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Sub-chronic agmatine treatment modulates hippocampal neuroplasticity and cell survival signaling pathways in mice

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ABSTRACT

Agmatine is an endogenous neuromodulator which, based on animal and human studies, is a putative novel antidepressant drug. In this study, we investigated the ability of sub-chronic (21 days) p.o. agmatine administration to produce an antidepressant-like effect in the tail suspension test and examined the hippocampal cell signaling pathways implicated in such an effect. Agmatine at doses of 0.01 and 0.1 mg/kg (p.o.) produced a significant antidepressant-like effect in the tail suspension test and no effect in the open-field test. Additionally, agmatine (0.001–0.1 mg/kg, p.o.) increased the phosphorylation of protein kinase A substrates (237–258% of control), protein kinase B/Akt (Ser^{473}) (116–127% of control), glycogen synthase kinase-3β (Ser^{9}) (110–113% of control), extracellular signal-regulated kinases 1/2 (119–137% and 121–138% of control, respectively) and cAMP response elements (Ser^{133}) (127–152% of control), and brain-derived-neurotrophic factor (137–175% of control) immunocontent in a dose-dependent manner in the hippocampus. Agmatine (0.001–0.1 mg/kg, p.o.) also reduced the c-jun N-terminal kinase 1/2 phosphorylation (77-71% and 65-51% of control, respectively). Neither protein kinase C nor $p38^{MAPK}$ phosphorylation was altered under any experimental conditions. Taken together, the present study extends the available data on the mechanisms that underlie the antidepressant action of agmatine by showing an antidepressant-like effect following sub-chronic administration. In addition, our results are the first to demonstrate the ability of agmatine to elicit the activation of cellular signaling pathways associated with neuroplasticity/cell survival and the inhibition of signaling pathways associated with cell death in the hippocampus.

1. Introduction

Major depressive disorder (MDD) is a serious public health problem. It is the leading cause of disability in the U.S. for individuals aged 15–44 (WHO, 2008). It is well known that the pathophysiology of MDD involves a monoaminergic dysfunction (Elhwuegi, 2004; Heninger et al., 1996). Although most antidepressant drugs act acutely on the monoaminergic system by increasing its synaptic availability, the clinical effects of the antidepressant drugs are only observed 2–3 weeks after the onset of treatment (Gourion, 2008). This phenomenon is explained by the neurotrophic hypothesis of depression, which proposes that the long-term antidepressant treatment modulates signal transduction survival pathways. This modulation, in turn, induces the expression of neurotrophic factors, primarily the Brain-Derived Neurotrophic Factor (BDNF), promoting neurogenesis and restoring the neural networks altered in depressed subjects (Masi and Brovedani, 2011; Neto et al., 2011). Several kinases such as protein kinase A (PKA), phosphatidylinositol 3kinase (PI3K)-Akt, protein kinase C (PKC), and the extracellular signal-regulated kinases (ERK)/1-2 are able to activate by phosphorylating the transcriptional regulator cyclic-AMP responsive-element binding protein (CREB) (Lonze and

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived-neurotrophic factor; CaMK, Ca^{2+}/calmodulin-dependent protein kinase; CRE, cAMP response elements; CREB, cyclic-AMP responsive-element binding protein; ERK, extracellular signal-regulated kinases; FST, forced swimming test; GSK-3β, glycogen synthase kinase-3β; JNK, c-jun N-terminal kinase; PI3K, phosphatidylinositol 3kinase; MAPK, mitogen-activated protein kinase; MDD, Major depressive disorder; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C; PVDF, polyvinylidene fluoride; ROS, reactive oxygen species; RNS, reactive nitrogen species; TNF-α, tumor necrosis factor-α; TrkB, tropomyosin-related kinase B; TST, tail suspension test.

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Ginty, 2002; Shaywitz and Greenberg, 1999). Once phosphorylated, CREB binds to the BDNF promoter, up-regulates BDNF expression and lifts the depressive mood (Fišar and Hroudová, 2010; Numakawa et al., 2010).

There is a growing amount of evidence showing that the activation of the stress activated pathways c-jun N-terminal kinase (JNK) and $p38^{MAPK}$ play important roles in neuronal cell death, suggesting that JNK and $p38^{MAPK}$ inhibitors could constitute potential therapeutic drugs for neural diseases, including MDD (Borsello and Forloni, 2007; Harper and LoGrasso, 2001; Yasuda et al., 2011). Additionally, several studies have proposed that glycogen synthase kinase-3 β (GSK-3β) inhibitors have the potential to augment the efficacy of antidepressants or to be used as monotherapy (Beaulieu et al., 2009; Maes et al., 2012; Vidal et al., 2011).

Agmatine is a neuromodulator in the brain with antidepressant properties (Piletz et al., 2013). Our group was the first to demonstrate that agmatine is able to produce an antidepressant-like effect in the mouse forced swimming test (FST) and in the tail suspension test (TST), accompanied by modulation of the monoaminergic and opiod systems, NMDA receptors and the L-arginine-NO pathway (Zomkowski et al., 2002, 2004, 2005). More recently, we showed that agmatine produces an antidepressant-like effect that was paralleled by its capability to maintain the pro-/anti-oxidative homeostasis in the hippocampus (Freitas et al., 2013a), and we showed that agmatine is able to abrogate the depressive-like behavior induced by tumor necrosis factor-α (Neis et al., 2014). In addition, agmatine's ability to produce a clinical antidepressant effect was shown by Shopsin (2013). Despite agmatine's potential for use as a coadjuvant or monotherapy in the management of MDD, there is no study reporting its ability to produce an antidepressant-like effect following sub-chronic administration as well as the molecular mechanisms underlying such an effect. Therefore, the aim of the present study was to investigate the effect of sub-chronic agmatine treatment on the regulation of hippocampal signaling targets associated with neuronal survival, namely PKA, PKC, ERK1/2, Akt, GSK-3β, JNK1/2, $p38^{MAPK}$, CREB and BDNF.

