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# Correlations between behavioural and oxidative parameters in a rat quinolinic acid model of Huntington's disease: Protective effect of melatonin

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ABSTRACT

The present study was designed to examine the correlations between behavioural and oxidative parameters in a quinolinic acid model of Huntington's disease in rats. The protective effect of melatonin against the excitotoxicity induced by quinolinic acid was investigated. Rats were pre-treated with melatonin (5 or 20 mg/kg) before injection of quinolinic acid (240 nmol/site; 1 μl) into their right corpora striata. The locomotor and exploratory activities as well as the circling behaviour were recorded. The elevated body swing test was also performed. After behavioural experiments, biochemical determinations were carried out. Melatonin partially protected against the increase of circling behaviour caused by quinolinic acid injection. No alteration was found in the number of crossings and rearings of animals treated with melatonin and/or quinolinic acid. Melatonin decreased the percentage of contralateral biased swings induced by quinolinic acid. Melatonin protected against the increase in reactive species and protein carbonyl levels as well as the inhibition of superoxide dismutase activity resulting from quinolinic acid injection. Melatonin was partially effective against the inhibition of striatal catalase activity and a decrease of non-protein thiol levels induced by quinolinic acid. Melatonin was not effective against the inhibition of $Na^+$, $K^+$ ATPase activity caused by quinolinic acid injection. There were significant correlations between circling behaviour and oxidative parameters. The antioxidant property of melatonin is involved, at least in part, in its neuroprotective effect. The results reinforce the idea that melatonin could be useful in overwhelming neurotoxicity caused by quinolinic acid, a rat model of Huntington's disease.



## 1. Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is a natural hormone primarily secreted by the pineal gland during darkness. It is known to produce a receptor-independent mitochondrial protective effect due to its antioxidant and free radical scavenging properties (Huang et al., 2009). In addition, melatonin promotes neurogenesis (Ramírez-Rodríguez et al., 2009) and maintains or attenuates a reduction in neurogenesis after irradiation (Manda et al., 2009). Melatonin readily crosses the blood–brain barrier, concentrates in the nuclear and mitochondrial compartments of the cell, and has relatively high efficacy and low toxicity (Acuna-Castroviejo et al., 1995).

This hormone has important regulatory effects on the central nervous system (Wan et al., 1999). In fact, researchers have demonstrated neuroprotective effects of melatonin in 6-hydroxydopamine- (Sharma et al., 2006), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)- (Patki and Lau, 2011) and rotenone-induced models of Parkinson's disease (Saravanan et al., 2007). Additionally, melatonin improves D-galactose-induced aging effects (Yoo et al., 2012) and has anticonvulsant action (Moezi et al., 2011).

The beneficial effects of melatonin on oxidative damage have also been demonstrated in a model of Huntington's disease. In fact, the effects of melatonin in protein carbonyl, superoxide dismutase (SOD) and succinate dehydrogenase activities in 3-nitropropionic acid rat model of Huntington's disease have been reported (Túnez et al., 2004). Moreover, the potential involvement

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of melatonin dysregulation (diurnal variations) in Huntington's disease pathogenesis has been demonstrated (Aziz et al., 2009).

Quinolinic acid is a neuroactive metabolite of tryptophan which has been implicated in the pathogenesis of a variety of degenerative, infectious and inflammatory human neurological diseases (Cabrera et al., 2000; Stone, 1993). Accordingly, the injection of quinolinic acid into the rat striatum produces striatal lesions similar to those observed in Huntington's disease (Bruyn and Stoof, 1990), such as stereotypical motor alterations (Rossato et al., 2002).

The excitotoxicity caused by quinolinic acid is sustained by activation of *N*-methyl-D-aspartate (NMDA) receptor and is related to an increase of cytosolic $Ca^{2+}$ concentrations, ATP exhaustion, γ-aminobutyric acid (GABA) depletion, specific GABAergic and cholinergic neuronal death and oxidative cell damage (Foster et al., 1983; Santamaría and Ríos, 1993; Schwarcz et al., 1984). Free radical formation and oxidative stress are associated to neurotoxicity caused by quinolinic acid (Santamaría et al., 2001), supporting the hypothesis that antioxidants may represent potential therapeutic tools against quinolinic acid toxicity.

