

Effects of Agmatine on Depressive-Like Behavior Induced by Intracerebroventricular Administration of 1-Methyl-4-phenylpyridinium (MPP⁺)

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Abstract Considering that depression is a common non-motor comorbidity of Parkinson's disease and that agmatine is an endogenous neuromodulator that emerges as a potential agent to manage diverse central nervous system disorders, this study investigated the antidepressant-like effect of agmatine in mice intracerebroventricularly (i.c.v.) injected with the dopaminergic neurotoxin 1-methyl-4-phenylpyridinium (MPP⁺). Male C57BL6 mice were treated with agmatine (0.0001, 0.1 or 1 mg/kg) and 60 min later the animals received an i.c.v. injection of MPP⁺ (1.8 µg/site). Twenty-four hours after MPP⁺ administration, immobility time, anhedonic behavior, and locomotor activity were evaluated in the tail suspension test (TST), splash test, and open field test, respectively. Using Western blot analysis, we investigated the putative modulation of MPP⁺ and agmatine on striatal and frontal cortex levels of tyrosine hydroxylase (TH) and brain-derived neurotrophic factor (BDNF). MPP⁺ increased the immobility time of mice in the TST, as well as induced an anhedonic-like behavior in the splash test, effects which were prevented by pre-treatment with agmatine at the three tested doses. Neither drug, alone or in combination, altered the locomotor activity of mice. I.c.v. administration of MPP⁺ increased the striatal immunocontent of TH, an effect prevented by the three tested doses of agmatine. MPP⁺ and

agmatine did not alter the immunocontent of BDNF in striatum and frontal cortex. These results demonstrate for the first time the antidepressant-like effects of agmatine in an animal model of depressive-like behavior induced by the dopaminergic neurotoxin MPP⁺.

Keywords Parkinson's disease · Agmatine · Antidepressant · Depression · MPP⁺

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease affecting about 1 % of the population after the age of 65 years (Mayeux 2003). Typically, PD is considered a motor disorder, with resting tremor, bradykinesia, muscle rigidity, and postural instability as main clinical features (Fahn 2003). Despite PD is classically diagnosed based on the motor symptomatology, it is now appreciated that most people with PD experience non-motor symptoms, sometimes even many years before the onset of motor signs. Studies have shown that non-motor manifestations of PD, such as constipation, sleep disorder, olfactory impairment, neuropsychiatric, and cognitive deficits may occur in up to 80 % of PD patients and they are not ameliorated by the current anti-parkinsonian pharmacotherapy (Bodis-Wollner 2003; Simuni and Sethi 2008; Shulman et al. 2001).

Among the non-motor manifestations of PD, depression presents the highest prevalence, with estimated rate from 2.7 to 68.1 % (Hantz et al. 1994; Martinez-Martin et al. 2015; Slaughter et al. 2001; Zach et al. 2004). The presence of depressive symptoms in PD patients increases direct and indirect costs of treatment (Nilsson et al. 2002) and contributes to reduce patient's health-related quality of life

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(Adler 2005). Although the etiology and pathophysiology of PD-associated depression is not entirely understood, studies suggest that reduced extracellular concentrations of serotonin or serotonergic receptor dysfunction are associated with the development of depression in PD (Ballanger et al. 2012; Paulus and Jellinger 1991). Importantly, serotonin is produced by raphe nuclei, which has an early involvement in the neurodegenerative process underlying PD according to Braak's hypothesis (Braak et al. 2004). Degeneration of dopaminergic pathways probably also contributes to depressive symptoms in PD (Brown and Gershon 1993). Especially anhedonia, a common symptom of depression, is supposed to be a consequence of altered dopaminergic reward mechanisms, which in turn, are associated with degeneration of the ventral tegmental area and limbic forecasts (Heinz et al. 1994). Importantly, neurodegeneration of the nigrostriatal dopaminergic pathway and depletion of dopamine are usually assessed by measuring striatal content of tyrosine hydroxylase (TH), the rate-limiting enzyme of dopamine synthesis (Lee et al. 2013; Kozina et al. 2014).

1-Methyl-4-phenylpyridinium (MPP⁺), the active metabolite of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is a parkinsonian mimetic compound which selectively destroys dopaminergic neurons in the substantia nigra (SN) (Javitch et al. 1985). The mechanisms involved in toxicity of MPTP and MPP⁺ include glutamatergic excitotoxicity, mitochondrial dysfunction, peroxynitrite production, oxidative stress, and inflammation, events which lead to neuronal and glial cell damage (for review see Yokoyama et al. 2008).

