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Antidepressant-like effect of ascorbic acid is associated with the modulation of mammalian target of rapamycin pathway

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ABSTRACT

The present study investigated the involvement of the PI3K, GSK-3β, heme oxygenase-1 (HO-1) and mTOR in the antidepressant-like effect of ascorbic acid in the tail suspension test (TST). Male Swiss mice were pretreated with ascorbic acid (1 mg/kg, p.o.) or vehicle and 45 min after, LY294002 (10 μg/site, i.c.v., reversible PI3K inhibitor), rapamycin (0.2 nmol/site, i.c.v., selective mTOR inhibitor), zinc protoporphyrin (ZnPP – 10 ng/site, i.c.v., HO-1 inhibitor) or vehicle was administered. We also investigated the synergistic effect of ascorbic acid (0.1 mg/kg, p.o., sub-effective dose in the TST) with lithium chloride (10 mg/kg, p.o., non-selective GSK-3β inhibitor), AR-A014418 (0.01 μg/site, i.c.v., selective GSK-3β inhibitor) or cobalt protoporphyrin (CoPP – 0.01 μg/site, i.c.v., HO-1 inducer) in the TST. The antidepressant-like effect of ascorbic acid (1 mg/kg, p.o.) was prevented by the treatment of mice with LY294002, rapamycin or ZnPP. In addition, sub-effective doses of lithium chloride, AR-A014418 or CoPP, combined with a sub-effective dose of ascorbic acid produced a synergistic antidepressant-like effect. We also demonstrated that 1 h after its administration, ascorbic acid increased the phosphorylation of p70S6K and the immunocontent of PSD-95 in the hippocampus of mice. These results indicate that the antidepressant-like effect of ascorbic acid in the TST might be dependent on the activation of PI3K and mTOR, inhibition of GSK-3β as well as induction of HO-1, reinforcing the notion that these are important targets for antidepressant activity and contributing to better elucidate the mechanisms underlying the antidepressant-like effect of ascorbic acid.

1. Introduction

Worldwide, depression is a common, recurrent and incapacitating psychiatric disorder associated with significant morbidity and mortality (Nemeroff, 2007). Treatment of depression has usually focused on alleviating symptoms and preventing recurrence of episodes, however a considerable number of patients exhibit partial, refractory or intolerant responses to the pharmacological treatment and less than one third of them achieve remission after 10–14 weeks of treatment with a standard antidepressant (Trivedi et al., 2006). The recognition of the clear need for the development of new, effective, and better-tolerated therapeutic approaches with a more rapid onset of action has conducted to the investigation of the putative roles of intracellular signaling cascades and non-monoaminergic systems in the pathophysiology and treatment of depression (Coyle and Duman, 2003).

Modulation of glutamatergic neurotransmission has emerged as a promising strategy for next-generation fast-acting antidepressants. Of particular significance is the demonstration that low doses of the N-Methyl-D-aspartate (NMDA) receptor antagonist, ketamine, induces a rapid (within hours), long lasting (up to 1 week), and robust antidepressant response in treatment-resistant cases of depression in humans (Berman et al., 2000; Zarate et al., 2006). Accordingly, it was demonstrated that a single dose of NMDA receptor antagonists (ketamine or Ro 25-6981) completely reversed the behavioral deficits in a rat 21-day chronic unpredictable stress model (Li et al., 2011), reinforcing the antidepressant potential of these compounds.

Although the mechanisms underlying the fast and robust effects of ketamine are not completely understood, recent animal studies showed that they are mediated by rapid, but transiently activation of the mammalian target of rapamycin (mTOR) pathway (Li et al., 2010). Activation of mTOR signaling stimulates mRNA translation

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and new protein synthesis by activating p70 S6 kinase (p70S6K) and by inhibiting eukaryotic initiation factor 4E-binding protein 1. In rats, it was demonstrated that mTOR signaling results in rapid and sustained elevation of synapse-associated proteins and spine number in the prefrontal cortex (Li et al., 2010). The rapid antidepressant action of ketamine was also dependent on the inhibition of glycogen synthase kinase-3β (GSK-3β) activity through its phosphorylation at Ser9 residue. It was shown that a subthreshold dose of ketamine was potentiated by a single dose of lithium chloride (a non-selective GSK-3β inhibitor) or by a preferential GSK-3β inhibitor, an effect associated with rapid activation of the mTORC1 signaling pathway and increased phosphorylation of GSK-3β (Liu et al., 2013). Conversely, inhibition of mTOR attenuated GSK-3β phosphorylation and increased its kinase activity in lipopolysaccharide (LPS)-stimulated cells (Wang et al., 2011). Corroborating the notion that the activation of mTOR pathway and inhibition of GSK-3β by phosphorylation are implicated in the antidepressant responses of rapid antidepressants, the behavioral responses to ketamine were blocked in mice expressing constitutively active GSK-3β (Beurel et al., 2011). Stimulation of protein kinase B (PKB/Akt), possibly by activity-dependent release of brain-derived neurotrophic factor (BDNF), is probably implicated in the increased phosphorylation of GSK-3β induced by ketamine (Jourdi et al., 2009). Of note, the inactivation of GSK-3β induced by phosphorylation at Ser9 is linked to activation of the NF-E2-related factor 2 (Nrf2). This is a nuclear transcription factor which controls redox homeostasis by regulating several stress responsive genes, including heme oxygenase-1 (HO-1), which plays a pivotal role in cell protection against inflammatory insult and oxidative/nitrosative stress (Surh et al., 2009).

