Neuropharmacology 75 (2013) 304–311

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Differential effects of modafinil on memory in naïve and memory-impaired rats

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ARTICLE INFO

Article history:
Received 19 February 2013
Received in revised form
23 July 2013
Accepted 30 July 2013

Keywords:
Modafinil
Recognition memory
Iron
Inhibitory avoidance
Memory deficits

ABSTRACT

Modafinil is a wake-promoting drug and has been approved for the treatment of excessive daytime sleepiness in narcolepsy and obstructive sleep apnea. Modafinil was shown to improve learning and memory in rodents, and to reverse memory deficits induced by sleep deprivation or stress. However, depending on the memory paradigm used, modafinil might also impair memory. We aimed to investigate the effects of modafinil on memory consolidation and retrieval for object recognition and inhibitory avoidance in naïve adult rats. We also investigated whether acute or chronic administration of modafinil would reverse memory deficits induced by iron overload, a model of memory impairment related to neurodegenerative disorders. Adult naïve rats received modafinil (0.0, 0.75, 7.5 or 75 mg/kg) either immediately after training or 1 h prior to testing in object recognition or inhibitory avoidance. Iron-treated rats received modafinil immediately after training in object recognition. In order to investigate the effects of chronic modafinil, iron-treated rats received daily injections of modafinil for 17 days, and 24 h later they were trained in object recognition or inhibitory avoidance. Acute modafinil does not affect memory consolidation or retrieval in naive rats. A single injection of modafinil at the highest dose was able to recover recognition memory in iron-treated rats. Chronic modafinil completely recovered iron-induced recognition memory and emotional memory deficits. Additional preclinical and clinical studies are necessary in order to support the applicability of modafinil in recovering memory impairment associated with neurodegenerative disorders.

1. Introduction

Modafinil [(diphenyl−methyl) sulphinil-2-acetamide] is a psychostimulant that acts as a wake-promoting drug and has been approved for the treatment of excessive daytime sleepiness in narcolepsy and obstructive sleep apnea (Ballon and Feifel, 2006; Minzenberg and Carter, 2008). Though modafinil has been classified as a psychostimulant, evidence suggests that it acts on a neural pathway different from amphetamine and cocaine (Ballon and Feifel, 2006). While its precise mechanism of action is still not well identified, human and animal research suggest that it directly or indirectly activates the dopaminergic (de Saint Hilaire et al., 2001; Volkow et al., 2009), glutamatergic (Ferraro et al., 1999), noradrenergic (de Saint Hilaire et al., 2001; Minzenberg et al., 2008) and serotonergic (de Saint Hilaire et al., 2001) systems in several brain regions, including the prefrontal cortex, hippocampus, hypothalamus and striatum, whereas it inhibits GABAergic pathways in the same regions (Ferraro et al., 1999).

Evidence suggests that modafinil improves visual discrimination/attention in rodents (Morgan et al., 2007). Moreover, modafinil facilitates learning and memory in spatial and contextual tasks, in healthy adult rats (Burgos et al., 2010; Tsanov et al., 2010) and mice (Beracochea et al., 2008; Shuman et al., 2009). In addition, it was demonstrated that modafinil recovers memory deficits induced by sleep deprivation (He et al., 2011; Moreira et al., 2010; Piérard et al., 2007, 2011) or by chronic stress (Piérard et al., 2006). However, depending on the learning paradigm used, dose and time window of drug administration, contradictory results were found. For instance, modafinil was shown to impair emotional memory in rodents (Burgos et al., 2010; Fernandes et al., 2013).