It is well recognized that administration of MPTP or MPP⁺ by different routes elicits an increased immobility time of rats and mice in the forced swimming test (FST) and tail suspension test (TST) (Castro et al. 2013; Moreira et al. 2010; Santiago et al. 2010; Vuckovic et al. 2008), two widely accepted predictive models of depression in rodents. Furthermore, systemic administration of MPTP in rats decreased the preference for the sucrose over water, an indicator of anhedonia (Kryzhanovskii et al. 1995). Dopaminergic degeneration induced by MPTP in rats is also associated with lack of trophic support in neurons or brain areas implicated in the pathophysiology of PD (Rekha et al. 2013; Sathiyaraj et al. 2013). Reinforcing these results, decreased MPP⁺-induced toxicity in cultured hippocampal and mesencephalic slices were associated with increased brain-derived neurotrophic factor (BDNF) immunoccontent and the activation of BDNF-dependent signaling pathways (Jourdi et al. 2009). Outstandingly, PD patients present a reduction of BDNF serum levels, an effect exacerbated by depression, suggesting a common role for this neurotrophin in both PD and depression (Ricci et al. 2010).

Since depression in PD patients is highly prevalent and the conventional pharmacotherapy is unsatisfactory to manage this medical condition, the research of new drugs or alternative therapies could help improve PD-associated symptoms. Agmatine, a cationic polyamine synthesized from L-arginine, is an endogenous neuromodulator that emerges as a promising agent to manage diverse central nervous system disorders, including PD and depression (for review see Moretti et al. 2014). It was shown that agmatine promotes beneficial effects against MPTP-induced dopaminergic neurotoxicity in mice (Gilad et al. 2005; Matheus et al. 2012), suggesting a neuroprotective effect of this compound. Corroborating this finding, agmatine also prevented cognitive and motor impairments induced by intranasal administration of MPTP in mice (Matheus et al. 2012). It was also demonstrated that agmatine produces an antidepressant-like effect in the mouse FST and TST by modulating L-arginine–nitric oxide pathway, K⁺ channels, α_2 -adrenoceptors, serotonergic system, and NMDA receptors (Budni et al. 2007; Zomkowski et al. 2002, 2004).

Considering the substantial amount of literature data showing positive effects of agmatine as a neuromodulator and neuroprotective agent, this study aimed to investigate the acute antidepressant-like effects of this polyamine in mice i.c.v. injected with the neurotoxin MPP⁺.

Materials and Methods

Animals

This study was performed using adult male C57BL6 mice (3 month, 30–35 g) provided by the animal facility of the Universidade Federal de Santa Catarina (Florianópolis, Brazil). The animals were maintained at 20–22 °C with free access to water and food, under a 12/12-h light–dark cycle (lights on at 07:00 h). Mice were caged in groups of 15 animals in a 41 × 34 × 16 cm cage. The cages were placed in the experimental room 24 h before the test for acclimatization. All manipulations were carried out between 9:00 and 17:00 h. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Universidade Federal de Santa Catarina (Protocol Number: PP00795). All efforts were made to minimize animal suffering.

Drugs

The following drugs were used: agmatine sulfate salt and MPP⁺ iodide, both obtained from Sigma Chemical Co., St.

Louis, U.S.A. Agmatine and MPP⁺, freshly prepared before administration, were dissolved in distilled water and saline solution (0.9 % NaCl), respectively. Appropriate vehicle-treated groups were also assessed simultaneously.

The primary antibodies anti-TH (sc-25269) and anti-BDNF (sc-20981) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti- β -actin (ab8226) was bought from ABCAM. The secondary antibodies anti-mouse IgG (horseradish peroxidase, #7076) and anti-rabbit IgG (horseradish peroxidase, #7074) were purchased from Cell Signaling Technology (Beverly, MA, USA).

Treatments

Agmatine (0.0001, 0.1 or 1 mg/kg) was administered once orally (p.o.) by gavage in a constant volume of 10 mL/kg body weight. Sixty minutes after administration of agmatine, the animals received an intracerebroventricular (i.c.v.) injection of MPP⁺ (1.8 μ g/site) in a volume of 3 μ l per mouse. Twenty-four hours after administration of MPP⁺, immobility time, anhedonic-like behavior, and locomotor activity were evaluated in two independent cohorts of animals in the TST, splash test, and open field test, respectively. The treatment schedule and the doses of the drugs used in the present study were chosen based on experiments previously performed in our laboratory (Neis et al. 2014).

