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Physical exercise down-regulated locomotor side effects induced by haloperidol treatment in Wistar rats

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ABSTRACT

Extra-pyramidal symptoms (EPS) such as akinesia, dystonia, gait alteration and tremors are observed when dopamine D2-receptors are blocked by pharmacological agents such as haloperidol. These alterations produce a Parkinson disease-like state (PLS). Physical exercise has been proven to improve gait and locomotor symptoms in Parkinson's disease; we sought to elucidate the effects of physical exercise on PLS induced by chronic administration of haloperidol in rats. We used 48 rats distributed into four groups: Control, Exercise, Haloperidol, and Hal + Exe. All the animals received a daily injection of saline or haloperidol for 30 days, and the exercise groups underwent a daily 30-minute exercise protocol for 20 days. The animals were subjected to the ink–paw test, bar test and open-field test throughout the training period. The haloperidol-induced akinesia increased throughout the days of injections, but exercise was shown to alleviate it. The assessment showed shortened stride length and increased stance width with the use of haloperidol, which were significantly alleviated by exercise. These results indicate that exercise could be an interesting approach towards reducing unwanted EPS caused by haloperidol.

1. Introduction

Extrapyramidal symptoms (EPS) are a collection of motor side-effects that can arise with the use of dopamine D2-receptor blockers. Drugs of this nature are widely used in the treatment of psychotic illnesses such as schizophrenia and bipolarity (Inada et al., 2002). Haloperidol is an example of a dopamine antagonist and, although it belongs to the first generation of antipsychotics drugs (APD), it is still the reference treatment for schizophrenia (McCue et al., 2006). EPS presents a very specific set of motor deficits such as tremors, akinesia, dystonia and gait alterations (Lieberman et al., 2005; Miyamoto et al., 2005), which greatly resemble the motor characteristics observed in Parkinson's Disease (PD) patients and animal models (Amende et al., 2005; Guillot et al., 2008; Kurz et al., 2007). For this reason, APD is said to cause Parkinsonism (Peluso et al., 2012) or, as it will be referred to in this study, a Parkinson's-like state (PLS).

Physical exercise is widely prescribed to PD patients in an attempt to improve motor control and enhance life quality (Uitti, 2012). Treadmill training, in particular, has been shown to greatly improve the gait quality of PD patients (Herman et al., 2008) and in PD animal models (Pothakos et al., 2009). On the other hand, very little has been written about gait alterations in PLS induced by APD, with some studies merely mentioning the presence of a gait deficit in this state (Hansen et al., 1997; Lieberman et al., 2005). Additionally, previous studies have shown that physical exercise has some beneficial effects on EPS induced by haloperidol in rats (Teixeira et al., 2011).

Given that APD induces PLS, generating important gait alterations that are not completely understood, and that physical exercise has a beneficial effect on EPS (Herman et al., 2008; Uitti, 2012), the main goals of this study were to improve the knowledge about the motor gait deficit induced by D2 blockers and to investigate the effects of physical exercise in PLS induced by haloperidol.

2. Materials and methods

2.1. Animals

For this study, 48 male Wistar rats, three months old and weighing 200–300 g were obtained from the Institute of Basic Health Sciences (ICBS) — UFRGS. They were maintained in a controlled environment and housed in groups of five with food and water ad libitum, in a 12:12 h dark:light schedule. The animals were allocated into four groups (twelve each): 1 — Saline and Sedentary (Control), 2 — Saline and

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Exercise (Exercise), 3 — Haloperidol and Sedentary (Haloperidol) and 4 — Haloperidol and Exercise (Hal + Exe). All procedures were approved by our institution's ethics committee and were in accordance with the National Institute of Health (USA). All efforts were made to reduce number and suffering of animals used.

2.2. Drug and exercise program

Saline solution (0.9% NaCl) or Haloperidol (0.3 mg/kg/day — Janssen-Cilag Farmaceutica Ltda., Brazil) was administered intraperitoneally in a single daily dose during 30 days. This dose was chosen based as previous studies (Iwahashi et al., 1996; Kapur et al., 2003; Miyamoto et al., 2005; Putzhammer et al., 2005) in which the plasmatic drug level was approximately 1 nM. The first 4 days of drug injections, prior to initiating the exercise protocol, were used for treadmill habituation and to establish the speed to be used in the study. The exercise protocol consisted of treadmill walking for 30 min, 5 days a week for 4 consecutive weeks (a total of 20 days of exercise). All injections were applied 20–30 min before the exercise session. The exercise load consisted of a low-intensity treadmill walk at a speed of 4 m/min for 5 min, and then 6 m/min for the remaining 25 min. This protocol was adapted from a previous study using PD models (Yoon et al., 2007).

