicle or iron in the neonatal period, respectively).

DISCUSSION

In the present study we have demonstrated that rats treated neonatally with iron present severe long-term recognition memory deficits, in accordance with previous studies performed in our laboratory [18, 32-34]. Although we have consistently reported that iron induces memory impairments in different learning paradigms in mice [16] and rats [14, 18], the precise mechanisms underlying iron-induced memory deficits still remain to be clarified. Here we show that drugs that modulate cholinergic neural transmission improved recognition memory deficits associated with iron loading, as both galantamine and oxotremorine administered immediately after training increased recognition indexes in iron-treated rats, without affecting recognition memory in control rats. The training protocol used in the present study, in which animals are trained to reach a criterion of time exploring the objects, excludes the possibility that the effects of iron neonatal treatment as well as those of galantamine or oxotremorine could be attributed to differential memory acquisition induced by the treatments. Moreover, we can rule out the possibility that the deleterious effect induced by iron neonatal treatment on recognition memory task could be explained by motor, exploratory or motivational impairments.

It has been suggested that a disruption of cholinergic systems may contribute to cognitive deficits observed in Alzheimer's disease, schizophrenia, age-associated mild cognitive impairment, among other CNS disorders [48-50]. Galantamine is an inhibitor of AChE, approved for use in AD [51]. The main rationale for using AChE inhibitors such as galantamine would be increasing acetylcholine availability at the synaptic cleft, thus positively modulating cholinergic neural transmission. More recently, it has been proposed that galantamine could also act as an allosteric

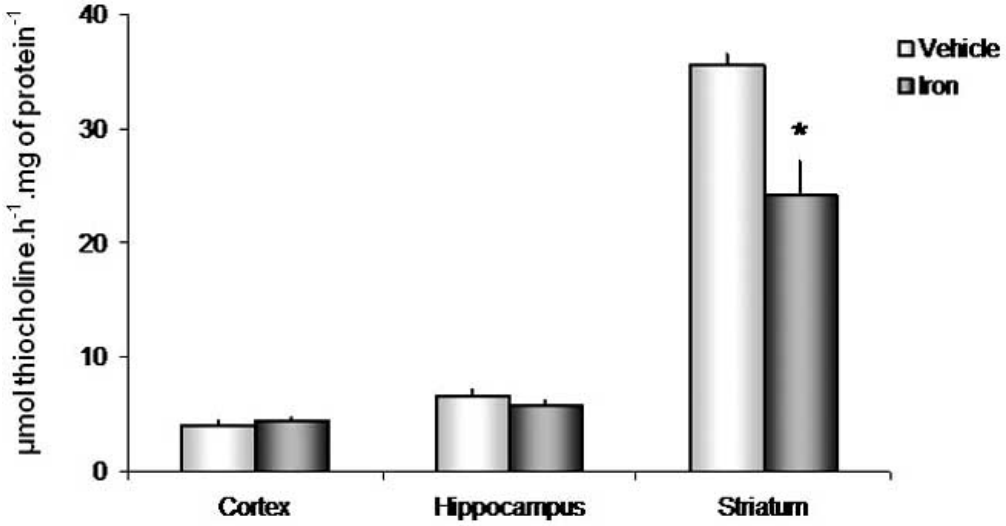

Fig. (3). Effect of neonatal iron treatment on AChE activity in brain regions of adult rats. AChE activity was determined using the method of Ellman *et al.* (1961) in the cortex, hippocampus and striatum of five vehicle-treated and five iron-treated rats, and was expressed as micromole of thiocholine released per hour per milligram of protein. Bars represent mean ± S.E.M. Comparisons between groups were performed by independent samples t-tests and are indicated as * $p < 0.01$.

modulator of nicotinic acetylcholine receptors [52, 53], and as an agonist at *N*-methyl-D-aspartate (NMDA) glutamate receptors [54]. A number of recent studies have investigated the effects of galantamine in preclinical tests using animal models of cognitive dysfunction. For instance, galantamine has been proved to be protective against memory loss induced by ischemia [55], scopolamine [56], β-amyloid peptide [53], MK-801 [57], and aging [29]. In our study, galantamine was able to completely reverse iron-induced memory impairment. Besides inhibition of AChE, cholinergic replacement therapeutic strategies include the use of nicotinic and muscarinic acetylcholine receptor agonists. In the present study, we used oxotremorine, a muscarinic agonist that has been effective in reversing memory impairment induced by the chronic administration of the anticonvulsant drug, gabapentin [58]. Other studies have also shown that oxotremorine is effective in facilitating memory [37, 59].