The i.c.v. injections were performed by employing a “free hand” method under light ether anesthesia according to the procedure previously described (Haley and McCormick 1957; Neis et al. 2014). Briefly, a 0.4-mm external diameter hypodermic needle attached to a 5- μ l Hamilton syringe by polyethylene tubing was inserted perpendicularly through the skull. The drugs were then administered in a volume of 3 μ l into the left lateral ventricle, according to the following coordinates from bregma: AP: -0.6 mm, ML: +1.1 mm, DV: -1.0 mm (Paxinos and Franklin 2004). The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. I.c.v. injections were performed by an experienced investigator, and after dissection of the brain of the animal, the success of the injection was examined, macroscopically, discarding results from mice presenting misplacement of the injection site or any sign of cerebral hemorrhage (<5 %).

Tail Suspension Test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive

tape placed approximately 1 cm from the tip of the tail. Mice were considered immobile only when they hung passively and completely motionless. Immobility time was manually recorded during a 6-min period by an experienced observer. The observer was in the room where experiments were performed and was blind to the animal condition.

Open Field Test

Ten minutes after TST, mice were evaluated in the open field paradigm as previously described (Neis et al. 2014). Animals were individually placed in a wooden box measuring 40 \times 60 \times 50 cm high with the floor of the arena divided into 12 rectangles. The number of rectangles crossed with all paws (crossing) was counted in a 6-min session. The apparatus were cleaned with a solution of 10 % ethanol between tests in order to hide animal clues.

Splash Test

The splash test was carried out with an independent group of animals. This test consists of squirting a 10 % sucrose solution on the dorsal coat of a mouse placed individually in clear Plexiglas boxes (9 \times 7 \times 11 cm) (Moretti et al. 2012). Because of its viscosity, the sucrose solution dirties the mouse fur and animals initiate grooming behavior. After applying sucrose solution, the time to start the first grooming and the total amount of time spent grooming were manually recorded for a period of 5 min as an index of self-care and motivational behavior, considered to be parallel with some symptoms of depression such as apathetic behavior (Willner 2005). The apparatus was cleaned with a solution of 10 % ethanol between tests in order to hide animal clues.

Western Blot Analysis

Immediately after behavioral evaluation, the animals were killed by decapitation and the striata and frontal cortices were removed, placed in liquid nitrogen and stored at -80 °C until used for biochemical evaluation.

Briefly, samples were mechanically homogenized in 300 μ l of TRIS 50 mM pH 7.0, EDTA 1 mM, NaF 100 mM, PMSF 0.1 mM, Na₃VO₄ 2 mM, Triton X-100 1 %, glycerol 10 %, Sigma Protease Inhibitor Cocktail (P2714), and then incubated for 10 min on ice. Lysates were centrifuged (10,000 \times g for 10 min, at 4 °C) to eliminate cellular debris. The supernatants were diluted 1/1 (v/v) in TRIS 100 mM pH 6.8, EDTA 4 mM, SDS 8 %, and boiled for 5 min. Thereafter, sample dilution (40 % glycerol, TRIS 100 mM, bromophenol blue, pH 6.8) in the ratio 25:100 (v/v) and β -mercaptoethanol (final concentration 8 %) were added to the samples. Protein content

was estimated at 620-nm wavelength using a standard curve with bovine serum albumin as standard (Peterson 1977).

The same amount of protein (40 µg per lane) for each sample was electrophoresed in SDS–PAGE minigels (10 % acrylamide) and transferred to nitrocellulose membranes using a tank transfer system at 100 V and 270 mA for 1 h (Mini-PROTEAN Tetra cell Electrophoresis System, Bio-Rad, Hercules, CA). To verify transfer efficiency process, gels were stained with Coomassie blue and membranes with Ponceau S.

The membranes were blocked with 5 % skim milk in TBS (TRIS 10 mM, NaCl 150 mM, pH 7.5). TH and BDNF were detected using specific antibodies incubated overnight diluted in TBS-T (Tris 10 mM, NaCl 150 mM, 0.1 % Tween-20, pH 7.5) containing 2.5 % BSA in the dilutions 1:5000 and 1:500, respectively. All membranes were incubated with mouse anti-β-actin (1:2000) antibody to verify that equal amounts of proteins were loaded on the gel. Next, the membranes were incubated with peroxidase-linked secondary antibody (1:2500) for 1 h and the reactions developed by chemiluminescence (LumiGLOH, cell signaling, Beverly, MA, USA). All blocking and incubation steps were followed by three washes (5 min) of the membranes with TBS-T. The optical density (O.D.) of the bands was quantified using Scion Image™ (Frederick, MD, USA). The phosphorylation levels of proteins were determined as a ratio of the O.D of the phosphorylated band over the O.D. of the total band and β-actin was used as a loading control. Data were expressed as percentage of the control (considered as 100 %).

