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Research report

The activation of adenosine monophosphate-activated protein kinase in rat hippocampus contributes to the rapid antidepressant effect of ketamine

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HIGHLIGHTS

- The down-regulation of the phosphorylated form of AMPK is observed in the depressed rats.
- Ketamine could reserves this down-regulation in the depressed rats, along with a rapid antidepressant effect during FST.
- BDNF is involved in the rapid antidepressant effect of ketamine, and maybe partly through the activation of AMPK.
- The phosphorylated form of mTOR is not the direct mechanism underlying the rapid antidepressant effect of ketamine.

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ABSTRACT

Recent studies have shown a rapid, robust, and lasting antidepressant effect of ketamine that makes ketamine a promising antidepressant drug. However, the mechanisms underlying this rapid antidepressant effect remain incompletely understood. The goal of the present study was to determine whether adenosine monophosphate-activated protein kinase (AMPK) was involved in ketamine's rapid antidepressant effect during the forced swimming test (FST). In the first stage of experiment, a lower level of phosphorylated form of AMPK (p-AMPK) in the hippocampus and a longer immobility time were observed in the depressed rats during FST; whereas ketamine reversed these changes at 30 min after the administration. In the second stage of experiment, we observed that, ketamine up-regulated the levels of p-AMPK and brain-derived neurotrophic factor (BDNF) in the hippocampus of the depressed rats. Moreover, AMPK agonist strengthened the antidepressant effect of ketamine with an up-regulation of BDNF, while AMPK antagonist attenuated the antidepressant effect of ketamine with a down-regulation of BDNF. In conclusion, our results suggest that the activation of AMPK in rat hippocampus is involved in the procedure of ketamine exerting rapid antidepressant effect through the up-regulation of BDNF.

1. Introduction

Major depressive disorder (MDD) is a serious and heterogeneous disease that has major negative impacts on public health [1,2], which affects about 120 million people worldwide [3]. Unfortunately, currently prescribed antidepressant drugs display a hysteresis effect and a low remission rate for MDD [4–6], therefore new antidepressant agents with short onset time and exact efficacy are urgently needed.

Accumulating clinical evidences suggest that ketamine, an antagonist of non-competitive N-methyl-D-aspartate acid (NMDA) receptors, can exert a rapid, robust, and lasting antidepressant effect even in treatment-resistant patients [7–9]. Similarly, preclinical studies in various rodent models of depression also show an effective and quick antidepressant property of ketamine [10–12].

However, the mechanisms underlying this rapid antidepressant effect of ketamine remain incompletely understood. It has been suggested that the expressions of serum and hippocampal brain-derived neurotrophic factor (BDNF) in depressed subjects are decreased, which can be reversed by antidepressant therapies [13,14]. Ketamine does not exert an antidepressant effect on BDNF-knockout mice, indicating that the fast-acting antidepressant action of ketamine requires BDNF involvement [11]. In addition, the rapid antidepressant effect of ketamine depends on the up-regulated expression of phosphorylated form of mammalian target of rapamycin (p-mTOR), and infusion of rapamycin, a selective inhibitor of mTOR, may completely block the antidepressant action of ketamine [15].

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Adenosine monophosphate-activated protein kinase (AMPK) is important for metabolic control, which is common to all eukaryotic cells [16], and can be activated by metabolic stresses, such as hypoxia, glucose deprivation and so on [17–19]. AMPK is expressed in neurons throughout the developing and adult brain, and more active brain regions have higher AMPK expressions [16]. The activation of AMPK up-regulates the expression of BDNF [20], but switches off mTOR signaling pathway [21]. Therefore, we suggested AMPK may play a key role in the rapid antidepressant effect of ketamine; however, there is no report regarding this aspect.

In the present study, we aimed to determine whether AMPK was involved in the rapid antidepressant effect of ketamine, and to explore the underlying mechanisms.

