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MAPK signaling correlates with the antidepressant effects of ketamine

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ABSTRACT

Studies have pointed to a relationship between MAPK kinase (MEK) signaling and the behavioral effects of antidepressant drugs. So, in the present study we examined the behavioral and molecular effects of ketamine, an antagonist of the N-methyl-D-aspartate receptor (NMDA), which has been shown to have an antidepressant effect after the inhibition of MEK signaling in Wistar rats. Our results showed that acute administration of the MEK inhibitor PD184161, produced depressive-like behavior and stopped antidepressant-like effects of ketamine in the forced swimming test. The phosphorylation of extracellular signal-regulated kinase 1/2 (pERK 1/2) was decreased by PD184161 in the amygdala and nucleus accumbens, and the effects of ketamine on pERK 1/2 in the prefrontal cortex and hippocampus were inhibited by PD184161. The ERK 2 levels were decreased by PD184161 in the nucleus accumbens; and the effects of ketamine were blocked in this brain area. The p38 protein kinase (p38MAPK) and proBDNF were inhibited by PD184161, and the MEK inhibitor prevented the effects of ketamine in the nucleus accumbens. In addition, ketamine increased pro-BDNF levels in the hippocampus. In conclusion, our findings demonstrated that an acute blockade of MAPK signaling lead to depressive-like behavior and stopped the antidepressant response of ketamine, suggesting that the effects of ketamine could be mediated, at least in part, by the regulation of MAPK signaling in these specific brain areas.

1. Introduction

Depression can be a chronic and disabling psychiatric disorder, possibly associated with unfavorable outcomes over time. In addition, patients with depression present brain dysfunctions in morphology and activity (Kessler and Wang, 2008). Although antidepressant drugs used for its treatment show a clinical response in a good percentage of patients, around 40% of depressed patients do not respond to any antidepressant drugs (Fava, 2003). The fact that antidepressant drugs, which are known to increase synaptic concentrations of monoamines, require at least two weeks to achieve a clinical response, suggest that other neural adaptations are recruited to therapeutic efficacy (Duman et al., 2007; Di Benedetto et al., 2013). In addition to this, it is important to search for more effective drugs that provide more rapid actions. In this sense, research has paid special attention to the glutamatergic system and to the drugs with different mechanisms of action within this system (Li et al., 2010; Zarate et al., 2010; Réus et al., 2011, 2013a; Fraga et al., 2013). In fact, postmortem studies have shown altered glutamate levels in various brain areas of individuals with depression (Levine et al., 2000; Taylor et al., 2011). Also, elevated levels of cortical glutamate in young people have been shown to increase familial vulnerability to depression (Taylor et al., 2011). Moreover, classical antidepressant drugs, such as fluoxetine and citalopram were able to decrease the microglial release of glutamate (Dhami et al., 2013), which points to a relationship between antidepressant action and the glutamatergic system.

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More recently, a number of important studies have highlighted antidepressant effects for antagonists of the N-methyl-D-aspartate receptor (NMDA), mainly ketamine, which has shown antidepressant properties in several animal models of depression. In fact, acute or chronic administration of ketamine (5, 10 and 15 mg/kg) lead to an antidepressant response in rats subjected to the forced swimming test (Garcia et al., 2008a, 2008b). Garcia et al. (2009) also demonstrated that ketamine (15 mg/kg) treatment was able to reverse the anhedonic behavior induced by chronic mild stress in rats (Garcia et al., 2009). In addition, ketamine at dose of 5 and 10 mg/kg showed synergistic antidepressant effects when given jointly with imipramine (Réus et al., 2011); further to this, a previous study showed that deprived rats treated with saline increased their immobility time, and treatments with imipramine (30 mg/kg) and ketamine (15 mg/kg) reversed this alteration (Réus et al., 2013). With regard to human studies, a single dose of ketamine resulted in an antidepressant response, and this effect lasted for at least a week (Berman et al., 2000; Sahay and Hen, 2007). Furthermore, it has presented a rapid antidepressant action in patients with refractory depression to conventional treatments (Berman et al., 2000; Zarate et al., 2006). NMDA receptor abnormalities have been observed in patients with major depression and also in suicide victims. In this context, Lapteva et al. (2006) showed that patients who met DSM-IV criteria for major depression had higher levels of anti-NR2 antibodies, suggesting that antibodies to NMDA receptors may represent a mechanism for cerebral dysfunction in patients with depression.

