Life Sciences 143 (2015) 187–193

Contents lists available at ScienceDirect

Life Sciences

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

Involvement of the monoamine system in antidepressant-like properties of 4-(1-phenyl-1h-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester

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ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form 5 November 2015
Accepted 10 November 2015
Available online 10 November 2015

Keywords:
Antidepressant-like effect
BDNF
MAO activity
AMPT
PCPA

ABSTRACT

Aims: Piperazinic derivatives have therapeutic potential by acting as analgesic, antidepressant-like, anticonvulsant and antipsychotic in preclinical studies. In order to develop new drugs to treat mental disorders, we designed and synthesized the 4-(1-phenyl-1H-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester (PPMP), a new piperazine derivative with putative activities on central nervous system that seems to involve serotonergic system.
Materials and methods: In order to investigate the antidepressant-like activity of PPMP, mice were treated acutely and tested in the forced swimming test (FST) and tail suspension test. Pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (PCPA, 100 mg/kg, i.p., 4 days), and the non-selective blocker of catecholamine synthesis α-methyl para-tyrosine (AMPT, 100 mg/kg, i.p.) were used to assay the involvement of serotonergic and catecholaminergic systems. "Ex vivo" monoamine oxidase (MAO) enzymatic assay and quantification of hippocampal level of brain derived neurotrophic factor (BDNF) were carried out.
Key findings: PPMP reduced the immobility time in both tests. PCPA or AMPT (100 mg/kg, i.p.) pretreatment blocked the effects of PPMP, thereby suggesting the involvement of serotonergic and catecholaminergic systems in the antidepressant-like effect of PPMP. PPMP did not inhibit the activity of MAO. Moreover, after 14 days of treatment, PPMP 15 mg/kg/day induced antidepressant-like effect and increased hippocampal level of BDNF. None of the treatments in this study altered the locomotor activity in the open field test.
Significance: In conclusion, PPMP demonstrates antidepressant-like effect that involve both serotonergic and catecholaminergic systems without inhibition of MAO activity. PPMP administration increased the hippocampal levels of BDNF.

1. Introduction

Depressive disorders are among the most prevalent psychiatric illness, affecting approximately 16% of the population [1]. Lifetime prevalence of major depressive disorders has estimate that ranged from 1.0% (Czech Republic) to 16.9% (United States), with midpoints at 8.3% (Canada) and 9.0% (Chile). The 12-month prevalence estimates ranged from 0.3% (Czech Republic) to 10% (United States), with midpoints at 4.5% (Mexico) and 5.2% (West Germany) [48].

Although the neuropathophysiology of major depressive disorders remains unclear, the monoaminergic hypothesis of depression is still valid. According to this hypothesis, the depletion of serotonin, noradrenaline, and dopamine in the central nervous system (CNS) is associated with depression. The antidepressant drugs, such as tricyclic antidepressants, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors exert their therapeutic effects by increasing the availability of these monoamines [2].

As this hypothesis could not explain fully the depression complexity and the delay in therapeutic effect of antidepressant drugs, other complementary theories such as the neurotrophic hypothesis, which postulates that low levels of neurotrophic factors mainly the brain-derived neurotrophic factor (BDNF) is associated with depression.

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http://dx.doi.org/10.1016/j.lfs.2015.11.009
0024-3205/

Some antidepressants drugs seem to restore the levels of neurotrophic factor [3,4].

Previous preclinical activities (analgesic [5], antidepressant-like [6–8], anticonvulsant [9], antipsychotic [10], antiarrhythmic [11], antiplasmodial and leishmanicidal [12]) of piperazinic derivatives are indicators of its therapeutic potential. In order to develop new drugs to treat mental disorders, we designed and synthesized 4-(1-phenyl-1H-pyrazol-4-ylmethyl)-piperazine-1-carboxylic acid ethyl ester (PPMP), a new piperazine derivative with putative activities on CNS. In the previous study, PPMP showed anxiolytic-like activity in different animal models without altering motor performance. This activity seems to involve serotonergic system but not benzodiazepine receptors [13].

The present study sought to evaluate antidepressant-like effect of PPMP and the possible contribution of serotonergic and catecholaminergic systems. The activity of monoamine oxidase (MAO), hippocampal levels of BDNF and acute toxicity of this compound were investigated.

2. Material and methods

2.1. Animals

Experiments were conducted using 300 male Swiss mice, weighing 25–35 g (3–4 months old), provided by the Central Animal House of Federal University of Goiás (Universidade Federal de Goiás — UFG). Animals were maintained at a constant room temperature of 21–23 °C under a 12:12 h light:dark cycle (lights on at 7:00 h) with free access to water and food. Mice were housed in standard laboratory cages in groups of 16–20. All experimental procedures were carried out between 09:00 and 16:00 h. All efforts were made to minimize animal suffering and to reduce the number of animals used. All studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals as published by the National Institutes of Health. The experimental protocols were approved by the Ethics Commission of the UFG (n° 104/11) in compliance with the Brazilian legislation (#11.794—10/08/2008).