2. Materials and methods

2.1. Animals

Healthy male Wistar rats weighing 200–280 g were purchased from the Shanghai Animal Center, Shanghai, China. The animals were 6 per cage, maintained in a standard condition with a 12 h light/dark cycle (lights on at 8:00 a.m.), and *ad libitum* access to food and water. Animal uses were in accordance with the National Institutes of Health Guidelines and the study protocol was approved by the Animal Care and Use Committee of Jinling Hospital.

2.2. Drugs and treatments

Ketamine was obtained from Gutian Pharmaceutical Company (China). 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR, an AMPK agonist) and Compound C (An AMPK antagonist) were purchased from Tocris (UK).

In the first stage of experiment, 18 rats were equally randomized into 3 groups: normal group (N group), saline group (S group), and ketamine group (K group). The rats in S and K groups were forced to swim for 15 min. Twenty-four hours later, the rats in the three groups were intraperitoneally injected with the same volume of saline, saline, and 10 mg/kg ketamine, respectively.

In the second stage of experiment, 54 rats were randomly divided into 6 groups ($n = 9$): saline + saline (S group), saline + ketamine (10 mg/kg) (K group), AICAR (10 mg/kg) + saline (A group), AICAR (10 mg/kg) + ketamine (10 mg/kg) (AK group), Compound C (1 mg/kg) + saline (C group), and Compound C (1 mg/kg) + ketamine (10 mg/kg) (CK group). Rats were handled according to the first experiment to establish the acute depression model on the first day. Regarding the co-administration of the drugs, the second drug (treatment) was administrated 30 min after the first drug administration (pretreatment).

Thirty minutes after the second drug administration, the rats in the two experiments were forced to swim for 6 min, and the immobility time of rats in the late 5 min was recorded. Immediately after that, rats were anesthetized (chloral hydrate, 400 mg/kg, i.p.) and hippocampus was removed and stored at −80 °C for later use.

2.3. Forced swimming test (FST)

FST is a widely used depression model to rapidly identify new antidepressant drugs and phenotype genes that manipulate the antidepressant effect in mice [22], an immobility posture of rat is interpreted as an expression of behavioral despair or helplessness [23,24]. We applied this model according to the previous study [15,25]. Animals were subjected to the FST on days 1 and 2. On the first day, the rat was placed in a plexiglas cylinder (65 cm height, 30 cm diameter) filled with 25 °C water to a height of 45 cm for 15 min. After that, the rat was removed, dried, and then placed back to its home cage. On the second day, 30 min after the drug administration, the rat was immersed in water to swim for 6 min. Swimming behavior is interpreted as movement throughout the cylinder, and immobility behavior is defined as a floating or immobile posture, and no additional activity other than that requires to let the rats head above the water is observed [26]. The FST activity of the rat is video recorded for later analysis [27,28].

2.4. Tissue preparation and ELISA

In the first stage of experiment, the level of phosphor Thr172-AMPK (p-AMPK, the activation form of AMPK [29]) in hippocampus ($n = 6$ rats in each group) was detected by sandwich-ELISA. In the second of experiment, the levels of p-AMPK, BDNF, and activity-regulated cytoskeletal associated protein (ARC) in hippocampus ($n = 6$ rats in each group) were also measured by sandwich-ELISA, according to the manufacturer instructions (Chemicon, USA). Generally speaking, hippocampus of the rat was homogenized in saline. Microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in diluent. The plates were washed with PBST buffer (2.0 g NaCl, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4{\cdot}12H_2O$, 0.2 g KCl, 0.2 g NaN_3, 1000 ml distilled water, 0.5 ml Tween 20) for 5 times, and each time 3 min. The levels of proteins were determined by absorbance in 450 nm. The standard curve demonstrated a direct relationship between optical density (OD) and protein concentration. Total proteins were measured by Lowry's method using bovine serum albumin as a standard.