Some findings have pointed towards ketamine exerting its antidepressants properties through the activation of NMDA receptors. Ketamine is also shown to have antidepressant properties for the hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) (Koike et al., 2011; Yang et al., 2012), and also beyond the regulation of intracellular pathways, such as in the regulation of the mammalian target of rapamycin (mTOR), in the induction of synaptogenesis, in the regulation of histone deacetylation induced by stress (Li et al., 2010; Réus et al., 2013), and in the increase of phosphorylated and activated forms of extracellular signal-regulated kinase (ERK) and protein kinase B (PKB/Akt) (Hoeffer and Klann, 2010). However, the exact mechanisms by which ketamine exerts its antidepressant effects are still not fully elucidated. So, further studies are needed to characterize these effects.

ERK 1 and ERK 2 are part of mitogen-activated protein kinase (MAPK) signaling pathway and play an important role in synaptic plasticity (Patterson and Yasuda, 2011). Moreover, some studies have highlighted ERK signaling playing a part in major depression (H Qi et al., 2009; Duric et al., 2010), and also in the antidepressant action (Zhanga et al., 2012; Di Benedetto et al., 2013). After the activation of ERK, other proteins involved with cell survival are phosphorylated, activated or expressed, including the brain-derived neurotrophic factor (BDNF) (Tao et al., 1997). BDNF is formed from its precursor form, pro-BDNF, and both pro-BDNF and mature BDNF have been shown to be altered in animal models of depression and in humans with this mood disorder (Tsai et al., 2010; Adlam and Zaman, 2013; Réus et al., 2013b; Zhou et al., 2013). In addition, BDNF is a neurotrophin with known importance in neuroplasticity, and presents a role in the regulation of the glutamatergic synapses (McAllister et al., 1999; Carvalho et al., 2008).

As there is a relationship between the signaling pathways of MEK and BDNF, and between the glutamatergic system and the action of antidepressant drugs, the principal aim of this study was to verify if the antidepressant effect of ketamine could be changed by modifying the function of the MAPK pathway. For this we used an inhibitor of MEK to investigate a potential role for MAPK signaling on the behavioral and molecular antidepressant-like effects of ketamine. pERK ½, ERK 1/2, p38MAPK and proBDNF were evaluated in the prefrontal cortex, hippocampus, amygdala and nucleus accumbens, which are brain areas that are important to mood regulation and which are involved with major depression (Duman and Monteggia, 2006).

2. Material and methods

Adult (60 days old) Wistar rats were housed five to a cage with food and water available ad libitum and were maintained on a 12-h light/dark cycle (lights on at 7:00 a.m.). In vivo studies were performed in accordance with the National Institute of Health guidelines and also with the approval of the ethics committee from Universidade do Extremo Sul Catarinense (UNESC) under protocol number 35/2012.

3. Animals

3.1. Experimental design and treatment

Firstly, all rats underwent a surgical procedure to receive a pharmacological inhibitor of MAPK (PD184161) at a dose of 0.1 mg/uL or vehicle (control group) intracerebroventricularly (i.c.v.) 24 h before the behavioral tests. The animals were then divided into four experimental groups: 1) vehicle + saline; 2) saline + PD184161; 3) PD184161 + ketamine 15 mg/kg; and 4) vehicle + ketamine 15 mg/kg. The intraperitoneal administration of ketamine was performed at 23.5, 05 and 01 h before behavioral testing (Duman et al., 2007; Réus et al., 2013a). The immobility, swimming and climbing times were assessed via the forced swimming test ($n = 12$) and exploratory motor activity was assessed using the open field test ($n = 12$), as described in Fig. 1. After the behavioral tests, the animals were killed by decapitation and the prefrontal cortex, hippocampus, amygdala and nucleus accumbens were removed for western blotting analysis ($n = 4$).