Iron, the most abundant metal in the human body, is essential for many key biological processes related to the development of the

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0028-3908/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuropharm.2013.07.038

nervous system, including myelination, neurotransmitter synthesis, and mitochondrial energy production (Connor et al., 1995). Over the last decades, many studies in humans as well as in animal models have shown that iron deficiency during neurological development leads to permanent cognitive deficits (Fretham et al., 2011; Lozoff, 2011). However, clinical and experimental evidence suggest a role of iron excess in neurodegenerative diseases. An abnormal iron homeostasis might cause severe cellular dysfunction or death, and it has been recognized as a triggering factor for different neurodegenerative disorders such as Parkinson's (PD), Alzheimer's (AD), and Huntington's (HD) diseases as well as amyotrophic lateral sclerosis (ALS) (Mills et al., 2010). Noteworthy, studies using brain imaging techniques show that iron concentrations, particularly in the parietal and temporal cortex, hippocampus and basal ganglia, positively correlate with poor performance in a variety of cognitive tests, both in healthy elderly individuals (Pujol et al., 1992; Sullivan et al., 2009; Bartzokis et al., 2011; Penke et al., 2012; Rodrigue et al., 2013) and in patients with dementia (Brass et al., 2006; House et al., 2006; Ding et al., 2009; Zhu et al., 2009).

In agreement with the observations that iron accumulation strongly correlates with poor cognitive performance in healthy and demented aged human subjects, we have demonstrated that brain iron accumulation induces persistent memory deficits in rodents (de Lima et al., 2005a; Fagherazzi et al., 2012; Schröder et al., 2001, 2013; Silva et al., 2012) and increases oxidative stress in brain regions related to memory formation in rats (de Lima et al., 2005a). Recently, we found that iron loading produces an increase in glial acidic protein (GFAP), which is an astrocyte marker, suggesting the presence of reactive gliosis in adult mice (Fernandez et al., 2010) and rats (Fernandez et al., 2011), and that the apoptotic markers, Par-4, and caspase-3 are also increased in the brains of adult animals treated with iron in the neonatal period (Miwa et al., 2011). Taken together, these results suggest that memory dysfunction associated with iron treatment may be viewed as a model of cognitive decline related to neurodegenerative disorders. Thus, over the last years we have been using this model in order to investigate drugs with potential use as cognitive enhancer for the treatment of memory deficits associated to aging and/or neurodegenerative disorders (de Lima et al., 2005b, 2007, 2008; Fagherazzi et al., 2012; Silva et al., 2012).

Thus, we aimed to evaluate whether the acute or chronic administration of modafinil would be able to reverse memory deficits induced by iron overload. Additionally, in order to better characterize the properties of modafinil on memory consolidation and retrieval, we tested healthy naïve adult rats using two different learning and memory paradigms, object recognition and the aversively motivated emotional memory task inhibitory avoidance.

2. Material and methods

2.1. Animals

For the experiments investigating the effects of acute modafinil on memory consolidation and retrieval in adult naïve control rats, male adult Wistar rats (200–250 g) were obtained from the State Health Science Research Foundation (FEPPS-RS, Porto Alegre, Brazil). For iron-induced memory impairment experiments, pregnant Wistar rats were obtained from FEPPS-RS. After birth each litter was adjusted within 48 h to eight rat pups 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 21 ± 1 °C and a 12/12-h light/dark cycle. At the age of 3 weeks, pups were weaned and the males were selected and raised, maintained in groups of three to five in individually ventilated cages with sawdust bedding.

In order to reduce the possibility of litter effects, rat pups were randomly assigned to treatment groups and each treatment group (iron or vehicle, as described below) consisted of rats derived from 9 different litters. For postnatal treatments, animals were given standardized pellet food and tap water *ad libitum*. All behavioral experiments were performed at light phase between 9:00 and 16:30 h. All experimental procedures were performed in accordance to the principles of laboratory animal care and with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 80–23 revised 1996) and approved by the Institutional Ethics Committee for the Use of Animals (CEUA) of the Pontifical Catholic University. All efforts were made to minimize the number of animals used and their suffering.

