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Melatonin attenuates tyrosine hydroxylase loss and hypolocomotion in MPTP-lesioned rats

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ABSTRACT

Parkinson's disease is a chronic neurological disease characterized by dopaminergic neuron degeneration in the substantia nigra pars compacta. Melatonin is a powerful antioxidant agent secreted by the pineal gland which has numerous physiological functions and seems to exert an important neuroprotective effect. The 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model has been used to understand the pathophysiology of the disease because of its capacity to mimic biochemical and histological features observed in Parkinson's disease. This study investigated the effect of pretreatment with melatonin (50 mg/kg) on MPTP-lesioned animals 24 h and 7 days after neurotoxin infusion using the open-field test, two-way avoidance task and immunohistochemistry. Twenty-four hours after lesioning, the MPTP+vehicle group exhibited hypolocomotion and significant loss of tyrosine hydroxylase-immunoreactive cells, whereas no differences in these parameters were observed in lesioned animals receiving melatonin. Seven days after surgery, the MPTP-lesioned rats did not show hypolocomotion compared to control animals, while there was a significant dopaminergic neuronal loss. In the two-way avoidance task, MPTP-treated animals presented a cognitive deficit compared to the control groups and melatonin administration did not repair this defect. The present results suggest that melatonin reduces neuronal loss in the MPTP animal model of Parkinson's disease.

1. Introduction

Parkinson's disease is the second most common neurodegenerative disease (Kidd, 2000) and is characterized by progressive loss of substantia nigra neurons accompanied by the occurrence of Lewy bodies (Klockgether, 2004). The cause of idiopathic Parkinson's disease is still unknown but aging, environmental factors, oxidative stress, neuroinflammation and genetic factors may be involved in the development of the disease (Dauer and Przedborski, 2003; Gao et al., 2003; Takahashi and Wakabayashi, 2005).

Research into the pathogenesis of Parkinson's disease has been rapidly advancing as a result of the development of animal models which also permit the investigation of new treatments. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was discovered in 1982, when a group of drug addicts developed subacute severe parkinsonism (Di Filippo et al., 2006). The MPTP animal model is a useful model for the study of neurodegeneration in Parkinson's disease because it produces clinical, biochemical and neuropathological changes similar to those observed in human Parkinson's disease (Schulz and Falkenburger, 2004).

Numerous studies have shown that the administration of MPTP causes cell loss in the substantia nigra pars compacta (SNpc) (Heikkila et al., 1984; German et al., 1996; Gevaerd et al., 2001; Lima et al., 2006) and decrease locomotion and/or rearing frequencies (Reksidler et al., 2007; 2008). In addition to these deficits in motor function, this toxin produces cognitive impairment in rats tested in the active avoidance task (Da Cunha et al., 2001; Gevaerd et al., 2001) and in the Morris water maze (Miyoshi et al., 2002; Perry et al., 2004; Reksidler et al., 2007).

Several cell death mechanisms involved in the neurotoxicity of MPTP have been suggested, including the inhibition of complex I in the mitochondrial electron transport chain, inflammation and the generation of reactive oxygen species, among others (Schulz and Falkenburger, 2004). Reactive oxygen species are produced normally by metabolism and exert important physiological functions, including their participation in the defense against intrusion of foreign bodies. However, the accumulation of these oxygen species may damage cells by oxidative degradation of critical molecules such DNA, lipids and proteins (Cui et al., 2004).

Melatonin (*N*-acetyl-5-methoxytryptamine) is an indolamine neurohormone produced from the amino acid tryptophan which is rhythmically secreted by the pineal gland (Cardinali and Pévet, 1998;

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Hardeland et al., 2006). This neurohormone reaches peak concentrations at night and this variation in melatonin concentration with time seems to be an important factor for circadian rhythmicity (Tan et al., 2007). Studies have demonstrated that melatonin exhibits immunomodulatory properties such as the activation of T cells, B cells, natural killer cells and monocytes, thymocyte proliferation and release of cytokines (IL-1, IL-2, IL-6, IL-12 and IFN-γ). The anti-inflammatory properties of melatonin are mediated by downregulation of cyclooxygenase type 2 (COX-2) and inhibition of nitric oxide (Hardeland et al., 2006). In addition to these actions, melatonin is also a potent free radical scavenger and broad-spectrum antioxidant (Tan et al., 2007).

The antioxidant effect of melatonin has been described in in vitro and in vivo studies. Using brain homogenates, Sewerynek et al. (1995) demonstrated that melatonin (0.1–5 mM) protected against H_2O_2-induced lipid peroxidation in a concentration-dependent manner. The involvement of melatonin in antioxidant enzyme activity has been demonstrated by Barlow-Walden et al. (1995), who observed an increase of glutathione peroxidase activity in the brain of rats after melatonin administration. In this respect, Baydas et al. (2002) reported that a reduction in melatonin by pinealectomy leads to a decrease in glutathione peroxidase activity levels. More recently, Subramanian et al. (2007) reported that daily administration of melatonin for 45 days at pharmacological concentrations (0.5 and 1.0 mg/kg) increased the activity of the brain and liver antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase and resulted in increased glutathione levels. Thus, melatonin seems to act as an electron donor for molecules deficient in one electron, reducing oxidative damage and still being able to increase the activity of antioxidant enzymes such as glutathione peroxidase (Reiter, 1998; Rodriguez et al., 2004).