3.2. Surgical procedure

The animals were anesthetized with thiopental 25 mg/kg and xylazine 30 mg/kg intraperitoneally. Using a stereotaxic surgical procedure, the skin and scalp of the rat in the skull region were removed. A 1 mm cannula was placed through the brain tissue at the following coordinates: 0.9 mm behind bregma, 1.5 mm to the right of bregma; with the cannula being located 2.6 mm deep in to the ventricle. The fixation of the cannula tube was made using acrylic cement. The i.c.v injection of inhibitor PD184161 (0.1 μg/μl) or saline occurred 72 h after surgery (Machado-Vieira et al., 2004; Duman et al., 2007).

3.3. Open field test

Locomotor activity was measured using the task of habituation to the open field, which is performed in a 40 × 60 cm arena,

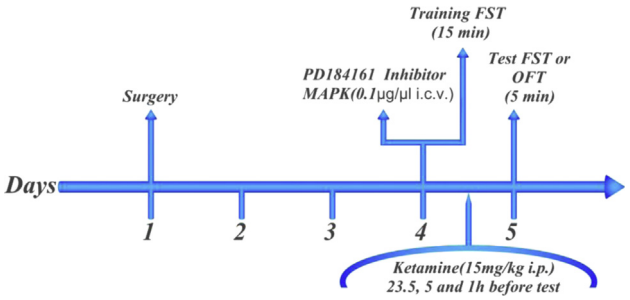

Fig. 1. The time-line and schedule for the behavioral tests and treatments used in the experiment. FST, forced swimming test; OFT, open-field test.

surrounded by 50 cm high walls which are made of plywood, and which has a frontal glass wall. The floor of the open field is divided into 9 rectangles by black lines. The animals were gently placed in the left rear quadrant, and left to explore the arena for 5 min. The number of crossings, where the rat crosses the black lines between the intersections marked in the arena and the number of rearings, which are the number of times the rat supports itself on its hind legs in order to explore the environment were then counted. The open field test was performed on the 5th day after the surgical procedure and 24 h after application of the inhibitor. This test was measured in order to assess spontaneous locomotor activity of the animals, so to avoid false-positive results.

3.4. Forced swim test

This test consists of procedures that are undertaken on two separate days, during which each rat was placed in a cylinder containing water at 23 °C, the water depth being such that the animal cannot touch the bottom of the cylinder. On the 4th day, the rats were forced to swim for an initial period of 15 min. On day 5, following the surgical procedure and 24 h after application of the inhibitor, each animal was again forced to swim for a period of 5 min. During the test session, some behavioral parameters were recorded in seconds, such as immobility time (i.e. no additional activity is observed other than that required to keep the rat's head above the water), the climbing time, which is defined as upward-directed movements of the forepaws along the side of the swim chamber, and swimming time (i.e. movement usually horizontal throughout the swim chamber) (Porsolt et al., 1977).

3.5. Western blotting analysis

To perform immunoblotting, tissue samples taken from the rats prefrontal cortex, hippocampus, amygdala and nucleus accumbens were homogenized in Laemmli buffer (62.5 mM Tris–HCl, pH 6.8, 1% (w/v) sodium dodecyl sulfate (SDS), 10% (v/v) glycerol) and equal amounts of protein (100 ug/well) were fractionated by polyacrylamide gel electrophoresis-sodium dodecyl sulfate (SDS-PAGE) and electro transferred to nitrocellulose membranes. The efficiency of the electro transfer was then verified by Ponceau S staining, and the membrane was then blocked in Tris-Tween buffer saline (TTBS: 100 mM Tris–HCl, pH 7.5, containing 0.9% NaCl and 0.1% Tween 20) with 5% albumin. The membranes were incubated overnight at 4 °C with rabbit polyclonal annti-pERK 1/2, anti-ERK 1/2, anti-proBDNF and anti-p38MAPK (1:1000). Secondary anti-rabbit IgG was incubated with the membrane for 2 h (1:1000). The membrane was then washed again with TTBS and immunoreactivity was detected by chemiluminescence using ECL. Densitometry analysis of the films was performed using the Image J® v.1.34 image analysis software. All results were expressed as a relative ratio between pERK 1/2, ERK 1/2, proBDNF and p38MAPK and immunocontent protein β-actin.

3.6. Statistical analysis

All data are presented as mean ± standard deviation. Differences among experimental groups in the behavioral tests and western blotting analysis were determined by one-way ANOVA, followed by Tukey *post-hoc* test when ANOVA was significant; *P* values <0.05 were considered to be statistical significant.

