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Antidepressant-like and antinociceptive-like actions of 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-hydrazinepyrimidine Mannich base in mice

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Abstract

This study investigated the possible antidepressant and antinociceptive action of CPMPH Mannich base, as well as the involvement of serotonergic, dopaminergic, noradrenergic and opioid systems and the L-arginine–nitric oxide pathway in the antidepressant-like effect of CPMPH in the forced swimming test (FST) in mice. The immobility time in the FST was significantly reduced by CPMPH (0.1–10 mg/kg, i.p.), without accompanying changes in the ambulation in an open-field. CPMPH at high doses (i.p. or s.c. routes) produced a significant inhibition of acetic acid-induced writhing. The antidepressant-like effect of CPMPH (1 mg/kg, i.p.) in the FST was prevented by pre-treatment of mice with methysergide (2 mg/kg, i.p., a non-selective serotonin receptor antagonist), sulpiride (32 mg/kg, i.p., a D_2 receptor antagonist) or yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist). In contrast, the antidepressant-like effect of CPMPH was not affected by pre-treatment (i.p.) with naloxone (1 mg/kg, a non-selective opioid receptor antagonist) or L-arginine (750 mg/kg, a nitric oxide precursor). The results demonstrate that CPMPH had an antidepressant-like action that appears to be mediated through its interaction with serotonergic, dopaminergic and noradrenergic systems.

Keywords: Mannich base; Antidepressant; Forced swimming test; Writhing test; Monoaminergic system

1. Introduction

Depression is a common, recurring disorder that ranges in severity from mild to very severe (Murray, 1996). The World Health Organization estimates that depression is now the fourth most important cause worldwide of loss in human disability adjusted life years. Although pharmacotherapy of depression includes a battery of drugs their efficacy is unsatisfactory, they exert multiple unwanted side effects and also their antidepressant mechanism remains incompletely understood (Murray and Lopez, 1997). However, it is clear that dysfunction of the brain monoamines systems (e.g., serotonin (5-HT), dopamine (DA) and noradrenaline (NA)) is likely to have a role in the pathophysiology of depressive disorders (Wong and Bymaster, 2002). Moreover, during the last decade, several studies have demonstrated that nitric oxide synthase inhibitors have an antidepressant-like effect in animal models of depression (Harkin et al., 1999; Da Silva et al., 2000; Volke et al., 2003).

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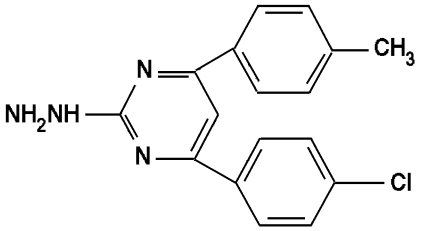

Fig. 1. Structure of the 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-hydrazinepyrimidine (CPMPH).

It is generally accepted that there is a relationship between chronic pain and depression (Pincus et al., 2004; Currie and Wang, 2004). In addition, several clinical and preclinical studies have found that antidepressant drugs are able to produce marked analgesia in humans and animals (Millan, 2002; Gutierrez et al., 2003; Rojas-Corrales et al., 2003).

Mannich bases are natural or synthetic compounds generally formed by the condensation of formaldehyde with ammonia or an amine (primary or secondary) and a compound containing, at least, a reactive hydrogen atom (Dimmock and Kumar, 1997). Mannich bases possess a broad spectrum of biological activities, including antibacterial, antihelmintic, antiviral, antiprotozoal, antifungal, cytotoxic and anticancer, anticonvulsant, analgesic and anti-inflammatory activities (Fillion et al., 1991; Erciyas et al., 1994; Dimmock et al., 1998; Pandeya et al., 2000; Gul et al., 2000, 2002; Sridhar and Ramesh, 2001). The biological activities of Mannich bases have been attributed to the α,β-unsaturated ketones liberated from these substances by a deamination process in vivo and under simulated conditions in vitro (Dimmock et al., 1992; Erciyas et al., 1994). However, studies regarding a possible antidepressant effect of Mannich bases, to our knowledge, do not exist.

The primary aim of the present study was to investigate the possible antidepressant-like effect of the 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-hydrazinepyrimidine (CPMPH) Mannich base (Fig. 1) in the forced swimming test (FST), a behavioral test which predicts the efficacy of antidepressant treatments (Porsolt et al., 1977), and the mechanisms underlying its antidepressant-like effect in mice. We also assessed the effect of CPMPH in the acetic acid-induced writhing, a classical chemical model of nociception.