Statistical Analysis

The Kolmogorov–Smirnov test was used to evaluate the normality assumption of behavioral and biochemical data. All variables in the present study showed a normal distribution. Comparisons between experimental and control groups were performed by two-way analysis of variance (ANOVA) with pre-treatment and treatment as independent variables followed by Duncan's multiple range post hoc test. All experimental results are given as the mean + S.E.M. A value of $p < 0.05$ was considered to be significant.

Results

Tail Suspension Test

Figure 1a shows the influence of pre-treatment of mice with agmatine on depressive-like behavior elicited by MPP⁺. Two-way ANOVA revealed significant effects for

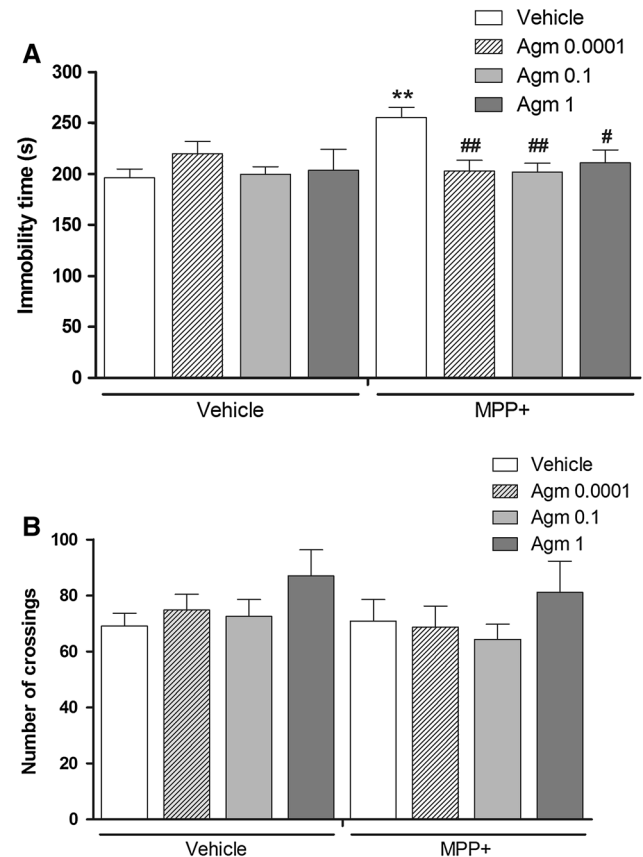


Fig. 1 Effects of treatment with MPP⁺ and/or agmatine on immobility time in the TST (*panel A*) and on locomotor activity in the open field test (*panel B*). Bars represent mean ± SEM of 7–9 mice. ** $p < 0.01$ versus control mice, ## $p < 0.01$ versus MPP⁺ + vehicle group, # $p < 0.05$ versus MPP⁺ + vehicle group, according to two-way ANOVA followed by Duncan's post hoc test

the interaction factor between pre-treatment vs. treatment [$F(3, 53) = 3.92$, $p < 0.05$], but not for agmatine pre-treatment [$F(3, 53) = 1.72$, $p > 0.05$] or MPP⁺ treatment [$F(1, 53) = 2.47$, $p > 0.05$]. Post hoc analysis indicated that the increase in the immobility time produced by MPP⁺ was prevented by agmatine treatment at doses of 0.0001, 0.1, and 1 mg/kg. Administration of agmatine had no per se effect on immobility time of mice treated with saline.

Open Field Test

Treatment with agmatine (0.0001, 0.1, and 1 mg/kg) and MPP⁺, alone or in combination, did not modify the locomotor activity in the open field (Fig. 1b). Two-way ANOVA revealed no significant effects for agmatine pre-treatment [$F(3, 53) = 1.88$, $p > 0.05$], MPP⁺ treatment [$F(1, 53) = 0.82$, $p > 0.05$], or their interaction [$F(3, 53) = 1.19$, $p > 0.05$] in the number of squares crossed in the open field test.

Splash Test

Regarding the time to start the first grooming, two-way ANOVA revealed no significant effects for agmatine pre-treatment [$F(3, 53) = 0.37, p > 0.05$], MPP⁺ treatment [$F(1, 53) = 0.97, p > 0.05$], or their interaction [$F(3, 53) = 1.05, p > 0.05$] (Fig. 2a).