4. Results

As depicted in Fig. 2A, the inhibitor PD184161 blocked the behavioral effects of the sub chronic administration of ketamine in the forced swimming test. Ketamine at the dose of 15 mg/kg reduced the immobility time, compared to the vehicle group, and the administration of PD184161 abolished the effects of ketamine ($F = 4.788$; $p = 0.007$; Fig. 2A), in the forced swimming test, we did not observe any alterations in recorded times for either the ketamine or PD184161 groups ($F = 2164$; $p = 0.110$; Fig. 2A), but the climbing time was reduced in the PD184161 group treated with ketamine ($F = 3.785$; $p = 0.019$; Fig. 2A). In the open-field test, we did not observe any alterations in the number of crossings ($F = 1.255$; $p = 0.307$; Fig. 2B) or rearings ($F = 1.748$; $p = 0.179$; Fig. 2B) in any of the groups.

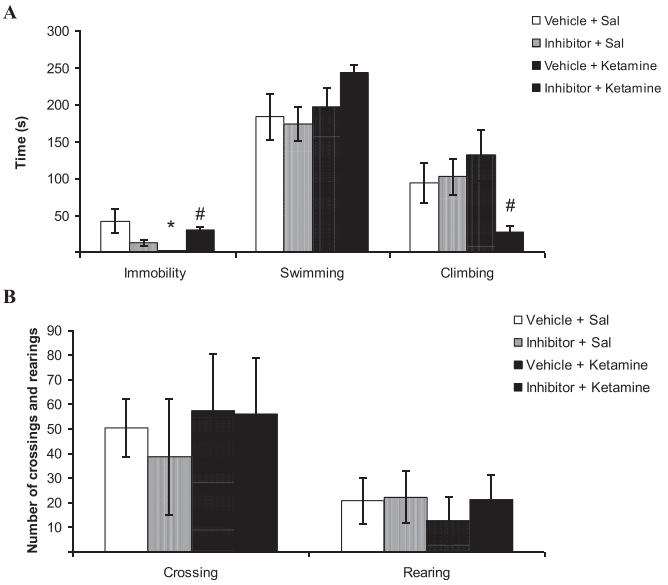

Fig. 2. The effects of ketamine administration after the infusion of inhibitor PD184161 on immobility, swimming and climbing times in the forced swimming test (A), and on number of crossings and rearings in the open-field test (B). Bars represent mean ± standard deviation. *$p < 0.05$ vs. vehicle + saline; #$p < 0.05$ vs. vehicle + ketamine according to ANOVA followed by Tukey post-hoc test.

Fig. 3 illustrates the effects of ketamine after the inhibition of MAPK with PD184161 on proteins involved with MAPK signaling pathways in the prefrontal cortex, hippocampus, amygdala and nucleus accumbens. The phosphorylation of ERK 1/2 decreased in the amygdala after infusion of the inhibitor or treatment with ketamine, when compared with the vehicle group, and with the inhibitor plus ketamine treatment group when compared to the ketamine group ($F = 17.994$; $p < 0.001$; Fig. 3A); in the nucleus accumbens, there was a decrease in pERK 1/2 in the group that received the inhibitor, ketamine or the inhibitor plus ketamine ($F = 3.961$; $p < 0.001$; Fig. 3A); in the prefrontal cortex, the pERK 1/2 levels decreased in the inhibitor plus ketamine group, when compared to ketamine treatment ($F = 3.741$; $p = 0.037$; Fig. 3A); and in the hippocampus, the pERK 1/2 levels increased after the administration of ketamine, when compared to the inhibitor group, but decreased in the inhibitor plus ketamine treatment group, when compared to the vehicle and ketamine groups ($F = 6.045$; $p = 0.007$; Fig. 3A).

Levels of ERK 1 were increased with ketamine administration, and also with inhibitor plus ketamine administration when compared with the inhibitor group in the prefrontal cortex ($F = 6.482$; $p = 0.006$; Fig. 3B), and compared with vehicle group in the hippocampus ($F = 10.697$; $p < 0.001$; Fig. 3B). In the amygdala ($F = 2.746$; $p = 0.082$; Fig. 3B) or nucleus accumbens ($F = 1.029$; $p = 0.410$; Fig. 3B) we did not observe any alterations in ERK 1 levels.