2. Methods

2.1. Animals

Swiss mice of either sex homogeneously distributed among groups, weighing 30–40 g were maintained at 22–27 °C with free access to water and food, under a 12:12 h light:dark cycle (light on at 7:00 h). The animals were bred in the Animal Facilities of the Federal University of Santa Catarina and were maintained according to the Animal Care Guidelines from the National Institute of Health of the United States of America. All manipulations were carried out between 9:00 and 17:00, with each animal used only once. The experiments were performed after approval of the protocol by the Ethics Committee of the Institution and all efforts were made to minimize animal suffering.

2.2. Drugs and treatment

The following drugs were used in the study: L-arginine, caffeine, methysergide, naloxone, sulpiride, yohimbine (Sigma Chemical Company, St. Louis, MO, USA). The 4-(4′-chlorophenyl)-6-(4″-methylphenyl)-2-hydrazinepyrimidine (CPMPH) Mannich base (Fig. 1) used in the present study was synthesized in our laboratory according to the methodology previously described (Pandeya et al., 1999). All drugs were dissolved in saline, except sulpiride (dissolved in saline with 5% DMSO) and CPMPH (dissolved in saline with 1% Tween 80) and administered by intraperitoneal (i.p.) or s.c. route 30 min before acetic acid-induced writhing in a constant volume of 10 ml/kg body weight. CPMPH or vehicle was administered i.p. 30 min before the FST, acetic acid-induced writhing or open-field test. Appropriate vehicle-treated groups were also assessed simultaneously.

2.3. Forced swimming test (FST)

Mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1 °C; the total duration of immobility during a 6-min test was scored as described previously (Zomkowski et al., 2002; Rosa et al., 2003). Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

To assess the possible involvement of the serotonergic system in the anti-immobility effect of the CPMPH, animals were pre-treated with methysergide (2 mg/kg, i.p., a non-selective serotonin receptor antagonist) or vehicle and 30 min later, they received CPMPH (1 mg/kg, i.p.) or a vehicle injection and were tested in the FST.

We also investigated the possible participation of the noradrenergic and dopaminergic systems in the antidepressant-like effect of CPMPH in the FST. Animals were pre-treated with yohimbine (1 mg/kg, i.p., an α_2-adrenoceptor antagonist), sulpiride (32 mg/kg, i.p., a selective D_2 receptor antagonist) or with vehicle and after 30 min they received CPMPH (1 mg/kg, i.p.) or a vehicle injection before being tested in the FST.

We also investigated the possible participation of the L-arginine–nitric oxide pathway and opioid system in the anti-immobility effects of the CPMPH. To this end, animals were pre-treated with L-arginine (750 mg/kg, i.p., a precursor of nitric oxide, a dose that produced no effect in the FST) or naloxone (1 mg/kg, i.p., a non-selective opioid receptor

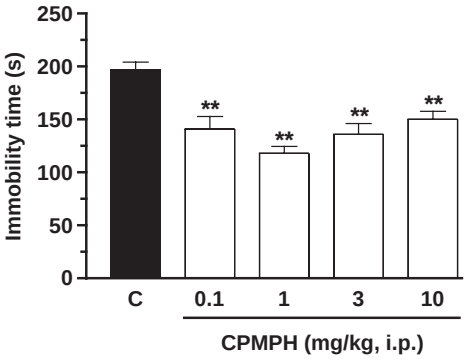

Fig. 2. Effect of acute administration of CPMPH on the forced swimming test (FST) in mice. CPMPH was administered 30 min before the test. Values are expressed as mean ± S.E.M. ($n = 8$), $^{**}p < 0.01$ compared with vehicle treated group (control; C).

antagonist) and, after 30 min, they received CPMPH (1 mg/kg, i.p.) or a vehicle injection before being tested in the FST.

2.4. Open-field behavior

The ambulatory behavior was assessed in an open-field test as described previously (Rodrigues et al., 1996). The apparatus consisted of a wooden box measuring 40 × 60 × 50 cm. The floor of the arena was divided into 12 equal squares. The number of squares crossed with all paws (crossing) was counted in a 6-min session. Mice were treated with CPMPH (0.1–100 mg/kg, i.p.), caffeine (15 mg/kg, i.p., positive control) or with vehicle 30 min beforehand.