Regarding the total time spent grooming, two-way ANOVA indicated significant effects for agmatine pre-treatment [$F(3, 53) = 3.44, p < 0.05$] and for the interaction between pre-treatment versus treatment [$F(3, 53) = 4.77, p < 0.01$], but not for MPP⁺ treatment [$F(1, 53) = 0.02, p > 0.05$]. As illustrated in Fig. 2b, post hoc analyses indicated that MPP⁺ significantly decreased the time spent grooming in mice treated with vehicle, an effect that was abolished by pre-treatment with agmatine at 0.0001, 0.1, and 1 mg/kg.

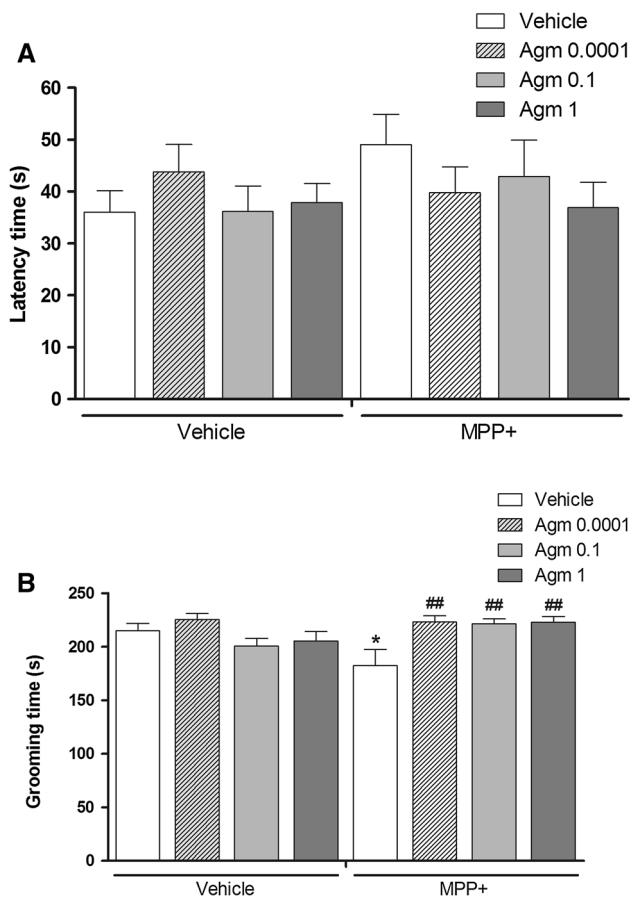


Fig. 2 Effects of treatment with MPP⁺ and/or agmatine on time to start the first grooming (*panel A*) and total time spent grooming (*panel B*) in the splash test. Bars represent mean \pm SEM of 7–9 mice. * $p < 0.05$ versus control mice, ## $p < 0.01$ versus MPP⁺ + vehicle group, according to two-way ANOVA followed by Duncan's post hoc test

Western Blot

Figure 3 shows a representative Western blot of the effect of treatment with MPP⁺ and agmatine on striatal TH immunocontent. Two-way ANOVA revealed significant effects of agmatine pre-treatment [$F(3, 24) = 3.85, p < 0.05$], MPP⁺ treatment [$F(1, 24) = 13.22, p < 0.1$], and for their interaction [$F(3, 24) = 3.03, p < 0.05$]. Post hoc analysis indicated that MPP⁺ administration significantly increased striatal TH immunocontent, an effect prevented by pre-treatment with agmatine at 0.0001, 0.1, and 1 mg/kg.