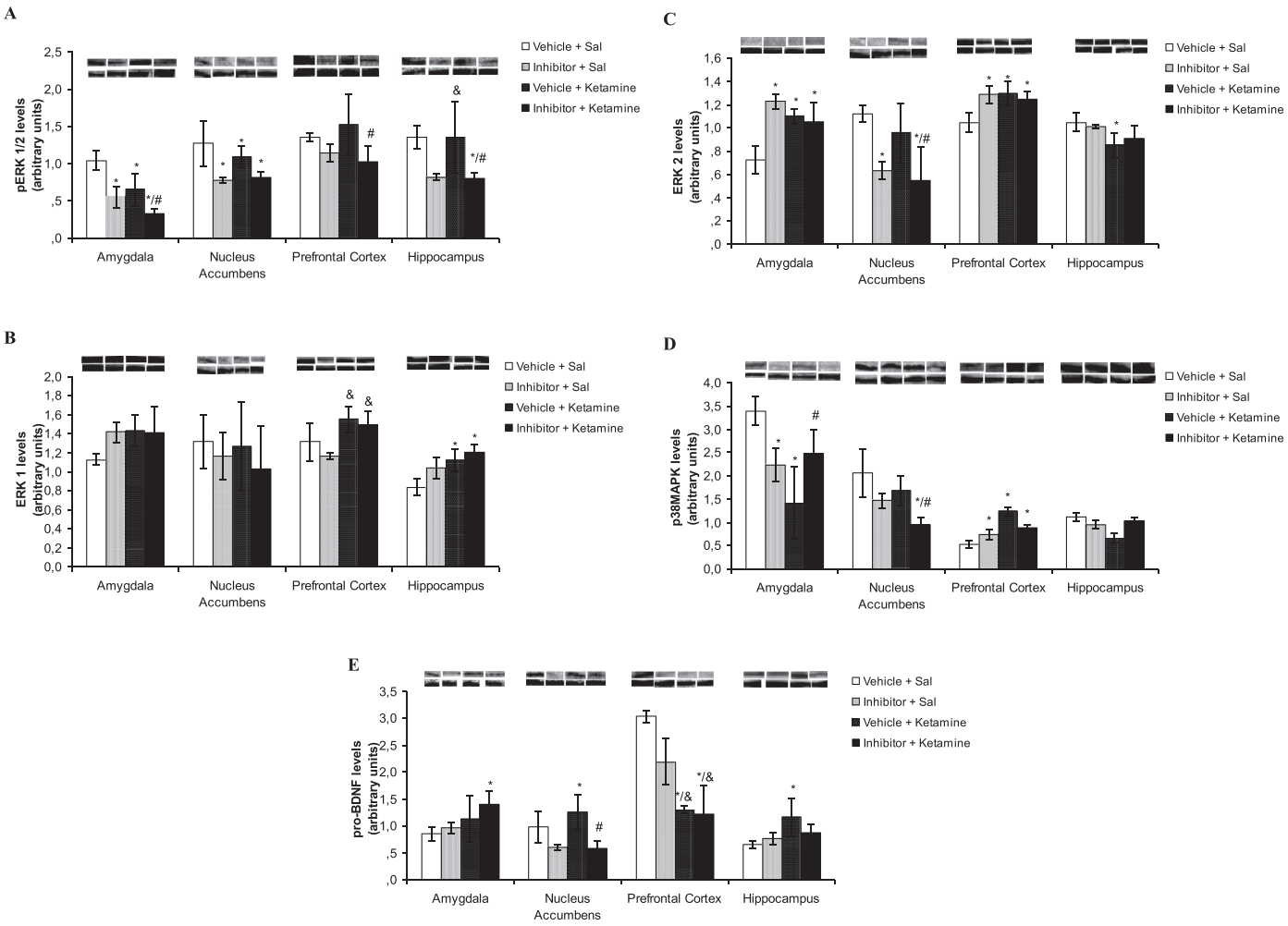

Fig. 3. The effects of ketamine administration after theinfusion of inhibitor PD184161 on pERK 1/2 (A), ERK 1 (B), ERK 2 (C), p38MAPK (D) and proBDNF (E) levels in the prefrontal cortex, hippocampus, nucleus accumbens and amygdala. Representative images of each protein and pERK 1/2 (A), ERK 1 (B), ERK 2 (C), p38MAPK (D) and proBDNF (E) and β-actin respectively are shown in the upper panels. Bars represent mean ± standard deviation. $^{*}p < 0.05$ vs. vehicle + saline; $\#p < 0.05$ vs. vehicle + ketamine; $^{\&}p < 0.05$ vs. inhibitor + saline according to ANOVA followed by Tukey post-hoc test.