2.3. Behavioral tests and measurements

The animals were weighed in the pre-training (PT) period and at the end of each week (1 week, 2 weeks, 3 weeks, and 4 weeks). The ink–paw test and the open-field test were performed in PT and in 4 weeks. The bar test was used in PT, 2 weeks and 4 weeks. All tests were performed before the daily injection to avoid the acute effects of the drug.

For the ink–paw test, the hind paws of the animals were dyed with non-toxic ink. Then, animals were individually placed on a catwalk with the floor covered by white paper. As the animals walked on the apparatus, paw prints were left on the paper. A set of six consecutive steps were analyzed using these parameters: 1 — Stride length (longitudinal distance between consecutive prints); 2 — Stance width (latitudinal distance between consecutive left and right paw prints); 3 — Paw length (distance between the print of the tip of the third toe and the ankle); and 4 — Paw width (distance between the tip of the first toe and the tip of the fifth toe) (Ilha et al., 2008) (Fig. 2).

In the bar test, akinesia was evaluated by placing both forelimbs on a 9 cm high horizontal bar and measuring the time taken by the animals to remove both paws from the bar (Ohno et al., 2010; Vasconcelos et al., 2003; Wu et al., 2009).

The open-field apparatus consisted of a 50 cm high, 60 cm × 40 cm box made of plywood, with one glass side. The floor was divided by drawn lines, composing 12 equal rectangles. Animals were individually placed in a corner of the apparatus and their behavior was recorded for 3 min. All behavior measurements were automated using ANY-Maze software (Version 4.70, Stoelting). The following parameters were analyzed: Lines crossed, total distance traveled, average speed, time mobile, time immobile, time in the center and immobile episodes. Rearing data was also analyzed. However, it is a rater-dependent variable.

A timeline with all experimental procedures can be seen in Fig. 1.

2.4. Statistics

To evaluate the ink–paw and open field tests, one-way ANOVA was performed followed by Tukey's post hoc test to evaluate the differences among the groups in the same period, while the differences in the same group in different periods (PT and 4 weeks) were analyzed using a t-paired test.

Repeated measures ANOVA followed by Tukey's post hoc test was used to evaluate the bar test. All procedures were performed in the SPSS 11.0 software ($P<0.05$).

3. Results

3.1. Weight

In this study no significant change to the animals' weight was observed (data not shown).

3.2. Ink–paw test

The ink–paw test demonstrated haloperidol induced gait alterations that were alleviated by exercise. After 4 weeks of treatment the Haloperidol group showed a reduced stride length ($F_{(3,46)}=4.45$, $P<0.05$) and larger stance width ($F_{(3,46)}=11.14$, $P<0.001$) when compared to all groups. These effects were not seen in the Hal + Exe group (Fig. 3A and B), hence, exercise alleviated gait alterations. However, the statistical analysis showed that there was no significant change in paw length and paw width in any of the groups (see Fig. 3C and D).

3.3. Bar test

The bar test showed a significant increase in akinesia associated with haloperidol treatment, as represented by the time spent on the bar at the different test intervals. The bar test results showed a significant effects in

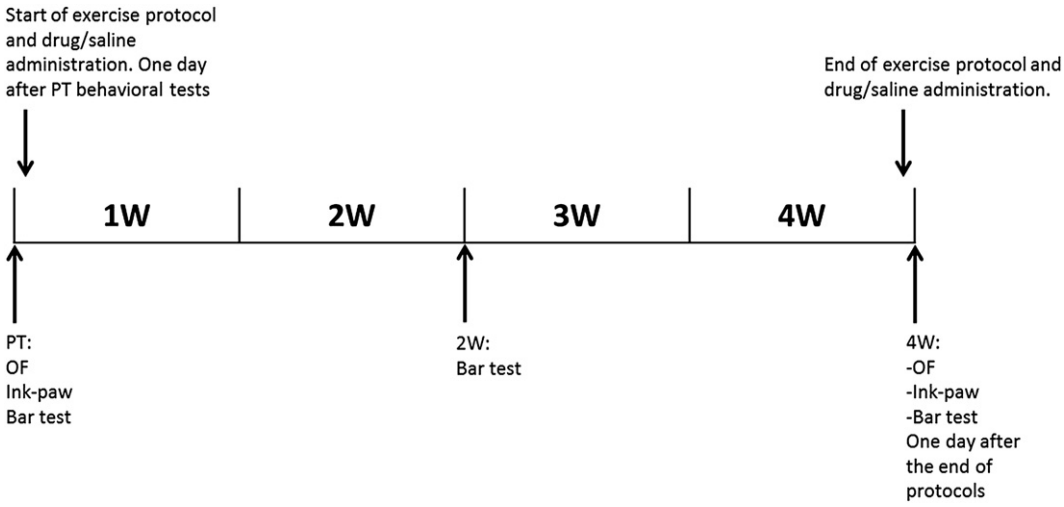

Fig. 1. A timeline of the experimental design. PT = pre-training, OF = open field test, W = weeks.