2.2. Drugs

PPMP was synthesized in the Laboratório de Química Farmacêutica e Medicinal (LQFM — Faculty of Pharmacy, Federal University of Goiás) as described by Brito et al. [13]. Clorgyline, *p*-chlorophenylalanine (PCPA), α-methyl para-tyrosine (AMPT), tyramine, vanillic acid, 4-aminoantipyrine, peroxidase, polyoxyethylenesorbitan monooleate (Tween 80®) were purchased from Sigma–Aldrich (Brazil). Imipramine (Trofanil® — Novartis, Brazil), fluoxetine (EMS, Brazil), tranylcypromine (Parnate® — GlaxoSmithKline, Brazil) were also used. PPMP, PCPA, AMPT were suspended in 2% Tween 80. Imipramine, fluoxetine, tranylcypromine, clorgyline were dissolved in a 0.9 *w*/*v* % NaCl solution. The other drugs were dissolved in the potassium phosphate buffer. The treatment doses were selected from previous studies [13,36–38].

2.3. Behavioral tests

2.3.1. Open field test (OFT)

The apparatus consisted of a circular wooden arena measuring 36 (diameter) × 20 cm (height), with the bottom divided into eight squares of equal area. For open field test, animals were placed individually at the center of the open field arena to freely explore it for 5 min [14]. During this time, the locomotor activity: defined as the number of total squares crossed/5 min was videotaped. The arena was cleaned with a 10% ethanol solution after each exposure of animal.

2.3.2. Forced swimming test (FST)

Each mouse was placed individually in a plastic cylinder (diameter 18 cm, height 42 cm) filled with water (25 ± 1 °C) at a height of 30 cm. Each session was recorded by a videocamera. The immobility time (floating while making only the movements necessary to keep the head above the water surface level) was scored during 6 min [15].

2.3.3. Tail suspension test (TST)

In a soundproof experimental room, mice were suspended individually by the tail at the height of 74 cm above the floor and affixed with adhesive tape placed approximately 1–2 cm from the tip of the tail. The immobility time was recorded for 6 min [16].

2.3.4. Acute treatment with PPMP

In order to investigate the acute effects of PPMP on locomotor activity and antidepressant-like effect, male mice were treated orally with this compound at five different doses (3.7, 7.5, 15, 30 and 60 mg/kg) 1 h prior to OFT and FST. TST was performed in independent groups of male mice, 1 h after the administration of PPMP at the doses of 3.7, 7.5 and 15 mg/kg. Imipramine 30 mg/kg was used as a positive control in these protocols.

2.3.5. Role of the serotonergic and catecholaminergic systems in antidepressant-like effect of PPMP

In order to investigate a possible contribution of 5-HT (serotonin) system in the antidepressant-like effect of PPMP, animals were pretreated (i.p) with PCPA (100 mg/kg, a tryptophan hydroxylase inhibitor) or vehicle (2% Tween 80), once a day, for 4 consecutive days in a separate series of experiments. Then, 1 h after the last PCPA or saline injection, animals were treated (p.o.) with PPMP 15 mg/kg or vehicle (2% Tween 80) and tested in the OFT and FST as described earlier.

In order to study a possible contribution of catecholaminergic system in the antidepressant-like effect of PPMP, animals were pretreated (i.p.) with AMPT (100 mg/kg, a tyrosine hydroxylase inhibitor) or vehicle (2% Tween 80). Then, 4 h later, animals were treated orally with PPMP 15 mg/kg or vehicle (2% Tween 80) and tested in the OFT and FST 1 h later, as described earlier.

2.3.6. Ex vivo MAO assay

In order to analyze a possible inhibition of MAO activity, animals were treated (p.o.) with PPMP 15 mg/kg, tranylcypromine 15 mg/kg p.o. (non-specific MAO inhibitor) and clorgyline 15 mg/kg p.o. (MAO-A inhibitor). After 1 h of treatments, animals were tested in the FST prior to brain dissection.

The dissected brains were washed in ice-cold potassium phosphate buffer (0.2 M, pH 7.6), and stored at −20 °C until MAO assays. Brains were thawed and homogenized (Turrax®) in 20 vol. of 0.32 M sucrose solution in potassium phosphate buffer. Homogenates were centrifuged (1200 ×*g*, 7 min, 4 °C). The supernatants were collected and recentrifuged (12,500 ×*g*, 15 min, 4 °C). The pellets obtained were centrifuged (12,500 ×*g*, 15 min, 4 °C) in 0.32 M sucrose solution in potassium phosphate buffer. The crude mitochondrial pellets were resuspended in 1 ml of 3.6 mM KCl solution in potassium phosphate buffer. Protein contents of crude mitochondrial solution were measured by the Bradford's method [17] using bovine serum albumin as standard. At the time of use, protein concentration was adjusted with phosphate buffer (0.2 M; pH 7.6) to 0.2 mg/mL. The continuous peroxidase-linked photometric assay was carried out in the 96-well microtiter format modified from Holt et al. [18] and Stafford et al. [19]. Each test well contained 120 μL amino substrate (2.5 mM tyramine) in potassium phosphate buffer, 40 μL chromogenic solution (2 mM vanillic acid, 1 mM 4-aminoantipyrine, 8 U/mL peroxidase in potassium phosphate buffer), 40 μL enzyme (rat liver homogenate) and 40 μL of sample. Background wells contained potassium phosphate buffer (0.2 M, pH 7.6) in place of chromogenic solution. The plate was incubated at 37 °C during 30 min and read at 498 nm. MAO activity was expressed as a percentage of absorbance of the vehicle-treated control ± S.E.M.