In the amygdala and the prefrontal cortex, the ERK 2 levels were increased in all groups, compared to the vehicle group ($F = 13.856$; $p < 0.001$; and $F = 8.189$; $p = 0.002$, respectively, Fig. 3C); the ERK 2 levels were decreased in the nucleus accumbens after infusion of the inhibitor, when compared to the vehicle group and also with inhibitor plus ketamine treatment, when compared to vehicle and ketamine groups ($F = 7.346$; $p = 0.003$; Fig. 3C); in the hippocampus the ERK 2 levels were decreased with ketamine administration, when compared to the vehicle group ($F = 4.643$; $p = 0.019$; Fig. 3C).

In the amygdala, we found a decrease in p38MAPK after administration of the inhibitor or ketamine, compared to vehicle group and also after infusion of the inhibitor plus ketamine treatment, when compared to the ketamine group ($F = 10.333$; $p < 0.001$; Fig. 3D); in the nucleus accumbens there was a decrease in p38MAPK after infusion of inhibitor plus ketamine treatment, when compared to the vehicle and ketamine groups ($F = 10.090$; $p = 0.001$; Fig. 3D); in the prefrontal cortex, we observed an increase in p38MAPK levels in all groups, when compared to the vehicle group ($F = 49.248$; $p < 0.001$; Fig. 3D; in the hippocampus the p38MAPK level did not alter in any of the groups ($F = 2.379$; $p = 0.144$; Fig. 3D).

There was an increase of proBDNF in the amygdala after infusion of inhibitor plus ketamine treatment, compared to the vehicle group ($F = 3.377$; $p = 0.49$; Fig. 3E); in the nucleus accumbens we observed an increase in proBDNF levels after ketamine administration, when compared to the vehicle group, and a decrease after infusion of inhibitor plus ketamine treatment ($F = 8.824$; $p = 0.002$; Fig. 3E); in the prefrontal cortex there was a decrease in proBDNF levels after treatment with ketamine or after the infusion of inhibitor plus ketamine, when compared to the vehicle and inhibitor groups ($F = 25.674$; $p < 0.001$; Fig. 3E); the proBDNF levels were increased in the hippocampus after treatment with ketamine, when compared to the vehicle group ($F = 4.613$; $p = 0.019$; Fig. 3E).

5. Discussion

In the present study, we concurred with previous research (Garcia et al., 2008a, 2008b; 2009), that ketamine decreased the immobility time of rats subjected to the forced swimming test. However, this is the first study to show that the antidepressant-like effects of ketamine are blocked by the inhibition of MEK signaling, without changing the levels of motor activity, suggesting that the behavioral effects of ketamine could be attributed, at least in part, by its action in regulating MAPK signaling. The present study also showed that the MAPK inhibitor itself (without ketamine) was also associated with reductions in immobility time. In contrast with these findings, Duman et al. (2007) presented that the acute administration of

PD184161 produced depressive-like behavior; however the MEK inhibitors were given as a single acute injection to BDNF mutant mice; which may explain the possible difference in results.