On the basis of these effects, many investigators have focused on an important aspect of melatonin, i.e., its neuroprotective effect. Melatonin has been shown to be neuroprotective in a number of Parkinson's disease models. In this respect, Sharma et al. (2006) observed neuroprotection mediated by melatonin in a 6-hydroxydopamine (6-OHDA) model of Parkinson's disease. Similarly, Saravanan et al. (2007) evaluated the effect of melatonin on rotenone-induced oxidative stress. The authors showed that melatonin (10, 20 and 30 mg/kg) treatment for 4 days restored the rotenone-induced decrease in glutathione levels and the changes in antioxidant enzyme activities (superoxide dismutase and catalase) in the substantia nigra of rats. Nam et al. (2005) showed that daily melatonin (10 mg/kg) treatment for 3 days significantly attenuated 3-nitropropionic acid-induced neuronal damage in rats, reducing the degree of asymmetric rotational behavior and restoring dopamine levels in the lesioned striatum. Furthermore, some studies on rodents have reported that the administration of melatonin protects dopaminergic neurons against MPTP-induced neurotoxicity (Jin et al., 1998; Khaldy et al., 2003). However, the mechanisms involved in the in vivo neuroprotective effect of melatonin have not been precisely determined.

All these lines of evidence suggest that melatonin may prevent or minimize the deleterious effects of toxic agents, including MPTP, by reducing the formation of reactive oxygen species which seem to be involved in the progression of several neurodegenerative diseases such as Parkinson's disease.

Thus, the aim of the present study was to investigate the effect of melatonin in MPTP-lesioned rats submitted to the open-field test and two-way active avoidance task. In addition, we evaluated the effect of melatonin administration on the number of tyrosine hydroxylase (TH)-immunoreactive neurons in the SNpc.

2. 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 six 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. 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 Universidade Federal do Paraná and was approved by the University Ethics Committee.

2.2. Drugs

MPTP-HCl (100 μg; Sigma, St. Louis, MO, USA) was dissolved in 1 μl saline and bilaterally injected intranigrally. Melatonin was purchased from Sigma and dissolved in a mixture of saline and propylene glycol at a proportion of 1:1, followed by sonication. The melatonin suspension was injected intraperitoneally at a dose of 50 mg/kg. Vehicle (1 ml/kg, a mixture of 0.9% NaCl and propylene glycol at a proportion of 1:1) was used as control solution for melatonin.

2.3. Experimental protocols

In this study, the rats were randomly divided into 6 groups: control+vehicle (propylene glycol and saline at a proportion of 1:1, 1 ml/kg; $n=18$), control+melatonin (50 mg/kg; $n=15$), sham+vehicle ($n=12$), sham+melatonin ($n=12$), MPTP+vehicle ($n=15$), and MPTP+melatonin ($n=17$). All animals received a single intraperitoneal injection of vehicle or melatonin 30 min prior to surgery. The animals were injected between 7:00 and 12:00 a.m.

2.4. Experimental procedures

2.4.1. Stereotaxic surgery

The MPTP and sham groups were anesthetized with equitesin (0.3 ml/kg) and received a bilateral intranigral infusion of 100 μg MPTP-HCl in 1 μl saline (0.35 μl/min) through a 30-gauge needle according to the following coordinates adapted from the atlas of Paxinos and Watson (1986): anteroposterior (AP): −5.0 mm from the bregma; mediolateral (ML): ±2.1 mm from the midline; dorsoventral (DV): 8.0 mm from the skull. Animals of the sham groups were submitted to the same general procedure but did not receive MPTP infusion, and control animals were not operated upon. After surgery, the animals were left in a temperature-controlled chamber until they had recovered from anesthesia.

2.4.2. Open-field

This test was used to determine motor alterations 24 h and 7 days after surgery. The apparatus consisted of a rectangular box (40×50×63 cm) whose floor was divided into 20 (10×10) small rectangles. The animals were gently placed in the right corner of the open field and were allowed to freely explore the area for 5 min. The frequency of locomotion (number of crossings from one rectangle to the other) and rearing (number of times the animals stood on their hind paws) was determined. Hand-operated counters were used to score immobility time (number of seconds of lack of movement during testing) and latency to start the movement (latency to leave the first rectangle). The latency to start the movement in the open field was used to evaluate akinesia as suggested by Sedelis et al. (2001). The open field was washed with a 5% water–alcohol solution before behavioral testing to eliminate possible bias due to odors left by previous rats.

2.4.3. Two-way active avoidance task

Seven days after surgery the rats were submitted to a two-way active avoidance task as described previously (Da Cunha et al., 2001; Perry et al., 2004). The active avoidance test was an automated shuttle