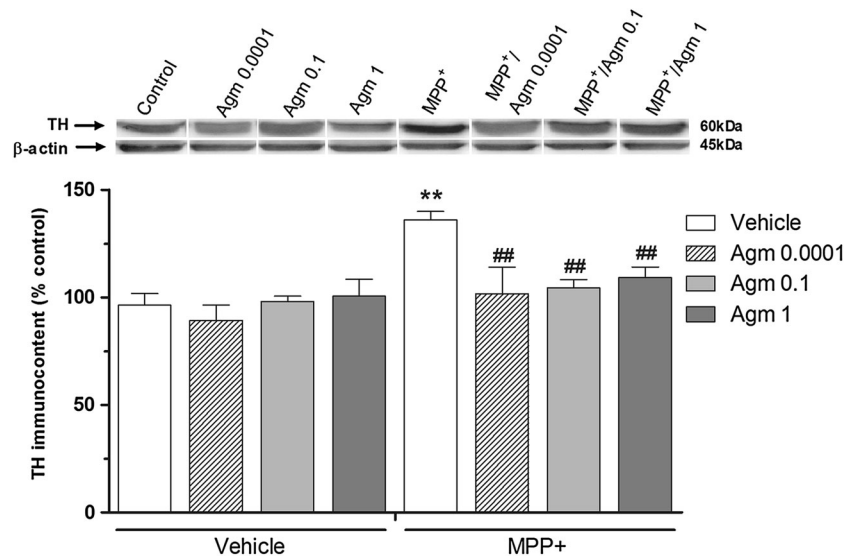
Figure 4 shows the effects of treatment of mice with MPP⁺ and agmatine on striatal and cortical BDNF immunocontent. No significant differences were observed in the experimental groups in the BDNF immunocontent in the striatum (Fig. 4a) or in the frontal cortex (Fig. 4b). For the striatum, two-way ANOVA showed no significant effects for agmatine pre-treatment [$F(3, 20) = 0.18, p > 0.05$], MPP⁺ treatment [$F(1, 20) = 0.07, p > 0.05$], and their interaction [$F(3, 20) = 0.13, p > 0.05$]. For frontal cortex, two-way ANOVA showed no significant effects for agmatine pre-treatment [$F(3, 16) = 0.80, p > 0.05$], MPP⁺ treatment [$F(1, 16) = 0.16, p > 0.05$], and their interaction [$F(3, 16) = 2.27, p > 0.05$].

Discussion

The main findings of this study include (i) the acute administration of different doses of agmatine prevented the depressive-like and anhedonic-like behaviors induced by MPP⁺ in mice; (ii) i.c.v. administration of MPP⁺ increased striatal immunocontent of TH, an effect prevented by pre-treatment with agmatine at 0.0001, 0.1, and 1 mg/kg; and (iii) no significant differences were observed in striatal or frontal cortex BDNF immunocontent in animals treated with MPP⁺ and/or agmatine.

An important aspect to be considered is that neither MPP⁺ nor agmatine affected the spontaneous locomotion of mice in the open field test, ruling out the possibility that an alteration in motor function might be responsible for the increased immobility time elicited by MPP⁺ or the anti-immobility effect of agmatine in the TST. It is well known that experimental administration of dopaminergic neurotoxins, including MPTP, 6-hydroxydopamine, and rotenone, induces some motor alterations due to massive dopaminergic cell loss (Bassani et al. 2014; Kumari et al. 2015; Matheus et al. 2012). Notably, these motor changes are only observed at high doses or after later periods of such toxin administration, consistent with a prominent reduction of striatal dopamine levels and TH protein deficiency (Patil et al. 2014).

Fig. 3 Effects of treatment with MPP⁺ and/or agmatine on striatal TH immunocontent. Results are expressed as % of control and bars represent mean \pm SEM of 3–5 mice. ** $p < 0.01$ versus control mice, ## $p < 0.01$ versus MPP⁺ + vehicle group, according to two-way ANOVA followed by Duncan's post hoc test