The MAPK signaling pathway regulates cellular responses, including neural plasticity, survival and apoptosis (Cross et al., 2000; Pearson et al., 2001), and this signaling pathway has been highlighted was playing an important role in depression. In fact, Jia et al. (2013) demonstrated that prolonged morphine withdrawal in mice induced a depressive-like behavior that was correlated with a decrease of phospho-ERK and an increase of MKP-1 levels in the hippocampus. On the other hand, the pharmacological blockade of MKP-1, while being selective for extracellular ERK, abolished the depressive-like behaviors induced by morphine withdrawal and correlated with increased hippocampal ERK phosphorylation (Jia et al., 2013). Another study, which evaluated the antidepressant effects of Curcumin, the major constituent of the plant *Curcuma longa*, found that it reduced depressive-like behaviors of mice in the forced swimming test. Moreover, Curcumin increased BDNF levels in the amygdala and this enhancement was suppressed by pre-treatment with the ERK inhibitor SL327 (Zhanga et al., 2012), indicating that, in line with our study, the inhibition of MAPK signaling can abolish antidepressant responses. Comparable with our findings, Duman et al. (2007) found that acute administration of the inhibitor PD184161 produced depressive-like behavior, and blocked the antidepressant-like effects of desipramine, sertraline and desipramine in several animal models of depression. Moreover, the authors also demonstrated that PD184161 produced a dose-dependent inhibition of ERK phosphorylation in the hippocampus (Duman et al., 2007). Furthermore, antidepressant drugs, such as imipramine and tianeptine activated MAPK signaling, reversing the decrease in MAPK signaling after stress (Svenningsson et al., 2007; Peng et al., 2008; X Qi et al., 2009; Szegedi et al., 2011).

MAPK has three identified subfamilies, which include c-Jun *N*-terminal protein kinases (JNK), ERKs and p38MAPK. Our results demonstrated that the MEK inhibitor PD184161 decreased the phosphorylation of ERK 1/2 in the amygdala and nucleus accumbens, as well as ketamine treatment, but in the amygdala, prefrontal cortex and hippocampus, the elevated levels of phosphorylation of ERK 1/2 caused by ketamine were blocked by PD184161. The ERK 1 levels were increased by ketamine in the prefrontal cortex and hippocampus, and the MEK inhibitor did not present any effect. The ERK 2 levels were also increased by both the inhibitor and ketamine in the amygdala and prefrontal cortex, however in the nucleus accumbens the PD184161 decreased the ERK 2 levels and blocked the effects of ketamine. The ERK activation of ERK 1/2 is important for plasticity and synaptic remodeling (Atkins et al., 1998; Patterson and Yasuda, 2011). So, we suggest that the therapeutic efficacy of ketamine in the present study could be caused, at least in part, by modulating MAPK signaling in the brain specific areas involved in mood regulation. Indeed, the ERK pathway is involved in glutamate-triggered excitotoxicity and responsible for the downstream signaling pathway of intracellular accumulation of Ca^{2+} (Jiang et al., 2000; Li et al., 2005; Hu et al., 2013), thus, the effect of ketamine caused by the blocking of the NMDA receptor could prevent the accumulation of Ca^{2+}, and alter ERK signaling. We also found evidence that AMPA receptor potentiation produced by the antidepressant tianeptine was modulated by three distinct MAPK pathways: p42/44, JNK and p38 signaling (Szegedi et al., 2011), relating a link between the glutamatergic system and MAPK signaling pathway. In addition to this, ketamine produced a rapid increase in the phosphorylated and activated forms of extracellular ERK1, ERK2 and protein kinase B (PKB/Akt), and the growth factor signaling pathways that have been linked to the activation of mTOR signaling (Li et al., 2010), thus screening the effects of ketamine in MAPK signaling.

p38MAPK has an important role in the regulation of the immune system, and in the microglia, it is activated in response to stress (Walton et al., 1998; Dong et al., 2002). Our results also showed that p38MAPK levels were reduced in the amygdala by inhibitor or ketamine administration, but infusion of PD184161 and ketamine treatment increased p38MAPK levels in this brain area, and decreased its level in the nucleus accumbens. This result could be related to a compensatory mechanism due the activation of p38MAPK in the amygdala, thus reducing its level in the nucleus accumbens. On the other hand, in the prefrontal cortex there was an increase in p38MAPK levels in all groups. Galeotti and Ghelardin (2012), showed elevated levels of phosphorylation of p38MAPK after stress events, but in contrast with our findings, these elevated levels were in the hippocampus, but not in the prefrontal cortex. In addition, the antidepressant amitriptyline prevented this increase in p38MAPK phosphorylation, suggesting that the inhibition of p38MAPK activity results in the induction of an antidepressant-like phenotype. On the other hand, Budziszewska et al. (2010), showed that prenatal stress decreased the levels of the active form of JNK and p38, and these changes were reversed by antidepressant drugs, suggesting that the decrease in pJNK and p-p38 could inhibit glucocorticoid receptor function and so enhance the glucocorticoid action. The alterations in p-38MAPK levels seen in the present study could lead to immune activation and alterations in glucocorticoid activity. In fact, both have been shown to be altered within depression (Réus et al., 2012, 2013a, 2013b; Lichtblau et al., 2013).