2.5. Abdominal constriction response caused by i.p. injection of acetic acid

The abdominal constrictions resulting from i.p. injection of acetic acid (0.6%), consisting of a contraction of the abdominal muscle together with a stretching of the hind limbs, were induced according to procedures described previously (Santos et al., 1999). Mice were pre-treated with CPMPH by i.p. (10–100 mg/kg) or s.c. (100 mg/kg) routes, 30 min prior to irritant injection. Control animals received a similar volume of vehicle. After the challenge, mice were individually placed into glass cylinders of 20 cm diameter, and the abdominal constrictions were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions, i.e. the difference between control animals (mice pre-treated with vehicle) and animals pre-treated with CPMPH.

2.6. Statistical analysis

The obtained data were presented as means ± S.E.M. and evaluated by one-way or two-way analysis of variance (ANOVA) followed by Duncan's test or unpaired t test when appropriate. A value of $p < 0.05$ was considered to be significant. The ID_{50} values (i.e., the dose of CPMPH reducing the nociceptive response by 50% relative to the control value) were reported as geometric means accompanied by their respective 95% confidence limits. They were determined by linear regression from individual experiments using linear regression GraphPad software (GraphPad software, San Diego, CA, USA).

3. Results

The effect of CPMPH in the FST is shown in Fig. 2. The one-way ANOVA revealed an overall significant effect on the time spent immobile [$F(4,35) = 11.04$, $p < 0.01$] and pairwise comparisons to the control group indicated that all the tested doses of CPMPH (0.1–10 mg/kg) significantly decreased the immobility time. In addition, one-way ANOVA revealed that CPMPH caused a significant [$F(6,40) = 6.39$, $p < 0.01$] change in ambulation scores in the open-field, and pairwise comparisons to vehicle controls showed that this was due to a reduction of the number of squares crossed by the 30 and 100 mg/kg doses (Table 1). As a positive control of a psychostimulant effect, caffeine administered at a dose which produced an anti-immobility effect in the FST (15 mg/kg, i.p.) caused an increase in the locomotor activity as compared to the control group (crossings: 121.2 ± 9.9 and 84.6 ± 5.2, respectively; [$F(1,8) = 10.63$, $p < 0.05$]).

With the purpose of investigating the mechanisms underlying the antidepressant-like effect of CPMPH in the FST, mice were pre-treated by i.p. route with methysergide (2 mg/kg), sulpiride (32 mg/kg), yohimbine (1 mg/kg), naloxone (1 mg/kg) or L-arginine (750 mg/kg). Fig. 3A shows the results of pre-treatment with methysergide on the antidepressant-like effect of CPMPH. The two-way ANOVA revealed a main effect of pre-treatment [$F(1,35) = 23.86$, $p < 0.01$], treatment [$F(1,35) = 6.56$, $p < 0.05$] and of pre-treatment × treatment interaction [$F(1,35) = 7.67$, $p < 0.01$]. Fig. 3B shows the influence of pre-treatment of mice with sulpiride on the antidepressant-like effect of CPMPH. The two-way ANOVA revealed a main effect of pre-treatment [$F(1,32) = 32.91$, $p < 0.01$], treatment [$F(1,32) = 5.13$, $p < 0.05$] and of pre-treatment × treatment interaction [$F(1,32) = 25.86$, $p < 0.01$]. Fig. 3C shows the results of pre-

Table 1
Effect of CPMPH in the locomotor activity in the open-field test

Groups	Number of crossings (mean ± S.E.M.)
Vehicle (control)	82.2 ± 3.4
CPMPH 0.1 mg/kg, i.p.	85.4 ± 8.9
CPMPH 1 mg/kg, i.p.	75.0 ± 6.1
CPMPH 3 mg/kg, i.p.	79.2 ± 10.6
CPMPH 10 mg/kg, i.p.	92.2 ± 2.8
CPMPH 30 mg/kg, i.p.	62.8 ± 4.6*
CPMPH 100 mg/kg, i.p.	57.9 ± 3.2**

CPMPH was administered at doses range 0.1–100 mg/kg, i.p. 30 min before the open-field test. Values are expressed as mean ± S.E.M. ($n = 6–8$), $^{*}p < 0.05$, $^{**}p < 0.01$ compared with vehicle treated group (control).

