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Effects of acute and chronic quercetin administration on methylphenidate-induced hyperlocomotion and oxidative stress

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ABSTRACT

Aims: Increases in protein kinase C (PKC) and oxidative stress have been related to mania. Drugs with antioxidant effects or inhibitory actions on PKC may have antimanic effects. The flavonoid quercetin has antioxidant and PKC-inhibiting effects that resemble those of lithium, the first-line treatment for mania in bipolar disorder. We hypothesized that quercetin may have antimanic-like effects in an animal model.
Main methods: In the present study, we investigated the effects of acute and chronic treatment with quercetin (2.5, 5, 10, and 40 mg/kg, i.p.) in male Swiss mice that were subjected to methylphenidate (5 mg/kg, i.p.)-induced hyperlocomotion, an animal model of mania. Lithium (100 mg/kg, i.p.) and diazepam (5 mg/kg, i.p.) were used as positive and negative controls, respectively. We also evaluated the effects of these treatments on methylphenidate-induced oxidative stress in the brain by measuring reduced glutathione (GSH) and lipid peroxidation (LPO) levels in the prefrontal cortex, hippocampus, and striatum.
Key findings: Acute and chronic (21-day) treatment with lithium and diazepam reduced methylphenidate-induced hyperlocomotion. Chronic but not acute treatment with quercetin (10 and 40 mg/kg) blocked methylphenidate-induced hyperlocomotion. These effects of lithium and quercetin occurred at doses that did not alter spontaneous locomotor activity, whereas diazepam reduced spontaneous locomotor activity. Chronic treatment with lithium and quercetin blocked the methylphenidate-induced increase in LPO levels in the striatum.
Significance: These results suggest that chronic quercetin treatment has antimanic-like and antioxidant effects, thus encouraging further studies of quercetin as a putative new antimanic drug.

1. Introduction

Manic episodes in bipolar disorder (BD) are treated with mood stabilizers (e.g., lithium), atypical antipsychotics (e.g., risperidone), and anticonvulsants (e.g., sodium valproate), but their management in clinical settings remains a challenge [1,2]. Mania has been related to oxidative stress [3,4] and increased activity of protein kinase C (PKC) [5,6]. Lithium and sodium valproate, which are the most frequently used antimanic drugs, exert antimanic effects via PKC inhibition and/or antioxidant activity [6,7,8,9,6,10].

One animal model that is employed to induce manic-like behavior involves the administration of psychostimulants, such as methylphenidate. The pharmacological induction of manic-like behavior (e.g., hyperlocomotion) is relatively easy to generate and test and has reliability and validity. Locomotor activity is known to increase in manic patients [11]. Mines et al. [12] showed that methylphenidate, which blocks dopamine and noradrenaline transporters (DAT and NET respectively) enhancing dopamine (DA) and noradrenaline (NA) synaptic levels, increased locomotor activity in mice, and this effect was blocked by sodium valproate, carbamazepine, and lithium at doses that did not impair spontaneous locomotor activity [13,14,15,16,17,18].

Quercetin is a flavonoid that possesses antioxidant properties [19] and inhibits PKC [20]. We hypothesized that quercetin might also have antimanic-like effects. A previous study found that acute administration of quercetin blocked both hyperlocomotion and oxidative stress that were induced by sleep deprivation [21]. However, this previous study administered quercetin only acutely and employed only one model, which may have resulted in false-positive results. Quercetin has already been tested in a clinical trial of its anti-inflammatory effects [22], indicating its therapeutic utility. The objective of the present study

Abbreviations: BD, bipolar disorder; CMC, carboxymethylcellulose; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); GSH, reduced glutathione; LPO, lipid peroxidation; PFC, prefrontal cortex; PKC, protein kinase C.

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was to evaluate the effects of acute and chronic quercetin administration on methylphenidate-induced hyperlocomotion and oxidative stress in the brain in mice.

2. Material and methods

2.1. Animals

The study included male Swiss mice, weighing 30–40 g, that were housed at 22 °C ± 2 °C under a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The animals were kept in polypropylene cages (41 cm × 34 cm × 16 cm) with food and water available ad libitum. All of the experiments were approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO-UFPR, protocol no. 733) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

2.2. Drugs

The mice were treated with saline (0.9% NaCl; 10 ml/kg, i.p.), lithium carbonate (Eurofarma, Brazil; positive control; 100 mg/kg, i.p., dissolved in saline, with the pH adjusted to 7.4 with hydrochloric acid), diazepam (Cristália, Brazil; 5 mg/kg, i.p., dissolved in distilled water), or quercetin (Sigma, St. Louis, MO, USA; 2.5, 5, 10, or 40 mg/kg, i.p., suspended in 0.5% carboxymethylcellulose [CMC]). Methylphenidate (Novartis, São Paulo, SP, Brazil) was dissolved in saline and administered subcutaneously (s.c.) at a dose of 5.0 mg/kg. The drugs were administered in a volume of 10 ml/kg of body weight. The doses were based on data from the literature [23] and previous studies by our research group [13,21,24]. Chronic treatment with lithium, diazepam, and quercetin was performed once per day for 21 days.