The activation of signaling cascades for MAP and ERK are regulated by BDNF (Huang and Reichardt, 2003). A series of studies have been related to the important role of BDNF in depression (Jiang and Salton, 2013; O'Keefe et al., 2014). BDNF is synthesized as a glycosylation precursor protein, proBDNF with molecular mass of 32–36 KDa. In the secretion process, the precursor protein is converted into two fragments, mature BDNF (mBDNF) and precursor peptide (propeptide of proBDNF, preBDNF). Some researchers have related biological differences between proBDNF and mBDNF. For example, mBDNF presents high affinity with the receptor of TrkB, while proBDNF binds with the receptors p75 and sortilin (Lee et al., 2001; Chao, 2003), so both mBDNF and proBDNF could present differences in intracellular signaling. The present study demonstrated that proBDNF levels were increased in the amygdala and prefrontal cortex, and were decreased after the infusion of inhibitor PD184161 and treatment with ketamine. However, levels of proBDNF were increased with ketamine treatment in the nucleus accumbens and hippocampus, but the inhibitor blocked the effects of ketamine in the nucleus accumbens. The differences found could be related to the variations in brain activity within the areas studied. In fact, unlike the prefrontal cortex and hippocampus, which have both activity and volume reduced in depression, the amygdala has increased activity and morphology (Drevets, 2003), and imaging studies have shown an increase in the volume of the amygdala in patients with depression (Lange and Irle, 2004). In addition, stress showed an increase in synaptic plasticity and function in the amygdala neurons, a distinct effect of atrophy found in the hippocampus and prefrontal cortex. These changes found in the amygdala may eventually contribute to the activation of neural circuits that control fear, anxiety and emotion (Pittenger and Duman, 2008). Duman et al. (2007) showed that the heterozygous deletion of BDNF alone did not influence behavior in the forced swimming test, but resulted in a depressive phenotype when combined with a low-dose MEK inhibitor or stress exposure. Thus, the MEK inhibitor could be altering the activity of proBDNF, as related in the present study. The effects of ketamine on BDNF levels, but not specifically proBDNF, were demonstrated in previous studies. Indeed, ketamine significantly increased plasma BDNF in patients with treatment-resistant depression (Haile et al., 2013).

Acute or chronic treatment with ketamine alone or in combination with AMPA or imipramine lead to an increase in hippocampal BDNF levels (Garcia et al., 2008a; Réus et al., 2011; Akinfiresoye and Tizabi, 2013), similar to the effects of ketamine in the present study on proBDNF in the hippocampus. The effects of ketamine on the nucleus accumbens could be related to the its effects on histone deacetylase, as previously demonstrated by our group, in which ketamine reduced the histone deacetylase activity induced by stress in the nucleus accumbens (Réus et al., 2013a, 2013b), suggesting that this inhibition can lead to increase in the transcription of genes involved in neuroplasticity and cellular survival, including BDNF.

In conclusion, we related that the anti-depressive like behavior of ketamine was attenuated by the MEK inhibitor PD184161. In addition, PD184161 blocked the effects of ketamine on pERK 1/2 in the amygdala, prefrontal cortex and nucleus accumbens, and on ERK 2, p38MAPK and proBDNF in the nucleus accumbens. Our results suggest that the effects of ketamine in the forced swimming test are mediated, at least in part, by a regulation of the MAPK signaling pathway, and this consequently causes an increase of proBDNF expression in the brain specific areas.

Conflicts of interest

The authors have declared that no conflicts of interest exist relevant to the current manuscript.

Author contributions

F.G.V., G.Z.R, E.L.S., and J.Q. designed, organized and coordinated the study. H.M.A., D.B.T., M.A.B.S., A.S.C., M.V.N., B.I.M., J.R.L., and M.M., participated in experimental protocols, surgical procedure, behavioral tests, and biochemical analysis. F.G.V., G.Z.R, M.M., F.D.P., and J.Q., contributed to data analysis, interpretation of data and wrote the manuscript.

Role of funding source

None.

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