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Propentofylline treatment on open field behavior in rats with focal ethidium bromide-induced demyelination in the ventral surface of the brainstem

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ABSTRACT

Propentofylline (PPF) is a xanthine derivative with pharmacological effects that are distinct from those of classic methylxanthines. It depresses the activation of microglial cells and astrocytes, which is associated with neuronal damage during neural inflammation and hypoxia. Our previous studies showed that PPF improved remyelination following gliotoxic lesions that were induced by ethidium bromide (EB). In the present study, the long-term effects of PPF on open field behavior in rats with EB-induced focal demyelination were examined. The effects of PPF were first evaluated in naive rats that were not subjected to EB lesions. Behavior in the beam walking test was also evaluated during chronic PPF treatment because impairments in motor coordination can interfere with behavior in the open field. The results showed that PPF treatment in unlesioned rats decreased general activity and caused motor impairment in the beam walking test. Gliotoxic EB injections increased general activity in rats that were treated with PPF compared with rats that received saline solution. Motor incoordination was also attenuated in PPF-treated rats. These results indicate that PPF reversed the effects of EB lesions on behavior in the open field and beam walking test.

1. Introduction

Important functional roles have been increasingly ascribed to glial cells in states of both health and disease [1, 2, 29]. Several in vitro and in vivo studies have shown that propentofylline (PPF; 3-methyl-1-[5′-oxohexyl]-7-propylxanthine), a xanthine derivative, exerts profound neuroprotective, antioxidant, and antiinflammatory effects [36]. It has shown clinical efficacy in degenerative vascular dementia [16] and as a potential adjuvant treatment for Alzheimer's disease [17], schizophrenia [32], and multiple sclerosis [35]. PPF depresses the activation of microglial cells and astrocytes, which is associated with neuronal damage during inflammation and hypoxia and consequently decreases the glial production and release of damaging proinflammatory factors [36].

In rats, 7 days of PPF administration significantly decreased both cue- and cocaine-induced reinstatement of cocaine seeking, effects that were attributable to its ability to restore glutamate transporter-1 expression in the nucleus accumbens [29]. Systemic treatment with PPF blocked both methamphetamine- and morphine-induced conditioned place preferences [24]. It also improved learning and memory deficits that were induced by β-amyloid protein infusion (1–40) in a rat model of Alzheimer's disease [39]. PPF also plays a modulatory role in pain [40] by blocking proinflammatory factors that are related to pain pathways in the central nervous system.

In aged dogs, repeated PPF administration did not affect locomotion in an open field [34]. However, in a model of ethidium bromide (EB)-induced gliotoxic injury in rats, PPF significantly increased both oligodendroglial and Schwann cell remyelination at 31 days [7]. Previous studies showed that EB-induced demyelination in the brainstem caused locomotor deficits in the beam walking test in rats 3–31 days post-injection, and remyelination was related to the recovery of function [6].

The open field test was initially developed to measure emotionality [38], but it has also been used to measure other behavioral responses, such as hyperactivity [11], exploratory behavior [10], locomotor activity [27], and anxiety-like behavior [28]. Several studies have shown that open field behavior is modulated by dopamine, particularly in the striatum [4, 18, 20, 26].

Glutamate aggravates neuronal damage associated with injury of the central nervous system, such as damage that is produced by ischemia. The striatum is richly innervated by both corticostriatal glutamatergic neurons and nigrostriatal dopaminergic neurons. The release of both transmitters is somewhat related to ischemic neuronal damage. PPF afforded protection against ischemic damage in striatal dopaminergic neurons [33].

The present study was performed to investigate the effects of long-term PPF administration on open field behavior in rats with EB-induced gliotoxic injury. The effects of PPF were first examined in

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naive rats that were not subjected to EB lesions. Behavior in the beam walking test was evaluated during chronic PPF treatment because motor incoordination can interfere with behavior in the open field.

2. Materials and methods

2.1. Ethics statement

This experiment was approved by the Ethics Commission of the University Paulista (protocol no. 182/13). All efforts were made to minimize suffering of the animals and reduce the number of animals used. The experiments were performed in accordance with good laboratory practice protocols.

2.2. Animals, treatments, and experimental design

A total of 35 male Wistar rats, 4–5 months of age, were used. They were housed in polypropylene cages (38 cm × 32 cm × 16 cm; 3–4 rats per cage) at a controlled temperature (22 °C ± 2 °C) and humidity (65–70%) with artificial lighting (12 h/12 h light/dark cycle, lights on at 6:00 AM). The animals had free access to Nuvilab rodent chow (Nuvital, Sao Paulo, SP, Brazil) and filtered water. Sterilized and residue-free wood shavings were used for animal bedding. All of the experiments, including treatments and behavioral observations, were performed between 9:00 AM and 1:00 PM to minimize the effects of circadian rhythms.