Studies have revealed that melatonin attenuates oxidative damage induced by quinolinic acid in the rat brain homogenate (Cabrera et al., 2000; Southgate and Daya, 1999) as well as in the brain tissue culture (Vega-Naredo et al., 2005). However, there are no data regarding the actions of melatonin on motor impairments induced by quinolinic acid in rats. Therefore, in the present study we sought to investigate the protective role of melatonin on motor deficits, such as circling behavior and rotational response, caused by quinolinic acid injection in striata of rats. We also extended our study to verify if melatonin reduces the striatal oxidative damage induced by quinolinic acid when evaluated in vivo. Lastly, to better characterize if motor deficits are linked to striatal oxidative damage, correlations between these factors were provided.

## 2. Materials and methods

### 2.1. Animals

All experiments were conducted using male adult Wistar rats (200–250 g) from our own breeding colony. Rats were kept in a separate animal room, on a 12 h light/dark cycle, with lights on at 7:00 a.m., at room temperature ($22 \pm 1$ °C), with free access to food and water. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil.

### 2.2. Drugs

Quinolinic acid and melatonin were purchased from Sigma Chemical Co, USA. Quinolinic acid was dissolved in saline and had pH adjusted to 7.4. Melatonin was dissolved in absolute ethanol and subsequently diluted with Milli-Q water (the final ethanol concentration was 0.5%). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

### 2.3. Surgical procedure

Rats were anesthetized with Equithesin (1% phenobarbital, 2% magnesium sulfate, 4% chloral hydrate, 42% propylene glycol, 11% ethanol; 3 ml/kg, intraperitoneally; i.p.) and then placed in a rodent stereotaxic apparatus. The body temperature was controlled during the surgery. The rectal temperature was measured by the insertion of a lubricated thermistor probe. Under stereotaxic guidance, a cannula was inserted unilaterally into the right striatum. The standard outer diameter measurements for guide cannula was of 22-gauge. The infusion of quinolinic acid was carried out using a microsyringe (model of Hamilton) connected with a plastic tube. After, we insert the internal cannula all the way down into the guide cannula. For rats, 1 μl of quinolinic acid was injected at a rate no greater than 1 μl/min.

Stereotaxic coordinates for the caudate putamen, relative to Bregma, were AP 0.5, L 2.6 and V 4.5, relative to the dura (Paxinos and Watson, 1984). The post-surgical recovery period was 7 days in all experiments.

### 2.4. Experimental design

Animals ($n=10$ animals per group) were pre-treated i.p. with melatonin (5 or 20 mg/kg) (Pei et al., 2003; Tang et al., 2002) or vehicle (1 ml/kg) 30 min before stereotaxic injection of 1 μl of quinolinic acid (240 nmol/site) or saline into their right corpora striata (Rodríguez-Martínez et al., 2000). Immediatelly after quinolinic acid injection, rats were subjected to behavioural assessment in an open-field. The doses of melatonin (5 and 20 mg/kg, i.p.) were chosen based on the literature (Agrawal et al., 2009; Pei et al., 2003; Tang et al., 2002).

### 2.5. Behavioural tests

The locomotor behaviour was assessed in the open-field test (OFT). The open-field was made of plywood and surrounded by walls 30 cm in height. The floor of the open-field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 20 min to record the locomotor (number of segments crossed with the four paws) and exploratory (number of rearings) activities (Walsh and Cummins, 1976). The circling behaviour (number of turns/20 min) was also measured. Rotational response was considered as a 360° movement.

After the open field session, the elevated body swing test (EBST) was performed as described by Borlongan and Sanberg (1995). The animal was elevated an inch above the ground by holding its tail. When the animal moved its head directed more than 10° to either side of the vertical axis a swing was counted. The direction and the frequency of each swing were observed. The test session for each animal lasted for 1 min. The results were expressed in % contralateral biased swings, calculated as follows: % contralateral biased swings $=[n^{\circ}$ contralateral swings$/(n^{\circ}$ ipsilateral swings $+ n^{\circ}$ contralateral swings$)] \times 100$.

The EBST has been shown to be a valuable behavioural tool to identify early effects of excitatory compounds and behavioural changes in unilateral neurotoxic animal models, since rats unilaterally injected with excitatory compounds display swing behaviour (Borlongan et al., 1995; Prauchner et al., 2004). The mechanisms involved in biased swing behaviour may be similar to those implicated in rotational behaviour (Borlongan and Sanberg, 1995). The ipsiversive and contraversive rotations were recorded by two observers who were blind to the treatment.

### 2.6. Biochemical determinations

After behavioural tests, biochemical determinations were carried out. Rats were killed by decapitation at 120 min post-injection of quinolinic acid. The brain was removed and striatum was separated. The ipsilateral (injected) and contralateral hemispheres were separated. The contralateral hemisphere served as an internal control. The analysis of contralateral side was done to evaluate the selectivity of the damage by quinolinic acid. The