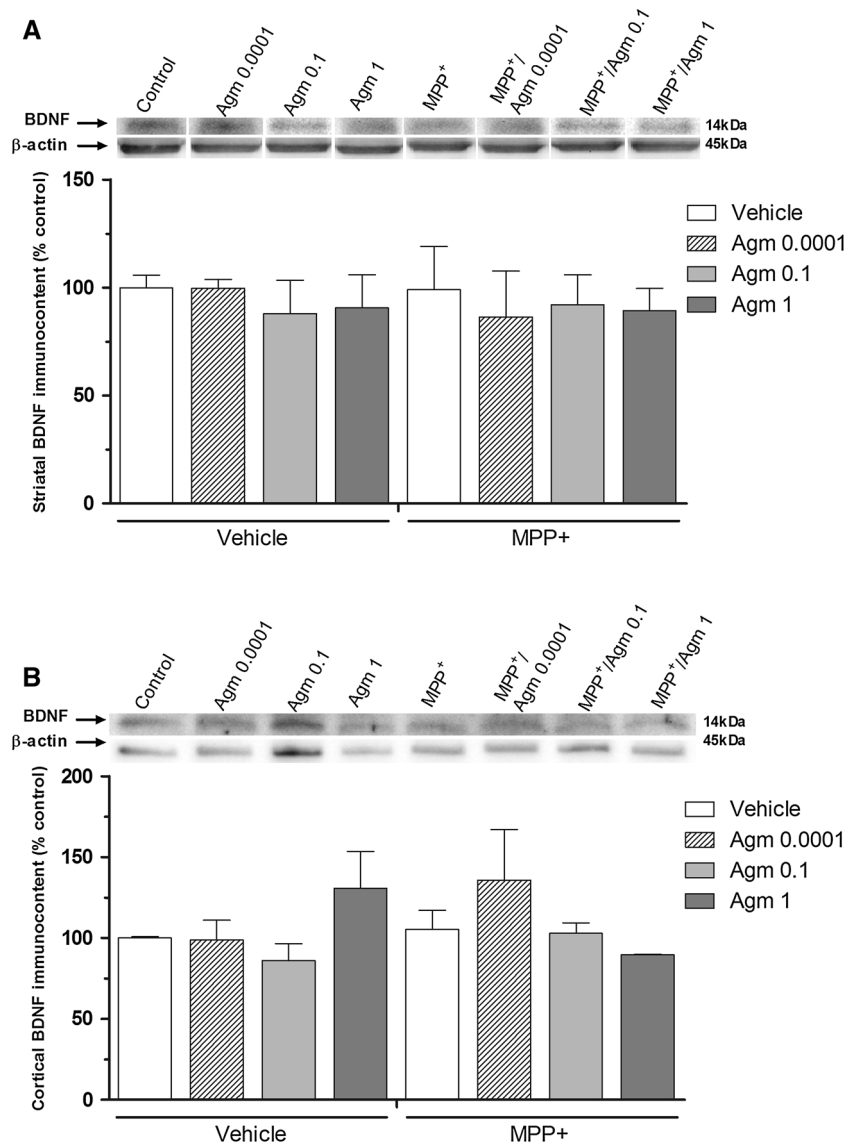
Some studies have demonstrated that before showing significant motor deficits, rats administered with MPTP present cognitive and emotional deficits (Castro et al. 2013; Moreira et al. 2010; Santiago et al. 2010). The results presented herein show that, 24 h after its central administration, MPP⁺ induces an increased immobility time in the TST, as well as an anhedonic-like behavior in the splash test. Previous studies showed that mice evaluated 1-day post-MPTP administration presented altered striatal interleukin-6 and interleukin-1 β levels (Kaku et al. 1999; Shen et al. 2005), as well as decreased striatal glutathione and dehydroascorbic acid/ascorbic acid ratio (an index of ascorbic acid oxidative status and ROS formation) (Serra et al. 2002). In addition, at the same time point (1 day after MPTP discontinuance), elevated glutamate levels in striatum of mice was reported (Serra et al. 2002). Considering that several lines of evidence support the association between depression and glutamatergic excitotoxicity, inflammation, and oxidative stress, it is plausible to suggest that the depressive phenotype observed in the present work may be related with the reported short-term biochemical modifications induced by MPP⁺.

A relevant finding of the present study is that agmatine (0.0001, 0.1, and 1 mg/kg), administered only once by oral route, was able to prevent the increased immobility time elicited by MPP⁺ in the TST. Moreover, agmatine pretreatment completely abolished the anhedonic-like effect induced by MPP⁺ in the splash test. Corroborating our findings, different research groups have consistently reported the antidepressant effect of agmatine in both animals and humans (Neis et al. 2015; Shopsin 2013; Taksande et al. 2013; Zomkowski et al. 2002). The antidepressant-like effect of agmatine depends on the antagonism of glutamate NMDA receptors (Zeidan et al.

2007; Zomkowski et al. 2002), inhibition of nitric oxide synthase in the brain (Raasch et al. 2001; Reis and Regunathan 2000), and the blockade of K⁺ channels (Budni et al. 2007). Moreover, agmatine may exert its antidepressant-like effect by modulating serotonergic and noradrenergic neurotransmission (Zomkowski et al. 2002, 2004), protecting against oxidative stress (Freitas et al. 2014b, c) and attenuating inflammatory response (Neis et al. 2014), targets also implicated in PD pathophysiology. Additionally, Freitas et al. (2015) demonstrated recently that agmatine prevents corticosterone-induced depressive-like behavior in mice by improving neuroplasticity markers (BDNF and CREB) and inducing (Erythroid 2-derived)-like 2 (Nrf2), a key regulator of the cellular antioxidant defenses. Furthermore, a recent study also revealed that the repeated treatment with agmatine during 21 days inhibited glycogen synthase kinase-3 β (GSK3 β) and up-regulated BDNF in the hippocampus, most likely through CREB activation (Freitas et al. 2014a), similar to the mechanism of the classical antidepressant fluoxetine (Hui et al. 2014). Interestingly, MPTP-induced apoptosis of dopaminergic neurons involves the activation of GSK-3 β , since it was demonstrated that inhibition of GSK-3 β activity prevented apoptosis of dopaminergic neurons and improved behavioral impairments induced by MPTP (Wang et al. 2007). These results indicate that these pathways may be important targets in the treatment of depression associated with PD and highlight agmatine as an attractive therapeutic strategy for the treatment of this clinical condition.

