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Dopaminergic D2 receptor is a key player in the substantia nigra pars compacta neuronal activation mediated by REM sleep deprivation

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ABSTRACT

Currently, several studies addresses the novel link between sleep and dopaminergic neurotransmission, focusing most closely on the mechanisms by which Parkinson's disease (PD) and sleep may be intertwined. Therefore, variations in the activity of afferents during the sleep cycles, either at the level of DA cell bodies in the ventral tegmental area (VTA) and/or substantia nigra pars compacta (SNpc) or at the level of dopamine (DA) terminals in limbic areas may impact functions such as memory. Accordingly, we performed striatal and hippocampal neurochemical quantifications of DA, serotonin (5-HT) and metabolites of rats intraperitoneally treated with haloperidol (1.5 mg/kg) or piribedil (8 mg/kg) and submitted to REM sleep deprivation (REMSD) and sleep rebound (REB). Also, we evaluated the effects of REMSD on motor and cognitive parameters and SNpc c-Fos neuronal immunoreactivity. The results indicated that DA release was strongly enhanced by piribedil in the REMSD group. In opposite, haloperidol prevented that alteration. A c-Fos activation characteristic of REMSD was affected in a synergic manner by piribedil, indicating a strong positive correlation between striatal DA levels and nigral c-Fos activation. Hence, we suggest that memory process is severely impacted by both D2 blockade and REMSD and was even more by its combination. Conversely, the activation of D2 receptor counteracted such memory impairment. Therefore, the present evidence reinforce that the D2 receptor is a key player in the SNpc neuronal activation mediated by REMSD, as a consequence these changes may have direct impact for cognitive and sleep abnormalities found in patients with PD.

This article is part of the Special Issue entitled 'The Synaptic Basis of Neurodegenerative Disorders'.

1. Introduction

Currently, several studies addresses the novel link between sleep and dopaminergic neurotransmission, focusing most closely on the mechanisms by which Parkinson's disease (PD) and sleep may be intertwined, whether as predictors or consequences of dopaminergic neurodegeneration (see Lima, 2013). Episodes of excessive daytime somnolence after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) – a well-known dopaminergic neurotoxin that mimics PD – injections in monkeys have been anecdotally reported as a prominent feature of Parkinsonism (Forno et al., 1986; Langston et al., 1984). The first report to provide evidence of sleep disruption by MPTP demonstrated a selective rapid eye movement (REM) sleep suppression that lasted 6–9 days after the last dose of the neurotoxin in cats (Pungor et al., 1990). Besides, it was demonstrated a strong relationship between motor impairment and rhythm disorganization in MPTP-treated monkeys (Almirall et al., 2001).

Moreover, it has been characterized that extracellular levels of dopamine (DA) changes in the terminal regions of ventral tegmental area (VTA) neurons over the course of the sleep-wake cycle (Lena et al., 2005). It was also observed that DA neurons exhibit enhanced c-Fos activity in bursts of spikes that are associated with REM sleep (Maloney et al., 2002). Furthermore, a robust increase in the firing of dopaminergic neurons of the VTA has been

Abbreviations: DI, discrimination index; DA, dopamine; DAT-KO, dopamine transporter knockout; DHPG, dihydroxyphenylglycol; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HIAA, 5-hydroxyindoleacetic acid; LDT, laterodorsal tegmental nuclei; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NA, noradrenaline; PD, Parkinson's disease; PPT, pedunculopontine tegmental nucleus; REM, rapid eye movement; REMSD, REM sleep deprivation; REB, rebound; 5-HT, serotonin; SNpc, substantia nigra pars compacta; VTA, ventral tegmental area.

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identified during REM sleep (Dahan et al., 2007). Moreover, clinical evidence has illustrated a transient restoration of motor control in PD patients during episodes of REM sleep (De Cock et al., 2007). Therefore, the notion that dopaminergic neurons purportedly present a static firing rate throughout sleep-wake cycles, a concept that was previously promulgated in the literature, is strongly refuted by these demonstrations (Lima, 2013).

