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Progesterone prevents depression-like behavior in a model of Parkinson's disease induced by 6-hydroxydopamine in male rats

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ABSTRACT

Hemiparkinsonism induced by 6-hydroxydopamine (6-OHDA) injected in left corpus striatum is a recognized model of motor deficits in rats. Some reports concerning motor deficits indicate a favorable response to steroid administration in hemiparkinsonian animals. However, there is no much information regarding progesterone administration in relation to cognitive and affective dysfunctions. Here we could confirm earlier reports regarding a mild deficit of memory and a noticeable depressive-like behavior 4 weeks after injecting 6-OHDA. We also present some evidence that progesterone could be – when administered 7 days after the injection of 6-OHDA – a possible neuroprotector concerning both motor deficits as well as cognitive — memory- and depression-like behaviors. The affective deficit was reverted by administering the tricyclic antidepressant imipramine. Since Parkinson's disease is a conspicuous cause of psycho-organic decline in human beings, it would be important to be able of dealing early with non-motor indicators in order to use prospective neuroprotectors to prevent the progression of the disease.

1. Introduction

Parkinson's disease was first described by James Parkinson in 1817 (cited in Dauer and Przedborski, 2003). It is a relatively common and serious impairment of health in developing countries all around the world, affecting about 1% of the population over 55 years, with a mean age of onset of 60 years (Hayes et al., 2010). The brains of patients suffering from Parkinson's disease show a profound deficit in dopamine levels, because of the loss of neurons of the substantia nigra. Accordingly, authors considered the disease primarily as a disorder of movement (Dauer and Przedborski, 2003). However, there are important cognitive symptoms that precede the motor stage, collectively known as pre-motor phase (Savica et al., 2010; Tadaiesky et al., 2008). These symptoms are responsible, at least in part, for the extreme disability that accompanies the motor stages as the disease progresses (Dauer and Przedborski, 2003).

Pre-motor symptoms precede the motor phase by several years (Hayes et al., 2010). This period – characterized by constipation, hyposmia, sleep disorders, depression, among others – is not only an interesting target for early diagnosis and treatment of the disease but also a critical period to evaluate the elusive impact of neuroprotectors and/or neuroregenerators. Since progesterone has been postulated to be a neuroprotector (Bourque et al., 2009; De Nicola et al., 2009; Djebaili et al., 2004, 2005; Garay et al., 2009; Liu et al., 2010; Singh et al., 2010) we used this steroid to examine whether it could eventually protect our subjects from non-motor and motor components of the model. In order to do so, we decided to use a 6-hydroxydopamine (6-OHDA) toxin-based model of parkinsonism in rats (modified from Dauer and Przedborski, 2003). Several reports informed that unilateral 6-OHDA-lesioned rats are suitable for behavioral and biochemical evaluation of models of Parkinson's disease (Kondo et al., 2004; Ahmad et al., 2005; Saravanan et al., 2005). Additionally, there exists some literature regarding asymmetric Parkinson's disease in human beings, which supports the use of the proposed model (Rafal et al., 1989; Tessitore et al., 2010).

It is well known that progesterone – when used in 6-OHDA injected subjects – affects motor components as well as reproductive behaviors in female rats and hamsters (Hansen et al., 1991; Frye et al., 2010). Here we centered our interest around two common non-motor–non-reproductive-symptoms (Tadaiesky et al., 2008): 1) cognitive disorders in an appetitive model of memory (by using a novel object recognition test); and 2) depression-like behaviors (by using a forced swimming

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test). Our results provide some interesting cues regarding what could eventually be a useful area of research in order to study non-motor stages in animal models, and the possible utility of using neuroactive steroids as neuroprotectors.

2. Materials and methods

2.1. Animals

We used male Sprague Dawley rats from our breeding colony. They were 60–120 days old at the beginning of the study, and their weight was 280–340 g. Experimental subjects were housed under controlled temperature (22 ± 2 °C) and lighting (12 hour light cycle beginning at 06.00 a.m.) conditions, with food and water made available ad libitum. Animals for these experiments were kept and handled according to the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academies, U.S.A., 8th edition, 2011). All efforts were made to minimize animal suffering.

2.2. Reagents

6-Hydroxydopamine hydrobromide, amphetamine, desipramine HCl, and progesterone were purchased from Sigma-Aldrich (St. Louis, MO, USA). Apomorphine hydrochloride was obtained from Research Biochemicals International (Natik, MA, USA). Chloral hydrate was purchased from Anedra (Buenos Aires, Argentina). Imipramine HCl was obtained from Farmacia Sevilla (Mendoza, Argentina).