Our results revealed an elevated striatal immunocontent of TH in mice i.c.v. administered with MPP⁺. These findings contrast with those of previous studies, which usually show that mice subjected to neurotoxin-based models of PD display decreased striatal TH activity (Serra

Fig. 4 Effects of treatment with MPP⁺ and/or agmatine on striatal (*panel A*) and frontal cortex (*panel B*) BDNF immunocontent. Results are expressed as % of control and bars represent mean \pm SEM of 3–4 mice



et al. 2002), immunocontent (Tsou et al. 2015), or number of TH-positive neurons (Chiu et al. 2015; Naskar et al. 2015; Ren et al. 2015). However, the aforementioned studies conducted these evaluations after multiple exposures to neurotoxin (at least 2 consecutive days) or at later periods (mostly 7 days) after neurotoxin administration, which differs from our experimental protocol. Remarkably, a previous work showed that MPP⁺ administration in PC12 cells causes a decrease in dopamine content at higher doses and a transient increase in dopamine levels at a lower concentration. Moreover, the time course of the increase in dopamine content corresponded with that of the increase in TH mRNA expression (Itano et al. 1995). These results suggest that MPP⁺ has a biphasic effects on dopamine and TH contents, being dependent on both concentration and treatment periods.

All doses of agmatine evaluated in the current study prevented the increase in striatal TH immunocontent produced by MPP⁺. These data suggest that agmatine produces protective effects against MPP⁺-induced neuroadaptations in striatum of mice. Indeed, the capacity of agmatine to regulate brain dopaminergic signaling has been reported in several studies. For example, it was demonstrated that agmatine pre-treatment reduced locomotor sensitization and blocked the elevation of dihydroxyphenylacetic acid and homovanillic acid (dopamine metabolites) induced by morphine administration in rats (Wei et al. 2007). Agmatine also prevented the locomotor impairments and the decrease on TH immunoreactivity in the SN of aging mice intranasally treated with MPTP (Matheus et al. 2012). Furthermore, agmatine showed neuroprotective properties in different animal and cellular

models of neuronal damage (Gilad and Gilad 2000; Kim et al. 2006; Lee et al. 2009), strongly supporting the use of this polyamine for central nervous system diseases.

There is strong evidence that modifications in BDNF expression play an important role in both depression (Haile et al. 2014; Lotrich et al. 2013) and motor diseases, including PD (He et al. 2013). Of high importance, a recent study showed that rats subjected to 6-hydroxydopamine-induced model of preclinical stages of PD displayed decreased levels of BDNF and trkB mRNA in the hippocampus and amygdala as well as lowered BDNF mRNA content in the habenula (Berghauzen-Maciejewska et al. 2015). All these impairments likely contribute to the depressive-like behavior observed in these animals (Berghauzen-Maciejewska et al. 2014). The results presented herein indicated no changes in the striatal or cortical levels of BDNF in our experimental groups. These findings conflict with those of previous studies, which showed that neurotoxicity induced by MPTP or MPP⁺ is mediated by reduction in BDNF levels (Jourdi et al. 2009; Patil et al. 2014; Sathiya et al. 2013). The lack of changes in the levels of BDNF after MPP⁺ and/or agmatine treatment is difficult to explain at this moment and can only be a matter of speculations. However, the drugs dose, treatment schedule, as well as the encephalic structures evaluated may be considered when ours and previous results are compared.

Considering the growing number of evidence indicating that agmatine modulates neuroplasticity and cell survival signaling pathways (Freitas et al. 2014a, c), we cannot discard that this polyamine could increase BDNF levels at higher doses or under other experimental conditions. Finally, it is also plausible to suggest that other mechanisms, including inhibition of excitatory glutamatergic transmission, through the blockade of NMDA receptors or improvement of cellular antioxidant defense system could contribute to the protective effects of agmatine against the depressive-like behavior induced by MPP⁺.

Conclusion

Altogether, the results of this study indicate that agmatine protects against the development of depressive- and anhedonic-like behaviors induced by the dopaminergic neurotoxin MPP⁺ in mice. Despite additional studies are required to better elucidate the beneficial effects of agmatine in this model, agmatinergetic system may become a valuable target for the development of new treatments for neurodegeneration-associated depression.

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