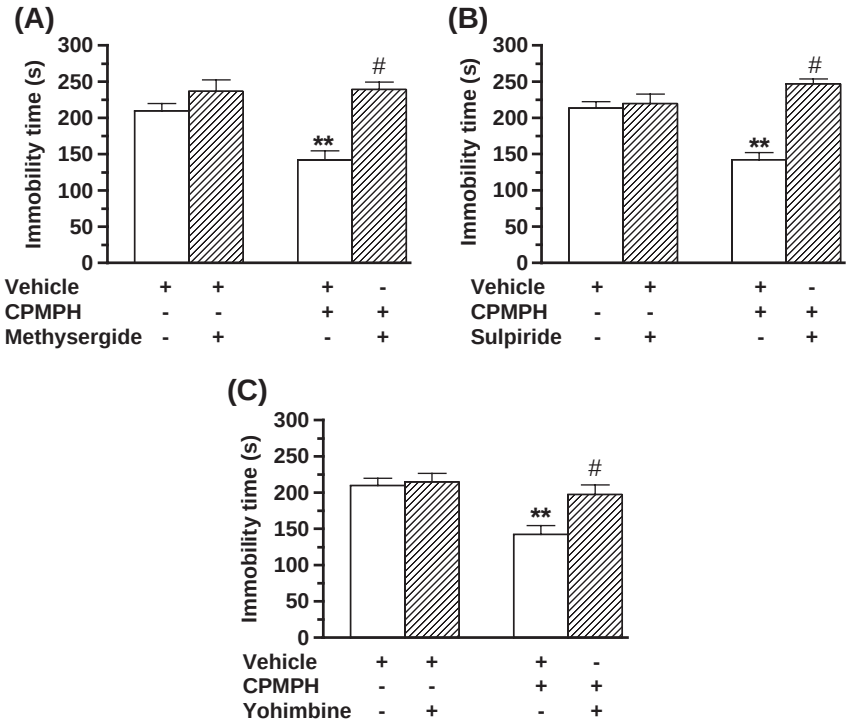

Fig. 3. Effect of pre-treatment of animals with (A) methysergide (2 mg/kg, i.p.), (B) sulpiride (32 mg/kg, i.p.) and (C) yohimbine (1 mg/kg, i.p.) on the CPMPH (1 mg/kg, i.p.)-induced reduction in immobility time in the FST in mice. Values are expressed as mean±S.E.M. ($n=8-11$), **$p<0.01$ compared with vehicle treated group (control) and $^{\#}p<0.01$ compared with CPMPH group pre-treated with vehicle.

treatment with yohimbine on the antidepressant-like effect of CPMPH. The two-way ANOVA revealed a main effect of pre-treatment [$F(1,33)=6.44$, $p<0.05$], treatment [$F(1,33)=12.63$, $p<0.01$] and of pre-treatment × treatment treatment interaction [$F(1,33)=4.38$, $p<0.05$]. Post hoc analyses indicated that the antidepressant-like effect of CPMPH was prevented by pre-treatment of animals with methysergide, sulpiride, or yohimbine. Fig. 4A shows the results of pre-treatment with naloxone on the antidepressant-like effect of CPMPH. The two-way ANOVA revealed a main effect of treatment [$F(1,34)=19.13$, $p<0.01$], but not of pre-treatment [$F(1,34)=0.70$, $p=0.41$ and of pre-treatment × treatment interaction [$F(1,34)=2.44$, $p=0.13$]. Fig. 4B shows the results of pre-treatment with L-arginine on the antidepressant-like effect of CPMPH. The two-way ANOVA revealed a main effect of treatment [$F(1,33)=29.46$, $p<0.01$], but not of pre-treatment [$F(1,33)=0.015$, $p=0.90$] and of pre-treatment × treatment interaction [$F(1,33)=0.12$, $p=0.73$]. Post hoc analyses indicated that the antidepressant-like effect of CPMPH was not prevented by pre-treatment of animals with naloxone and L-arginine.

The results depicted in Fig. 5 show that CPMPH (10–100 mg/kg, i.p.) produced dose-related inhibition of the acetic acid-induced abdominal constrictions in mice [$F(3,25)=102.80$, $p<0.01$], with an ID_{50} value (and its 95% confidence limits) of 13.2 (7.1–24.8) mg/kg and inhibition of 100%. Furthermore, when administered s.c. CPMPH (100 mg/kg) also induced a significant reduction of