From the results obtained in our behavioral study, indicating that enhanced cholinergic transmission was able to improve object recognition retention deficits associated with iron loading, we became interested in determining AChE activity in brain regions of iron-treated rats, aiming to examine whether iron would affect cholinergic functioning. Previous studies have indicated that iron accumulation following neonatal treatment occurs selectively in the basal ganglia, and not in cerebral cortex of rodents [14, 16, 17]. Accordingly, here we found that AChE activity, but not its mRNA expression, was significantly reduced in the striatum, but not in cerebral cortex and hippocampus of rats treated neonatally with iron. The precise mechanisms involved in the reduction of striatal AChE activity in adult rats induced by iron neonatal treatment are unknown. Because of the time interval between the treatment in postnatal life and the alteration found in adulthood, direct enzymatic inhibition is unlikely to be involved. We hypothesize that iron accumulation triggers a cascade of events that might result in impaired cholinergic transmission or even terminal degeneration that was revealed in our study by a decreased AChE activity. Because the main focus of the present work was to examine the consequences of iron loading on AChE, we did not examine AChE expression or activity in iron-treated rats that received acute administration of cholinergic drugs. However, stimulation of cholinergic transmission by galantamine and oxotremorine was able to reverse the cognitive deficit produced by iron, supporting the possibility that impaired cholinergic transmission revealed by decreased AChE is involved in iron-induced memory impairment. It is possible that this alteration is not caused by a direct neurotoxic effect producing neuronal death, since mRNA expression was not altered and previous findings have suggested that neonatal iron loading was not associated with overt neurotoxicity and oxidative stress in striatal neurons [18, 60]. Since galantamine and oxotremorine were given acutely at the time of behavioral training, it is unlikely that their beneficial effects on memory are related to structural alterations in the neural tissue. Galantamine and oxotremorine can prevent memory impairment by several direct and indirect mechanisms. For instance, galantamine has proven not only to inhibit AChE, but also to allosterically modulate nicotinic (nACh) receptors, protecting cortical neurons from apoptosis in AD mostly through the later mechanism [61]. Activation of nACh receptors by galantamine might lead to an increase in nitric oxide (NO) levels, which in turn might influence neural plasticity mechanisms and oxidative stress, having implications to iron-related memory dysfunction [62]. Oxotremorine has been shown to reverse behavioral deficits produced by chronic stress in rats by activating muscarinic cholinergic receptors, restoring brain AChE activity, and increasing hippocampal and cortical levels of norepinephrine [37].

Although the mechanisms and implications of the altered AChE activity observed remain unclear, previous evidence has indicated that a selective reduction of AChE without neuronal loss in the striatum might be associated with the pathogenesis of neurodegenerative disorders. Thus, a selective reduction of striatal AChE activity without cell death has been reported in both patients with HD and in the R6/1 transgenic mouse model of HD, in which reduced striatal cholinergic transmission was accompanied by spatial memory deficits [63]. The authors suggest that a defect in cholinergic transmission without cell loss in the striatum might play a role in cognitive dysfunction associated with HD. The reduced striatal AChE activity produced by brain iron accumulation is thus consistent with alterations observed in HD and adds to previous evidence that neonatal iron loading in rodents leads to several behavioral and neural features that parallel those associated with neurodegenerative disorders. Studies investigating possible changes in other biochemical parameters related to cholinergic integrity, such as choline acetyltransferase and the vesicular acetylcholine transporter, are warranted in our model.

According to the present results deleterious effects of iron loading in the neonatal period was selectively observed in the striatum of treated rats. Although studies have demonstrated that the hippocampus plays a key role in object recognition memory formation [39, 40], strong evidence has indicated that the striatum might be involved as well. For instance, a neurotoxic regimen of methamphetamine, known to induce dopaminergic terminal damage in the striatum produces severe short- and long-term recognition memory deficits, without affecting spatial memory in the Morris water maze task [41]. Recently it was demonstrated that the object recognition impairments of methamphetamine-treated rats correlate with monoaminergic transporter loss in ventral caudate-putamen, hippocampus, and perirrhinal cortex [42]. Another study has shown that connexin 31.1 (Cx31.1) knockout mice presented impaired object recognition and altered levels of AChE and cAMP response element-binding protein (CREB) in the striatum, but not in the hippocampus [64]. Object discrimination tasks might rely on the striatum rather than the hippocampus even in training procedures involving few learning trials [65]. Earlier studies have documented the role of tonically active cholinergic interneurons in the striatum during learning in monkeys [66, 67], including recognition learning [68]. In humans, fMRI experiments have indicated that brain areas mediating encoding and recognition of objects include the left putamen [69]. Taken together, these findings suggest that object recognition memory may depend, at least in part, on the integrity of the striatal circuitry, which is consistent with our finding that iron-induced impairment of recognition memory was associated with altered AChE activity in the striatum.

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 plays a causative role in neurodegeneration or is a consequence of the disease process. Taken together, the present findings suggest that iron accumulation in the brain disrupts cholinergic transmission which might have implications for the memory impairment observed in rats given neonatal iron overload. Based on the current knowledge that iron is accumulated in brain regions of patients with neurodegenerative disorders featuring cognitive dysfunction, our animal model may provide a tool for the investigation of multifactorial mechanisms that interact to produce memory decline observed in those patients.

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