European Journal of Pharmacology 762 (2015) 49–54

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

Physical exercise versus fluoxetine: Antagonistic effects on cortical spreading depression in Wistar rats

Heloísa Mirelle Costa Monteiro [a], Nathália Lima Barreto-Silva [b], Gracyelle Elizabete dos Santos [b], Amanda de Santana Santos [b], Mariana Séfora Bezerra Sousa [a], Ângela Amâncio-dos-Santos [b,*]

[a] Departamento de Nutrição, Universidade Federal de Pernambuco, Centro de Ciências da Saúde, 50670-901 Recife-PE, Brazil
[b] Departamento de Fisiologia e Farmacologia, Universidade Federal de Pernambuco, Centro de Ciências Biológicas, 50740-600 Recife-PE, Brazil

ARTICLE INFO

Article history:
Received 9 March 2015
Received in revised form
11 May 2015
Accepted 18 May 2015
Available online 21 May 2015

Keywords:
Body parameters
Brain excitability
Central nervous system
Treadmill

ABSTRACT

The antidepressant fluoxetine and physical exercise exert similar effects on the serotoninergic system by increasing brain serotonin availability, and both show antagonistic action on cortical excitability. Here we provide the first assessment of the interaction of the two together on cortical spreading depression (CSD) in young adult rats. Wistar rats (40–60 days of life) received fluoxetine (10 mg/kg/d, orogastrically) or an equivalent volume of water. Half of the animals from each condition were assigned to perform physical exercise in a treadmill, and the other half formed the sedentary (non-treadmill) control groups. Body parameters (Lee index and thoracic and abdominal circumferences) and the velocity of CSD propagation were investigated. Fluoxetine+exercise animals had less weight gain (78.68 ± 3.19 g) than either the fluoxetine-only (93.34 ± 4.77 g) or exercise-only group (97.04 ± 3.48 g), but body parameters did not differ among them. The velocity of CSD propagation was reduced in the fluoxetine-only and exercise-only groups compared to sedentary water controls (3.24 ± 0.39 mm/min). For the fluoxetine+exercise group, CSD velocity values were significantly lower (2.92 ± 0.22 mm/min) than for fluoxetine only (3.03 ± 0.35 mm/min); however, they were similar to values for the exercise-only group (2.96 ± 0.23 mm/min). These findings confirm the similar effects of fluoxetine and exercise and suggest a greater effect of physical exercise in reducing brain excitability.

1. Introduction

Serotonin, or 5-hydroxytryptamine (5-HT), is a neurotransmitter that is widely distributed in the central nervous system. This monoamine is important in many behaviors, including sleep, appetite, memory, sexual behavior, neuroendocrine function, and mood (Sghendo and Mifsud, 2012). It also interferes in food behavior, promoting satiety and reducing body weight ([Guimarães et al., 2006,Voigt and Fink, 2015]) and seems to decrease brain excitability ([Pardo-Peña et al., 2014,Tarlaci, 2009]).

The selective serotonin reuptake inhibitor (SSRI) fluoxetine is an antidepressant that increases brain serotonin availability (Qu et al., 2009). Because of some reports that physical exercise also increases brain serotonin (Lan et al., 2014; Meeusen et al., 1996), physical exercise could be suggested as a less expensive strategy for the same result.

A relationship between serotonin and brain excitability has been reported with other SSRIs (Amâncio-dos-Santos et al., 2006; Guedes et al., 2002). Citalopram can reduce the occurrence of spontaneous high-frequency oscillations that occur in epileptic seizures (Pardo-Peña et al., 2014). Escitalopram promotes a decreased frequency, duration, and intensity of episodes of migraine (Tarlaci, 2009). Additionally, serotonin exerts an antagonistic effect on cortical spreading depression (CSD) ([Amâncio-dos-Santos et al., 2006,Amâncio-dos-Santos et al., 2013,Guedes et al., 2002, Read and Parsons, 2000]). CSD has been described as a slowly propagating wave of depression of spontaneous cortical electrical activity that occurs with chemical, electrical, or mechanical stimulation of one point of cortical tissue (Leão, 1944). The CSD wave consists of reducing ("depression") of the electrical activity of brain tissue that spreads ("spreading") concentrically and reversibly (Leão, 1944). Simultaneous with the depression of spontaneous electrical activity, a slow direct-current (DC) potential change (SPC) appears in the cortical region where the CSD is observed, and this "all or none" signal clearly marks the beginning and the end of the phenomenon (Leão, 1947). Thus, the SPC is

* Correspondence to: Departamento de Fisiologia e Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco. Av. da Engenharia, S/N Cidade Universitária, 50740-600 Recife, PE-Brazil. Fax: +55 81 2126 8976.
E-mail addresses: helo-mcm@hotmail.com (H. Mirelle Costa Monteiro), angela.asantos@yahoo.com, angela.asantos@ufpe.br (Â. Amâncio-dos-Santos).

http://dx.doi.org/10.1016/j.ejphar.2015.05.027
0014-2999/

widely used to calculate the velocity at which the CSD propagates through the nervous tissue (Guedes et al., 2004).

We previously showed that administration of fluoxetine during development dose-dependently impairs CSD propagation. Wistar rats treated with fluoxetine (10 mg/kg, s.c.) showed a reduction in CSD propagation velocity. However, a dose of 20 mg/kg had an even stronger effect (Amâncio-dos-Santos et al., 2006). Additionally, the effect of physical exercise on CSD was also investigated. Rats performed physical exercise on a treadmill during early phases of development, at adulthood, or during senescence. At all ages, an antagonistic effect of exercise on CSD was observed (Batista-de-Oliveira et al., 2012; Monteiro et al., 2011). These data led us to ask the following questions: (1) Can physical exercise potentiate the effect of 10 mg/kg fluoxetine on CSD? (2) Do fluoxetine administration and physical exercise influence CSD propagation in young adulthood? (3) Do physical exercise and fluoxetine administration interact and influence body parameters?