2.5. Tissue preparation and Western blot

In the second stage of the experiment, hippocampus ($n = 3$ rats in each group) was homogenized in the RIPA lysis buffer (pH 7.4) containing 50 mM Tris–HCl, 150 mM NaCl, 1% NP40, 0.25% Na-deoxysodium cholate, and a mixture enzyme inhibitor (0.5 mM EDTA, 1 mM PMSF, 1 mM NaF, 1 mM Na_3VO) according to the instructions. The homogenate was centrifuged at 2.800 rpm at 4 °C for 10 min. After centrifugation, protein concentration was determined by the Pierce Bradford Protein Assay. Equal amount of proteins (15 μg) for each sample were loaded into running buffer for electrophoresis. The proteins were transferred to polyvinylidene difluoride (PVDF) membranes, then the PVDF membranes were incubated with 2% BSA in PBST (PBS and 0.1% Tween-20) at room temperature for 1 h. The incubated PVDF membranes were kept with mTOR and phosphoserine 2448-mTOR (p-mTOR, the activation form of mTOR [30]) antibodies (1:1000, CST, USA) overnight at 4 °C. The next day, blots were washed three times in PBS and incubated with horseradish peroxidase conjugated anti-mouse or anti-rabbit IgG (1:5000) for 1 h at room temperature. Blots were detected using enhanced chemiluminescence (ECL). The blots with specific antibodies were subsequently incubated in the stripping buffer at 50 °C for 30 min. The stripped blots were kept blocking for 1 h and incubated with the primary antibody against β-actin. Image J software was used for the densitometric analysis of each protein.

2.6. Statistical analysis

Statistical analysis was performed by the SPSS 16.0 software. Data are presented as mean ± SEM. Statistical differences among groups were determined by one-way analysis of variance (ANOVA) for the data in the first stage of experiment, and two-way ANOVA for the data in the second stage of experiment followed by least significant difference (LSD) or Dunnett's T3 tests for post hoc analysis. The correlation between the levels of BDNF and ARC was analyzed by Pearson's test. A p value <0.05 was regarded as statistically significant difference.

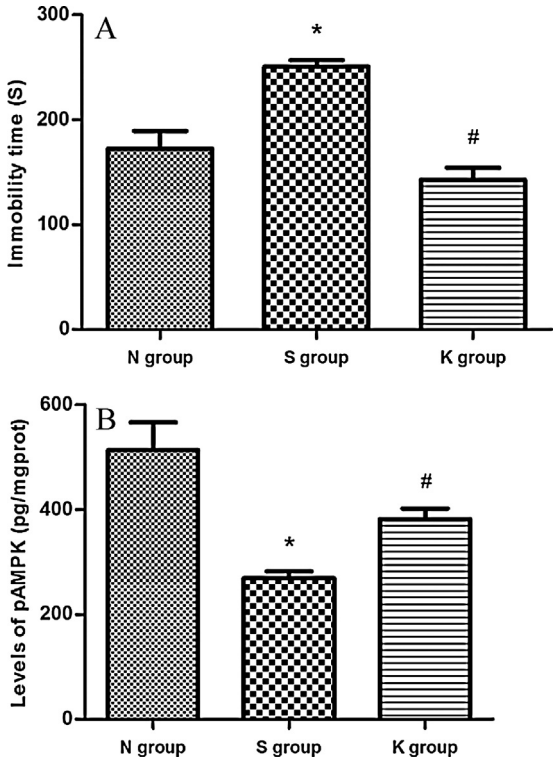

Fig. 1. (A) Effects of ketamine administration on the immobility time of the rats during FST ($n = 6$ rats). The results are expressed as mean ± SEM. Compared with N group, $^{*}p < 0.001$; compared with S group, $^{\#}p < 0.001$. (B) Effects of ketamine administration on the levels of p-AMPK in the rat hippocampus ($n = 6$ rats). The results are expressed as mean ± SEM. Compared with N group, $^{*}p < 0.05$; compared with S group, $^{\#}p < 0.01$.