In light of the relationship between dopaminergic neurotransmission and sleep, it was reported that dopaminergic D2 blockade may produce the reduction or even suppression of REM sleep after a period of REM sleep deprivation (Lima et al., 2008). Furthermore, electrophysiological data indicated that the absence of half of the substantia nigra pars compacta (SNpc) dopaminergic neurons, in rats, provoked a major impairment in the sleep-wake parameters, predominantly in REM sleep (Lima et al., 2007). In addition, REM sleep could be recovered in the dopaminergic transporter knockout (DAT-KO) mice by selective activation of the D2, but not the D1, suggesting a particular role of this receptor in the regulation of REM sleep (Dzirasa et al., 2006). Such involvement of DA has been previously reported subsequent to sleep deprivation protocols, as being directly involved in the generation of burly dopaminergic D2 supersensitivity (Nunes et al., 1994; Tufik, 1981; Tufik et al., 1978).

These observations, together with the theory that the pedunculopontine tegmental nucleus (PPT) and laterodorsal tegmental nuclei (LDT) – areas classically associated to REM sleep – are closely connected to the SNpc and VTA and consequently are directly affected by imbalances in DA levels (Lima, 2013) could explain the participation of DA in REM sleep. These changes could result from variations in the activity of afferents during the sleep cycles, either at the level of DA cell bodies in the VTA and/or SNpc or at the level of DA terminals in limbic areas (Lena et al., 2005) also impacting functions such as memory. To test this rationale we performed neurochemical quantifications of DA, serotonin (5-HT), as well as its metabolites levels within the striatum and hippocampus of rats treated with haloperidol (selective D2 antagonist) or piribedil (selective D2 agonist) and submitted to REM sleep deprivation (REMSD) and sleep rebound (REB). Then we evaluated the effects of REMSD on motor and cognitive parameters assessed through the open-field and object recognition tests. Lastly, c-Fos neuronal immunoreactivity was quantified within the SNpc was determined in both REMSD and REB paradigms.

2. Material and methods

2.1. Animals

Male Wistar rats from our breeding colony weighing 280–320 g at the beginning of the experiments were used. The animals were randomly housed in groups of five in polypropylene cages with wood shavings as bedding and maintained in a temperature-controlled room (22 ± 2 °C) on a 12-h light–dark cycle (lights on at 7:00 a.m.). The animals had free access to water and food throughout the experiment.

2.2. Ethics statement

The studies were carried out in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals, United States National Institutes of Health. In addition, the protocol complies with the recommendations of Federal University of Paraná and was approved by the Institutional Ethics Committee (approval ID #555).

2.3. REMSD procedure

REMSD was attained by means of the single platform method, in which each sleep deprived animal is placed onto a cylindrical platform, 6.5 cm in diameter and surrounded by water about 1 cm below the platform surface (Lima et al., 2008). At the onset of each REM sleep episode, the animal experiences a loss of muscle tonus and falls into the water, thus being awakened. When platforms of this size are used, REM sleep is completely eliminated (Machado et al., 2004). Throughout the study, the experimental room was maintained at controlled conditions (22 ± 2 °C, 12 h light/dark cycle, lights on 7:00 a.m.). The control group was kept in the same room as the REMSD rats during the study. Food and water were provided *ad libitum* by placing chow pellets and water bottles on a grid located on top of the tank.