2.2. Treatments

2.2.1. Modafinil

For the experiments investigating the acute effects of modafinil on memory consolidation and retrieval in naïve animals, adult (3 months old at arrival) rats were trained and tested in the novel object recognition task. Ten days later, groups were semi-randomized, in order to guarantee that a rat would not receive the same previous treatment, and were trained and tested in the inhibitory avoidance task. Vehicle (Tween 80—saline solution 1:16 v/v) or modafinil (Stavigile®, Libbs, USA) at the doses of 0.75, 7.5 and 75 mg/kg was administered intraperitoneally immediately after the training session for the investigation of its effects on memory consolidation and one hour before testing sessions for the investigation of effects on memory retrieval. The doses were selected based on pilot experiments performed in our laboratory and on a previous published study (Shuman et al., 2009).

For the investigation of the effects of modafinil on iron-induced memory impairments, adult (3 months old) rats treated neonatally with vehicle (Sorbitol 5%, Sorb) or iron (as described in detail below) received an acute intraperitoneal injection of vehicle or modafinil (at the doses of 0.75, 7.5 and 75 mg/kg) immediately after the training session of the object recognition task. For experiments investigating the chronic effects of modafinil (Mod) on iron-induced memory impairments, adult rats treated neonatally with vehicle (Sorb) or iron received a daily intraperitoneal injection of vehicle (Veh) or modafinil (Mod, at the doses of 0.75, 7.5, and 75 mg/kg) for 17 consecutive days. Training in the behavioral tasks of object recognition or inhibitory avoidance were performed 24 h after the last drug treatment. Drug solutions were freshly prepared immediately prior to administration.

2.2.2. Iron neonatal treatment

The neonatal iron treatment has been described in detail elsewhere (Fagherazzi et al., 2012; Silva et al., 2012). Briefly, 12-day-old rat pups received orally a single daily dose (10 ml/kg solution volume) of vehicle (5% sorbitol in water, control group) or 30 mg/kg of body weight of Fe^{2+} (iron carbonyl; Sigma–Aldrich, São Paulo, Brazil) via a metallic gastric tube, over 3 days (postnatal days 12–14).

2.3. Behavioral procedures

2.3.1. Inhibitory avoidance task

The inhibitory avoidance (IA) behavioral training and retention test procedures were described in previous reports (Schröder et al., 2001; Silva et al., 2012). The IA apparatus was a 50 × 25 × 25-cm acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consisted of parallel caliber stainless steel bars (1 mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. On the training trial, rats were placed on the platform and their latency to stepdown on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid, rats received a mild foot shock (0.5 mA) and were removed from the apparatus immediately afterwards. A retention test trial was carried out 24 h after the training trial. The retention test trial was procedurally identical to training, except that no foot shock was presented. Step-down latencies (in seconds) on the retention test trial (maximum 180 s) were used as a measure of IA retention.

2.3.2. Object recognition task

The object recognition task was performed as previously described (de Lima et al., 2005a). Briefly, the object recognition task took place in an open-field apparatus (45 × 40 × 60 cm) with sawdust covering its floor. On the first day, rats underwent 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). On the long-term memory (LTM) testing trial, performed 24 h after the training session, rats were allowed to explore the open field for 5 min in the presence of two objects: the familiar object A and a novel object B. These were placed in the same locations as in the training session. In long-term retention test trial, the novel object was placed in 50% of trials in the right side and 50% of trials in the left side of the open field. 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. Object exploration was measured by an experimenter blind to group treatment assignments using two stopwatches to record the time spent exploring the objects during the experimental sessions. Exploration was defined as follows: sniffing or touching the object with the nose. Sitting on the object was not considered as exploration. For the evaluation of the effects of modafinil on memory retrieval for the object recognition in naïve rats, animals were trained in this protocol, using two identical objects, and received Vehicle (Veh) or modafinil (Mod 0.75, 7.5, or 75 mg/kg) one hour before the test session. A recognition index calculated for each animal was expressed by the ratio TN/(TF + TN) [TF = time spent exploring the familiar object (A), TN = time spent exploring the novel object (B)].