2.3. Methylphenidate-induced hyperlocomotion protocol

In the acute treatment protocol, the animals were pretreated with lithium, diazepam, quercetin, or vehicle 15 min before the administration of either vehicle or methylphenidate (Fig. 1, top). In the chronic treatment protocol, the animals were pretreated with lithium, diazepam, quercetin, or vehicle once per day for 21 days. On the test day, vehicle or methylphenidate was administered 15 min after the last administration of lithium, diazepam, quercetin, or vehicle (Fig. 1, bottom).

Twenty minutes after vehicle or methylphenidate administration, the animals were individually placed in an automated activity box (40 cm × 20 cm × 26 cm) that was constructed from wood with a wire mesh floor. The box had three photoelectric sensors (10 cm apart) on the two longer lateral walls. The number of crossings was cumulatively recorded by photoelectric sensors over a 20 min period. The number of crossings was considered an index of locomotor activity. An increase in the number of crossings after methylphenidate administration indicated a stimulant effect. The blockade of the stimulant effect of methylphenidate at a dose that did not decrease spontaneous locomotor activity indicated an antimanic-like effect [24,25].

2.4. Evaluation of oxidative stress parameters in the mouse brain

2.4.1. Brain samples

The mice were euthanized by decapitation immediately after being exposed to the automated activity box. The prefrontal cortex (PFC), hippocampus, and striatum were dissected, frozen in liquid nitrogen, and stored at −80 °C until further analysis. The samples were homogenized in potassium phosphate buffer (0.1 M, pH 6.5) in a 1:10 dilution. One part of the homogenate was used to evaluate reduced glutathione (GSH) levels, and the other part was centrifuged at 9700 rotations per minute (rpm) in a micro-high-speed refrigerated centrifuge (VS-15000 CFNII, Vision Scientific, Daejeon, South Korea) for 20 min. The supernatant was used to evaluate lipid peroxidation (LPO) levels.

2.5. Evaluation of reduced glutathione levels

To evaluate GSH levels, 100 μl of the homogenate was mixed with 80 μl of 12.5% trichloroacetic acid and centrifuged at 6000 rpm for 15 min at 4 °C. Afterward, 20 μl of the supernatant was mixed with 280 μl of Tris buffer (0.4 M, pH 8.9) and 5 μl of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB; 0.01 M) according to the protocol of Sedlak and Lindsay [26], with minor modifications. Absorbance was read at 415 nm using a multi-mode microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA). The individual values were then interpolated in a standard curve of GSH (0.375–3 μg) to verify the linearity of the reaction (r^2 must be >0.99), and the values were divided by a correction factor. The results are expressed as μg of GSH per g of tissue.

2.6. Evaluation of lipid peroxidation levels

Lipid peroxidation levels were measured according to the method of Jiang et al. [27], with minor modifications. Initially, 100 μl of the supernatant was suspended in 100 μl of methanol, vortexed, and then centrifuged at 5000 rpm for 5 min at 4 °C. Afterward, 100 μl of the supernatant was added to 900 μl of FOX2 reagent (4 mM BHT, 250 μM $FeSO_4$, 250 mM H_2SO_4, and 100 mM xylenol orange). The samples were vortexed and incubated in the dark for 30 min at room temperature. Absorbance was read at 560 nm using a multi-mode microplate reader (BioTek Synergy HT, BioTek Instruments, Winooski, VT, USA). The results are expressed as mmol of hydroperoxides per mg of tissue.

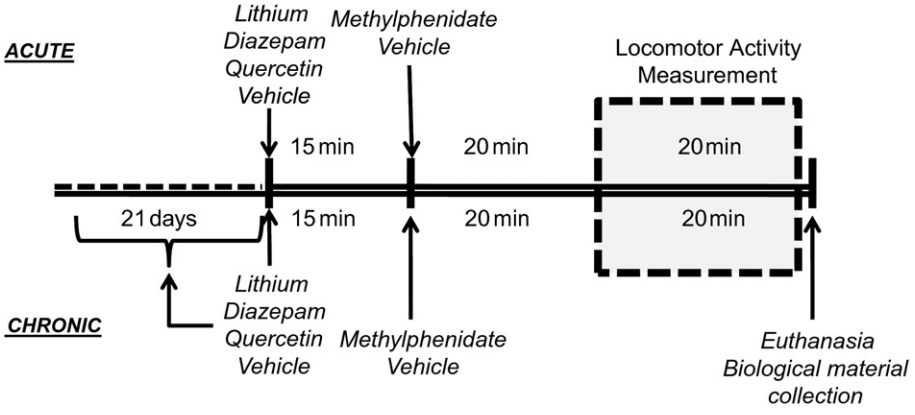

Fig. 1. Timeline of treatment schedule and methylphenidate-induced hyperlocomotion model of mania in mice.