2.4. Experimental design

The animals were distributed randomly in six groups for each experimental evaluation: control vehicle ($n = 10$), control haloperidol ($n = 10$), control piribedil ($n = 10$), REMSD vehicle ($n = 10$), REMSD haloperidol ($n = 10$), REMSD piribedil ($n = 10$). The rats from the sleep deprived groups underwent 24 h of REMSD (from 7:00 a.m. to 7:00 a.m) and subsequently the respective groups received a single intraperitoneal (i.p.) injection, at 7:01 a.m., of DMSO/saline 0.9% or haloperidol hydrochloride (1.5 mg/kg; Tocris Biosciences Bristol, UK) or piribedil dihydrochloride (8.0 mg/kg; Tocris Biosciences Bristol, UK) and 60 min after started the behavioral testing, including the open-field and the object recognition tests. At the end of these tests, the rats were allowed to sleep for 24 h (REB period) from 12:00 p.m. to 12:00 p.m. Afterward, the groups were re-tested for the same behaviors and immediately decapitated for tissue dissection of striatum and hippocampus for neurochemical purposes or intracardially perfused and the brains were processed for immunohistochemistry to assess c-Fos expression within the SNpc.

2.5. Open-field test

The apparatus consists of a circular arena (1 m of diameter) limited by a 40 cm-high wall and illuminated by four 60 W lamps situated 48 cm above the arena floor, providing illumination around 300 lx (Broadhurst, 1960). The animals were gently placed in the center of the arena and were allowed to freely explore the area for 5 min. During the experiments, the open-field was video recorded and the measures the locomotion and mean velocity were computed online by an image analyzer system (Smart junior, PanLab, Harvard Apparatus, Spain).

2.6. Object recognition test

The apparatus consists of an open box (width × length × height = 80 cm × 80 cm × 50 cm) made of wood and covered with a black opaque plastic film. The illumination on the floor of the box apparatus was around 186 lx. The objects to be discriminated were available in triplicate copies and were made of a biologically neutral material such as glass, plastic or metal. The objects were weighted so that the animals could not move them around in the arena. They are not known to have any ethological significance for the rats and they had never been associated with a reinforce (Ennaceur and Delacour, 1988).

The object recognition test consists of two phases, a sample phase (3 min duration) and a choice phase (3 min duration) with 15 min retention interval between the two phases (Ennaceur et al., 2005). In the sample phase two identical objects are exposed in the back corners of the open box, 10 cm away from the sidewall. The rat is placed in the open box facing away from the objects. The total time spent in exploring the two objects was video recorded. After 3 min of exploration, the rat is removed from the open box and returned to its cage. After a delay of 15 min elapsed the rat is reintroduced to the open box and the choice phase is started for a further 3 min. In the choice phase two different objects are exposed in the same locations that were occupied by the previous sample objects. One of the objects is identical to the object seen in the sample phase and the other is a novel object. The frequencies of approaches of each object are recorded.

The exploration is recorded only when the rat touches the object with its nose or that rat's nose is directed toward an object at a distance ≤2 cm. As a measure of discrimination, "discrimination index (DI)" was calculated by dividing the difference in number of explorations between the two objects (object N-object F) by the total amount of exploration for both objects (object N + object F). DI was then multiplied by 100 to express as a percentage.

2.7. Quantification of striatal and hippocampal neurotransmitters and metabolites

The striatum and hippocampus of the rats were rapidly dissected and stored at −80 °C until the neurochemical quantification. The endogenous concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) noradrenaline (NA) and dihydroxyphenylglycol (DHPG) were assayed by reverse-phase high performance liquid chromatography (HPLC) with electrochemical detection.

Briefly, the system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 × 4.6 mm i.d., 4 μm particle size) fitted with a 4 × 3.0 mm pre-column (Security Guard Cartridges Fusion-RP); an electrochemical detector (ESA Coulochem III Electrochemical Detector) equipped with a guard cell (ESA 5020) with the electrode set at 350 mV and a dual electrode analytical cell (ESA 5011A); an LC-20AT pump (Shimadzu) equipped with a manual Rheodyne 7725 injector with a 20 μL loop. The column was maintained inside in a temperature-controlled oven (25 °C). The cell contained two chambers in series: each chamber including a porous graphite coulometric electrode, a double counter electrode and a double reference electrode. Oxidizing potentials were set at 100 mV for the first electrode and at 450 mV for the second electrode. The tissue samples were homogenized with an ultrasonic cell