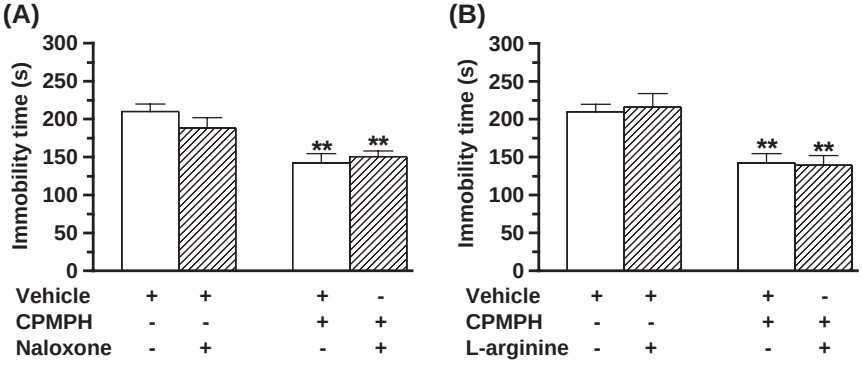

Fig. 4. Effect of pre-treatment of animals with (A) naloxone (1 mg/kg, i.p.) and (B) L-arginine (750 mg/kg, i.p.) on the CPMPH (1 mg/kg, i.p.)-induced reduction in immobility time in the FST in mice. Values are expressed as mean±S.E.M. ($n=7-11$), **$p<0.01$ compared with vehicle treated group (control).

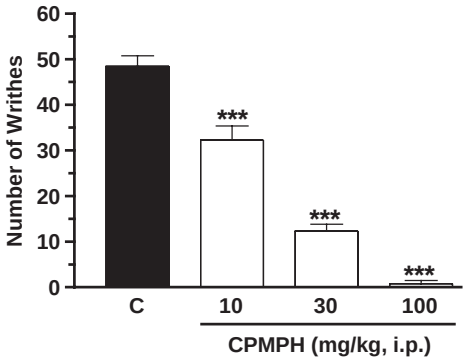

Fig. 5. Effect of CPMPH against acetic acid-induced writhing in mice. Values are expressed as mean ± S.E.M. ($n = 7–8$), **$p < 0.01$ compared with vehicle treated group (control; C).

the number of abdominal constrictions (controls: 43.5 ± 3.4; CPMPH: 17.5 ± 2.1, $t = 10.408$, $p < 0.01$).

4. Discussion

In the present study, we demonstrate that CPMPH, given systemically (i.p. route), is effective in producing antidepressant-like effects when assessed in the FST, as is evident from the significant reduction in the immobility time elicited by this compound. The FST is the most widely used model to screen new antidepressant drugs, a result of its ease of use, reliability and specificity, besides the fact that it is sensitive to acute antidepressant treatments (Porsolt et al., 1977; Cryan et al., 2002). A wide variety of antidepressants, and compounds with potential antidepressant-like activity reduce the duration of immobility in the FST (Porsolt et al., 1977; Da Silva et al., 2000; Zomkowski et al., 2002; Cryan et al., 2002; Rosa et al., 2003).

The antidepressant-like effect of CPMPH is not due to a psychostimulant action of this base, as no alteration in locomotor activity was found in the open-field test. This shows that increased motor activity is not involved in the action observed in the FST, indicating that the antidepressant-like effect of this compound is specific.

The mechanisms underlying the antidepressant-like effect of CPMPH were investigated in the present study. To this end, the possible involvement of serotonergic, dopaminergic, noradrenergic and opioid systems, as well as the L-arginine–nitric oxide pathway in the action of CPMPH in the FST was investigated. The main finding of this study is that the CPMPH elicits an antidepressant-like effect in the FST through its interaction with serotonin, dopamine and noradrenaline neurotransmission. Indeed, the monoamines are believed to be involved in the pathogenesis of depression. Thus, it has been demonstrated that all antidepressants in clinical use acutely increase the availability of these amines at the synapse, which may induce long-term adaptive changes via the modulation of the activity of these and maybe other neurotransmitter systems (Elhwuegi, 2004).

The hypothesis of the involvement of serotonergic neurotransmission in the pathophysiology of depression is supported by a multitude of studies that have demonstrated that measures of 5-HT function, including basal cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) levels, plasma tryptophan levels, blood platelet 5-HT function, and hormonal response to 5-HT specific neuroendocrine challenge agents, are reduced in depressed patients (Owens and Nemeroff, 1994; Meltzer et al., 1997). Moreover, pharmacological agents that facilitate 5-HT neurotransmission including precursors of 5-HT (L-tryptophan and/or 5-hydroxytryptophan), non-selective serotonin agonists, monoamine oxidase inhibitors and 5-HT reuptake inhibitors, elevate mood in depressed patients (Maes et al., 1995). In the present study, the pre-treatment with methysergide, a non-selective serotonin receptor antagonist, at a dose that produced no effect in the FST, reversed the CPMPH-induced antidepressant-like effect in the FST. This result suggests that CPMPH interacts with serotonin receptors and/or increases the serotonin availability in the synaptic cleft.