The rats were randomly divided into five groups ($n = 7$ per group). Two experiments were performed. In the first experiment, 14 naïve rats (rats not submitted to surgical procedures) were divided into two equal groups: PPF group (injected with 12.5 mg/kg PPF daily [20 mg/ml], intraperitoneal [i.p.]; Agener União Quíimica, São Paulo, SP) and control group (injected with 1 ml/kg of 0.9% saline solution, i.p., for the same period of time). The open field test and beam walking test were performed on days 3, 7, 11, 15, 21, and 31 of treatment. In the second experiment, 21 rats were divided into three equal groups: EB + SAL (injected with 10 μl of 0.1% EB solution into the *cisterna pontis* and treated with 0.9% saline solution, i.p., for 31 days), SAL + PPF (injected with 10 μl of 0.9% saline solution into the cisterna pontis and treated with 12.5 mg/kg PPF daily, i.p., for 31 days), and EB + PPF (injected with 10 μl of 0.1% EB solution into the cisterna pontis and treated with 12.5 mg/kg PPF daily, i.p., for 31 days). These rats were treated and observed similarly to rats in Experiment 1.

2.3. Surgical procedure

The rats were anesthetized with thiopental (50 mg/kg, i.p.), and a burr hole was drilled on the right side of the skull, 8 mm rostral to the fronto-parietal suture. They were submitted to a local injection of 10 μl of 0.1% EB into the cisterna pontis, an enlarged subarachnoid space below the ventral surface of the pons, performed freehand using a Hamilton syringe of 10 ml, fitted with a 35° angled polished gauge (26 s) needle.

2.4. Open field test

The open field apparatus was previously described by Bernardi and Palermo-Neto [4]. The test was performed in a small room with dim lighting. Each rat was individually placed in the center of the apparatus, and the following parameters were recorded over 5 min: total locomotion (one unit was defined as the animal entering one square of the floor with all four paws), peripheral locomotion (one unit was defined as the animal entering the peripheral areas with all four paws), rearing frequency (one unit was defined as the animal standing upright on its hindlimbs), immobility time (time, in seconds, without movement), and number of entries in the central area. The frequencies of locomotion and rearing and duration of immobility were determined to evaluate possible effects of the treatments on motor/exploratory behavior [3]. Peripheral locomotion is considered an index of anxiety [9]. The apparatus was washed with a 5% alcohol/water solution before placement of the animals to obviate possible bias caused by odor cues left by previous rats.

2.5. Beam walking test

Motor coordination was evaluated on a wooden beam as previously described by [30]. This model was adapted from the one described by Jeffery and Blakemore [15]. The apparatus was a wooden beam (18 mm width × 18 mm thickness × 2 m length) with a 100 mm^2, 18 mm thick platform at each end. The beam was elevated 20 cm above the floor and painted white with two black vertical marks delimiting 1 m in the central portion. Each rat was trained to walk on the beam in 5 min daily sessions. On the first day, positive reinforcement was employed, in which a small portion of condensed milk was placed on both platforms to habituate the rat to the environment and reinforcement. The next day, the animal was placed on the beam, close to the platform with the reinforcement, with the head facing the location of reinforcement. On subsequent days, the rat was placed on the beam but at progressively farther distances from the platform with the reinforcement, until the animal crossed the entire length of the beam to reach the platform with the reinforcement. It was then returned to the initial platform. The rats always received the reinforcement after each crossing. The training period (7–10 days) was considered complete when each rat reliably crossed the beam without stalling (i.e., four crossings). Few footstep errors were made during this training stage. The animals that were unable to walk the entire length of the beam after 10 days were excluded from the experiment. After training, the rats were subjected to their respective treatment regimens, and observations in the beam walking test were made on days 3, 7, 11, 15, 21, and 31 of treatment. In each observation, a score (Table 1) was attributed for each step of the pelvic member, turned for the observer, when the rat walked in the central portion of beam. The number of steps was also measured. At the end of each session, the scores for each animal for the four crossings were cumulated. The ratio of total score/total number of steps was also calculated. Before each animal was tested, the wooden beam was cleaned with a cloth that was moistened with water. After all of the animals that were housed together in one cage completed the test, the wooded beam was cleaned with a 5% ethanol solution before the next cage of animals was tested. Total scores represent the sum of all scores given to rats from the same group obtained during all periods of observation (day 3–31 days of observation).

2.6. Statistical analysis

Homogeneity was verified using the *F* test or Bartlett's test. Normality was verified using the Kolmogorov-Smirnov test. In both experiments, the two-way ANOVA followed by Bonferroni's multiple-comparison test was used to compare data in the open field test. In experiment 1, the Student's *t*-test was used to analyze differences between two groups for parametric data and for comparisons of total scores and the ratio of total score/total number of steps in the beam walking test between two groups, the Mann-Whitney test was

Table 1
Motor coordination scores in the beam walking test.

Score	Foot position
0	Normal foot position on top of beam, no slippage.
1	Minor error: foot slip so that part of the foot is visible below the lower surface of the beam.
2	Major error: whole foot slip below the lower surface of the beam.