2. Materials and methods

2.1. Animals

Female Swiss mice (3 months old, 40–45 g) were maintained at constant room temperature (20–22 °C) with free access to water and food, under a 12:12 h light:dark cycle (lights on at 07:00 h). 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, with each animal used only once. The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the local Ethics Committee. All efforts were made to minimize animal suffering and the number of animals used in the experiments.

2.2. Drugs and treatment

Agmatine (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in distilled water and administered once daily for 21 days via the oral route (p.o.) by gavage at doses of 0.001–0.1 mg/kg in a constant volume of 10 ml/kg body weight. A control group received distilled water as the vehicle. The number of mice per group was 8. To administer agmatine or vehicle, mice were first weighed to determine the dosing volume to be administered, following by the introduction of the gavage tube (feeding tubes approximately 31 mm in length with a rounded tip) in the diastema of the mouth by an experienced researcher. The tube was gently advanced along the upper palate, and the treatment was administered by a syringe attached to the end of the tube. After dosing, the tube was gently removed following the same angle as insertion. Finally, the animals were returned to their cage.

2.3. Tail suspension test (TST)

The tail suspension test was performed 24 h after the last sub-chronic drug administration. The total duration of immobility induced by tail suspension was measured using the method described by Steru et al. (1985). Acoustically and visually isolated mice were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was registered during a 6-min period (Freitas et al., 2013b, 2013c, 2010).

2.4. Open-field test

Five minutes after the tail suspension test, mice were evaluated in the open-field paradigm (Rodrigues et al., 1996) to assess the effects of agmatine on locomotor activity. The number of squares crossed with all paws (crossings) counted in a 6-min session. The apparatus were cleaned with a solution of 10% ethanol between tests to hide animal clues.

2.5. Western blot

Immediately after the behavioral observations, mice were decapitated. Brains were removed, and the hippocampus was rapidly dissected and placed in liquid nitrogen for storage at −80 °C until use. Western blot analysis was performed as previously described (Cordova et al., 2004; Freitas et al., 2013b, 2013d; Lopes et al., 2012, 2013). Briefly, hippocampal tissue was mechanically homogenized in 400 μl of Tris-base 50 mM pH 7.0, EDTA 1 mM, sodium fluoride 100 mM, PMSF 0.1 mM, sodium vanadate 2 mM, Triton X-100 1%, glycerol 10%, and protease inhibitor Cocktail; the tissue was then incubated for 30 min in ice. Lysates were centrifuged (10,000 × *g* for 10 min, at 4 °C) to eliminate cellular debris; the supernatants were diluted 1/1 (v/v) in Tris-base 100 mM pH 6.8, EDTA 4 mM, and 8% SDS and then boiled for 5 min. Subsequently, the loading buffer (glycerol 40%, Tris-base 100 mM, bromophenol blue, pH 6.8) at a ratio of 25:100 (v/v) and β-mercaptoethanol (final concentration 8%) were added to the samples. The protein content was estimated by the method described by Peterson (1977) using bovine serum albumin as protein standard. To compare the obtained signals, the same amount of protein (70 μg per lane) for each sample was electrophoresed in 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) minigels and transferred to nitrocellulose or polyvinylidene fluoride (PVDF) membranes using a semidry blotting apparatus (1.2 mA/cm2; 1.5 h). To verify the transfer efficiency process, gels were stained with Coomassie blue (Coomassie blue R-250 0.1%, methanol 50%, acetic acid 7%) and membranes with Ponceau S 0.5% in acetic acid 1%.

After this process, blots were incubated in a blocking solution 5% non-fat dry milk in Tris buffer saline solution (TBS) (Tris 10 mM, NaCl 150 mM, pH 7.5) for 1 h at room temperature, and targets were detected after overnight incubation (4 °C) with specific antibodies diluted in TBS with tween (TBS-T) that contained 2% BSA at the following dilutions: anti-phospho-PKA substrates (Cell Signaling Technology, Boston, MA, USA, 1:1000), anti-phospho-PKC substrates (Cell Signaling, 1:1000), anti-phospho-Akt (Sigma Chemical Co., 1:2000), anti-phospho-GSK-3β (Cell Signaling, 1:1000), anti-phospho-ERK1/2 (Sigma Chemical Co., 1:2000), anti-phospho-JNK1/2 (Cell Signaling, 1:5000), anti-phospho-$p38^{MAPK}$ (Millipore, Billerica, MA, USA, 1:10000), anti-phospho-CREB (Cell Signaling, 1:1000), anti-total-Akt (Cell Signaling, 1:1000), anti-total-GSK-3β (Cell Signaling, 1:1000), anti-total-ERK1/2 (Sigma Chemical Co.,