2. Methods

2.1. Animals and experimental groups

Male Wistar rats were handled in accordance with the "Principles of Laboratory Animal Care" (National Institutes of Health, USA) and the norms of the Ethics Committee for Animal Research of the Universidade Federal de Pernambuco. They were reared in polyethylene cages (51 cm × 35.5 cm × 18.5 cm) in a room maintained at 22 ± 1 °C with a 12- h light:dark cycle (lights on at 6:00 a. m.) and free access to water and rodent laboratory chow diet (Presence Nutrição Animal, São Paulo, Brazil). At 40–60 days of life, the animals were assigned to one of the following groups: sedentary and treated with vehicle (distilled water control; water-only group); exercised and treated with vehicle (exercised-only group); sedentary and treated with fluoxetine 10 mg/kg/d (fluoxetine-only group); and exercised and treated with fluoxetine (exercised + fluoxetine group).

2.2. Fluoxetine administration and the treadmill exercise protocol

Animals were administered orogastrically either 10 mg/kg fluoxetine hydrochloride (Medley®) or an equivalent volume of distilled water. The total volume administered in each gavage was 1.0 ml/d. Following gavage, rats in the two exercise groups were submitted to moderate exercise on a treadmill (model ET 2000, Insight Equipamentos Científicos, Ribeirão Preto, Brazil, 0° inclination) (Batista-de-Oliveira et al., 2012; Gomes da Silva et al. 2010). Periods of treadmill exercise lasted 3 weeks, 30 min per day, in 5 weekly sessions (one session per day). The treadmill running velocity was set at 5 m/min during the first week; during the second and third weeks, the velocity was increased to 10 m/min and 15 m/min, respectively (Monteiro et al., 2011). Rats from the sedentary groups (water-only and fluoxetine-only groups) were placed in the treadmill for the same period as the exercised animals, but the treadmill was turned off.

2.3. Body parameters

2.3.1. Weight gain

Weight gain was taken as the difference between weights at the age of 60 days and 40 days of life. Body weight measurements were made using a digital electronic scale, with 1010 g capacity and sensitivity of 0.5 g (ADF 1000 model, Marte Científica e Instrumentação Industrial Ltda, Minas Gerais, Brazil).

2.3.2. Lee index

This index was calculated at the beginning and end of the experiments (rats at 40 and 60 days old). It expresses the relationship between the cube root of body weight and the nose-to-anus length [$\sqrt[3]{}$ Weight (g)/NAL (cm)] (Novelli et al., 2007).

2.3.3. Abdominal (AC) and thoracic circumference (TC)

At 40 and 60 days, each animal was contained in a quiet and noise-free environment, and a tape measure was placed immediately anterior to the forefoot to obtain AC or immediately behind the foreleg for TC (Novelli et al., 2007). The ratio of AC/TC was calculated in the same period.

2.4. Cortical spreading depression (CSD) recording

In the four groups, CSD was recorded at 61–81 days of life. Before recordings, the animals were intraperitoneally anesthetized with a mixture of 1000 mg/kg urethane plus 40 mg/kg chloralose (both from Sigma Co., USA). This mixture was used because in the rat, it provides a very stable anesthesia that lasts for several hours and does not block CSD (Gorelova et al., 1987). Furthermore, a tracheal cannula was inserted to improve breathing. For the measurement, three trephine holes aligned in the anteroposterior direction and parallel to the midline were drilled on the right side of the skull (2–4 mm diameter). One hole was positioned on the frontal bone and used to apply the stimulus (KCl) to elicit CSD. The other two holes were drilled on the parietal bone (3–4 mm diameter) and used to record the propagating CSD wave. During surgery and CSD recording, animals breathed spontaneously, and rectal temperature was continuously monitored and maintained at 37 ± 1 °C. CSD was elicited at 20- min intervals by 1 min application of a cotton ball (1–2 mm in diameter) soaked in 2% KCl solution (approximately 270 mM) to the anterior hole drilled in the frontal region. The KCl application was repeated every 20 min for a total of 4 h. The slow potential changes accompanying CSD were recorded using two Ag/AgCl agar–Ringer electrodes (one in each hole) against a common reference electrode of the same type placed on the nasal bones. The CSD velocity of propagation was based on the time required for a CSD wave to pass the distance between the two cortical recording points. This time was measured using the beginning of the rising phase of the negative SPC as the initial point, as previously reported (Batista-de-Oliveira et al., 2012). At the end of the recording session, the animals, while still anesthetized, were killed by the introduction of a lesion in the bulbar region with a sharp needle, inserted through the cistern magna, promptly provoking cardio-respiratory arrest.

2.5. Statistical analysis

Body parameters and CSD velocity were expressed as mean ± standard error of the mean. Intergroup body parameter differences were compared by using a one-way analysis of variance (ANOVA) for repeated measures followed by the post-hoc Tukey test. CSD propagation differences were compared by employing a two-way ANOVA, including the factors of gavage treatment (fluoxetine or water) and exercise condition (exercised or sedentary) followed by a post-hoc (Holm–Sidak) test, when indicated. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Body parameters

The animals treated for 21 days with fluoxetine and submitted to physical exercise presented less ($P < 0.05$) weight gain