Our results also showed that the D_2 receptor antagonist sulpiride (at a dose that produced no effect in the FST) blocked the anti-immobility effect of CPMPH in the FST. This result is in line with the proposed role for dopamine in depressive symptoms and in the antidepressant effect of drugs (Willner, 1995). Results from several animal models of depression are consistent with dopamine-deficiency in depression (Parsey et al., 2001). It has been demonstrated that chronic treatment with antidepressants produced sensitization of behavioral responses to D_2 receptor agonists (Elhwuegi, 2004). Furthermore, it has been shown that sulpiride may induce a short-term suppression of serotonin release (Nakazato et al., 1998), which could account, at least in part, for the blockade of the CPMPH antidepressant-like effect in the FST by pre-treatment with sulpiride.

Moreover, yohimbine, an α_2-adrenoceptor antagonist, significantly prevented the CPMPH-induced antidepressant-like effect in the FST. This result suggests that CPMPH interacts with α_2-adrenoceptors to produce an antidepressant-like effect in the FST. The α_2-adrenoceptors are supposed to play a role in depressive illness. It is well established that some antidepressant drugs increase the synaptic concentration of noradrenaline (NA) and some of these drugs were found to act directly at noradrenergic receptors. The release of noradrenaline from noradrenergic neurons is under the negative control of α_2-adrenergic autoreceptors and the activation of α_2-adrenergic heteroreceptors on 5-HT neurons suppresses 5-HT release from serotonergic nerve terminal (Elhwuegi, 2004). Furthermore, presynaptic α_2-adrenoceptor function is decreased after repeated antidepressant drug treatment (Elhwuegi, 2004). It was recently demonstrated that clonidine, an α_2-adrenoceptor agonist, reduced the immobility time of mice in the FST by an α_2-adrenoceptor-mediated mechanism (O'Neill et al., 2001).

Our results showed that the activation of either the opioid system or the L-arginine–nitric oxide pathway seems unlikely to be involved in the antidepressant-like effect of CPMPH. This view derives from the fact that treatment of animals with naloxone, a non-selective opioid receptor antagonist, or L-arginine, a precursor of nitric oxide, at doses that are inactive on their own in the FST, had no effect on the CPMPH antidepressant-like action in the FST. A previous study by our group showed that the precursor of NO, L-arginine, and the inhibitor of nitric oxide synthase, L-NNA, depending on their dose, produced antidepressant-like effects in the FST (Da Silva et al., 2000).

Antidepressant drugs are reported to be used as analgesics in the clinical management of migraine and neuropathic pain (Singh et al., 2001; Millan, 2002). Thus, in the present study, we also carried out a preliminary investigation of the ability of CPMPH to produce anti-nociception in a classical model of nociception in mice, the acetic acid-induced writhing. The results showed that intraperitoneal administration of CPMPH elicited a marked and dose-dependent reduction of abdominal constrictions at high doses that otherwise produced a significant reduction in the locomotor activity in an open-field. On subcutaneous injection, CPMPH also produced significant reduction of acetic acid-induced writhings, though with a lower efficacy. Considering the present data, we can speculate that CPMPH may elicit a non-specific antinociceptive effect due to different factors [e.g. administration route (i.p. versus s.c.), metabolism of first-pass (metabolite of the CPMPH), absorption surface, or influence of the pH (direct interaction of the CPMPH with the acetic acid)] that may alter its bioavailability. Thus, the reduction of the abdominal constrictions produced by high doses of CPMPH (or its metabolites) may be confused with its locomotor altering effect.

In summary, results from the present study show, for the first time, that CPMPH exerts an antidepressant-like effect in the FST and also produces a non-specific (at high doses) antinociception in the acetic acid-induced abdominal constriction response in mice. The results also demonstrated that the antidepressant-like effect of CPMPH is mediated, at least in part, by an interaction with serotonergic, dopaminergic and noradrenergic (via α_2-adrenoceptors) systems. However, the opioid system and the L-arginine–nitric oxide pathway are unlikely to participate in the antidepressant-like effect of CPMPH. Finally, on the basis of the present results it may be speculated that CPMPH or its derivatives might be of interest as an antidepressant agent, with a mechanism of action deserving further study.

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