The discovery of compounds that can produce ketamine-like effects with a safer side-effect profile and decreased abuse liability may represent an important advance in the field of depression. Our group has demonstrated that ascorbic acid, a water-soluble vitamin with neuroprotective and antioxidant properties (Rice, 2000), exhibits an antidepressant-like effect in the mouse tail suspension test (TST), an animal model predictive of antidepressant activity (Binfaré et al., 2009). Additionally, this compound was able to reverse the depressive-like behavior and brain oxidative damage induced by acute and chronic unpredictable stress in mice (Moretti et al., 2012b, 2013). Considering that the antidepressant properties of ascorbic acid are mediated, at least in part, by inhibition of NMDA receptors (Moretti et al., 2011), we hypothesized that this compound could exhibit ketamine-like biochemical effects. Therefore, the goal of this study was to examine if the antidepressant-like effect of ascorbic acid in the TST is dependent on the modulation of mTOR and its down and upstream signaling targets: phosphatidylinositol 3-kinase (PI3K), GSK-3β, HO-1, p70S6K and PSD95.

2. Material and methods

2.1. Animals

The experiments were conducted using male adult Swiss mice (2 months, 30–40 g), maintained at 20–22 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 7:00 a.m. The animals were caged in groups of 15 in a 41 × 34 × 16 cm cage and were placed in the experimental room 24 h before the test for acclimatization. All behavioral tests were carried out between 09.00 a.m. and 04.00 p.m. The animals were used according to the NIH Guide for the Care and Use of Laboratory Animals. The protocol and experiments were approved by the local Ethical Committee of Animal Research (CEUA/UFSC). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

The following drugs were used: ascorbic acid, LY294002, AR-A014418, zinc protoporphyrin (ZnPP), cobalt protoporphyrin (CoPP) (Sigma Chemical Co., St Louis, USA) and lithium chloride (MERCK, Darmstadt, Germany). Lithium chloride and ascorbic acid were dissolved in distilled water. These solutions, freshly prepared before administration, were given orally (p.o.) by gavage in a volume of 10 ml/kg body weight. LY294002 and AR-A014418 were dissolved in saline at a final concentration of 1% DMSO and administered by i.c.v. route, in a volume of 5 μl per mouse. Rapamycin was dissolved in 100% DMSO and administered by i.c.v. route, in a volume of 3 μl per mouse. ZnPP and CoPP were dissolved in final concentration of 0.1% DMSO and administered by i.c.v. route, in a volume of 5 μl per mouse, respectively. Appropriate vehicle-treated groups were also assessed simultaneously.

The i.c.v. injections were performed by employing a "free hand" method under light ether anesthesia according to the procedure described previously (Kaster et al., 2012). Briefly, a 0.4 mm external diameter hypodermic needle attached to a cannula, which was linked to a 25 μl Hamilton syringe, was inserted perpendicularly through the skull (no more than 2 mm into the brain of each mouse). The drugs were then administered into the left lateral ventricle. 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. The injection site was 1 mm to the left from the mid-point on a line drawn through to the anterior base of the ears. 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%).

2.3. Pharmacological treatment

To investigate the involvement of PI3K in the antidepressant-like effect of ascorbic acid, mice were pre-treated with ascorbic acid (1 mg/kg, p.o., active dose in the TST) or vehicle 45 min before i.c.v. administration of LY294002 (PI3K inhibitor, 10 nmol/site) or vehicle. The TST was carried out 15 min after LY294002 administration.

To test the hypothesis that the antidepressant-like effect of ascorbic acid could be mediated by the inhibition of GSK-3β activity, mice were treated with a sub-effective dose of ascorbic acid (0.1 mg/kg, p.o.) or vehicle and immediately after, a sub-effective dose of lithium chloride (10 mg/kg, p.o., a non-selective GSK-3β inhibitor) or vehicle was administered. The TST was carried out 60 min later. In another set of experiments mice were treated with a sub-effective dose of ascorbic acid (0.1 mg/kg, p.o.) or water and 45 min after, were injected with a sub-effective dose of the selective GSK-3β inhibitor, AR-A014418 (0.01 μg/site, i.c.v.) or vehicle. A further 15 min were allowed to elapse before the animals were tested in the TST.

To investigate the involvement of HO-1 in the antidepressant-like effect of ascorbic acid in the TST, mice received an active dose of ascorbic acid (1 mg/kg, p.o.) or vehicle and after 45 min were treated with the HO-1 inhibitor ZnPP (10 ng/site, i.c.v.) or vehicle. In another set of experiments mice were treated with a sub-effective dose of ascorbic acid (0.1 mg/kg, p.o.) or water and 45 min after, received a sub-effective dose of the HO-1 inducer CoPP (0.01 μg/site, i.c.v.) or vehicle. Animals were tested in the TST 15 min after the i.c.v. administrations.

To evaluate the participation of mTOR in the anti-immobility effect of ascorbic acid in the TST, mice were treated with an active dose ascorbic acid (1 mg/kg, p.o.) or vehicle and after 45 min