2.3. Surgical procedures

In order to achieve unilateral lesions of the nigrostriatal system, rats received 6-OHDA injections into the left striatum. Animals were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed into a stereotaxic frame (David Kopf, USA). 6-OHDA was dissolved at a concentration of 2 μg/μl saline in 0.1% ascorbic acid. The lesion was performed by injecting the toxic with a Hamilton syringe at the following coordinates: AP: −1.2 mm; ML: ±1.1 mm; DV: −5.0 mm; TB at ±0 mm. The injection was conducted at a rate of 0.5 μl/min and the needle was left in place for another 5 min before it was slowly drawn back. To prevent uptake by noradrenergic neurons, animals were pretreated with desipramine (25 mg/kg, i.p.) 30–40 min before injection of 6-OHDA (Larramendy et al., 2008).

2.4. Experimental design and drug-induced behavioral tests

Behavioral records were all performed by an observer blinded to the experimental condition of the group as well as to any previous performance of the subjects in other behavioral tests. Procedures were performed according to the following outline: a) besides leaving some intact animals, adult rats were randomly selected in order to be surgically injected with the neurotoxic 6-OHDA, or received only a saline injection; b) seven days later the animals were randomly assigned to one of 4 experimental groups: 1) sham group: the animals were handled as in the other groups, but they were neither injected with 6-OHDA nor administered any experimental treatment at all; 2) progesterone control group: subjects in this group were administered only progesterone 4 mg/kg s.c. at noon for three consecutive days, according to Gonzalez et al. (2006); 3) hemiparkinsonian group: the subjects were injected with 6-OHDA in their left striatum during original surgery, and then received no additional treatments; and 4) hemiparkinsonian/progesterone group: as in the previous group, but here the subjects were administered progesterone 4 mg/kg s.c. at noon for three consecutive days, according to Gonzalez et al. (2006); c) two weeks after surgery all groups were tested for amphetamine-induced ipsilateral rotation according to the protocol described below; d) four weeks after surgery all groups were tested for apomorphine-induced contralateral rotation according to the protocol described below; e) finally, 2 days after testing contralateral rotation, the subjects were sequentially tested in the following behavioral tests: 2 days for the novel object recognition test (NORT) and 2 days for the forced swimming test (FST). The open field test was performed shortly before NORT and FST in order to avoid potential confounding variables. The behavioral tests are described below in detail (Sections 2.5, 2.6 and 2.7).

Each experimental group began with at least 12 animals. We dismissed animals not showing ipsilateral movements 2 weeks after injecting 6-OHDA or not showing contralateral movements 4 weeks after injecting 6-OHDA, according to the protocol described below. At least 8–10 animals were finally assigned to each group.

Amphetamine-induced rotation was measured at 2 week post-lesion. Rats received 1 mg/kg amphetamine i.p. (Larramendy et al., 2008), and were placed in individual plastic bowls with a diameter of 20 cm and attached via a specially adapted harness to an automated rotameter (Rotamex, Columbus Instruments, Columbus, OH). They were allowed to habituate to their dimly lit environment for 10 min before contralateral and ipsilateral turns – regarding the side of the lesion – were recorded over 90 min. Results were expressed as ipsilateral net turns/min (Galpern et al., 1996).

Apomorphine-induced rotation was tested at 4 weeks post-lesion. Apomorphine was injected s.c. at a dose of 2 mg/kg (Estrella et al., 2002) and rotation was monitored for 90 min using the same experimental set up as for amphetamine-induced rotation. Results were expressed as contralateral net turns/min.

2.5. Open field test

In order to assess the locomotive and exploratory activity of the animals – avoiding potentially confounding variables affecting the main memory results, i.e., the effect of fear, motivation and limitations regarding locomotive activity – we used an open field, according to Kaur et al. (2010), excepting for the fact that the floor of the box was painted black instead of white. The apparatus consisted of a wooden box 90.0 × 90.0 × 38.0 cm positioned in a dimly lit room. The floor was divided by 1 cm wide white lines into 25 squares 17.0 × 17.0 cm (16 peripheral squares and 9 central squares). Initially the animals were placed in the center of the box. For the following 10 min, the following measures were recorded with a computer according to a simplified ethogram: 1) ambulatory activity: all movements detected as displacement; 2) non-ambulatory activity: any activity performed by the animal while remains in the same place; 3) time spent by the animals adjacent to border squares or using center squares of the box during their displacement; 4) vertical activity: times the animal rise for at least 2 s on their rear feet in the air or against the walls. The ethogram was registered with the free software Etholog v2.0 (Ottoni, 2000).

2.6. Novel object recognition test

The apparatus consisted of a wooden box (70 × 45 × 30 cm) with a white acrylic floor. It was located in an isolated testing room that was dimly lit by constant indirect illumination from the main source, a 25 W light bulb suspended over the box. The objects utilized as familiar (previously experienced object) or unfamiliar (object not previously experienced, i.e. the novel one) were three copies of a pink truncated pyramid and a grayish-opaque candlestick — of approximately the same size, all of which were heavy enough to prevent displacement by the animals. Since rats are red color-blind, we compared grayscale values for both the pyramids and the candlestick, finding that the grayscale value for the pyramids was a composed red–green–blue (RGB) of 166 (the whole range extending from 0 to 255), while the corresponding value for the candlestick was R165:G169:B161, accounting for an average