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Moclobemide up-regulates proliferation of hippocampal progenitor cells in chronically stressed mice¹

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KEY WORDS antidepressants; moclobemide; neurogenesis; stress; brain-derived neurotrophic factor; *N*-methylaspartate

ABSTRACT

AIM: To explore the action mechanism of antidepressants. **METHODS:** The PC12 cell proliferation was detected by flow cytometry. The proliferation of hippocampal progenitor cells and level of brain-derived neurotrophic factor (BDNF) were measured by immunohistochemistry. **RESULTS:** Treatment with *N*-methylaspartate (NMDA) 600 $\mu\text{mol/L}$ for 3 d significantly decreased the percentage of S-phase in PC12 cells, while in the presence of classical antidepressant, moclobemide (MOC) 2 and 10 $\mu\text{mol/L}$, the percentage in S-phase increased. Furthermore, the proliferation of progenitor cells in hippocampal dentate gyrus (subgranular zone), as well as the level of BDNF in hippocampus significantly decreased in chronically stressed mice, while chronic administration with MOC 40 mg/kg (ip) up-regulated the progenitor cell proliferation and BDNF level in the same time course. **CONCLUSION:** Up-regulation of the proliferation of hippocampal progenitor cells is one of the action mechanisms for MOC, which may be closely related to the elevation of BDNF level at the same time. These results also extend evidence for our hypothesis that up-regulation of the hippocampal neurogenesis is one of the common mechanisms for antidepressants.

INTRODUCTION

There are three main kinds of classical antidepressants used in clinic for several decades, including: tricyclic antidepressants (TCAs), such as desipramine (DMI); selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine (FLU); monoamine oxidase inhibitors (MAOIs), such as moclobemide (MOC). Despite of their remarkable structural diversity, they all pos-

sess certain therapeutic effects, but their common action mechanisms are still unclear.

Chronic stress can induce depressive disorder, and animal stress models are also widely used in antidepressant evaluation^[1]. Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons, which may be mediated by activation of the hypothalamic-pituitary-adrenal (HPA) axis acting in concert with the endogenous excitatory amino acid release^[2,3]. More importantly, chronic stress decreases the hippocampal neurogenesis in adult hippocampus. Neurogenesis has been documented in adult brain of a number of different animals, including bird, rodent, and mammalian. In the hippocampus, progenitor cells are located in the subgranule zone where they divide and give rise to new neurons. Stress related psy-

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chotic diseases, such as depression and post traumatic stress disorder (PTSD), are closely associated with the down-regulation of hippocampal neurogenesis^[4-6].

The neurodegenerative lesion and down-regulation of neurogenesis in adult hippocampus may be caused by the hyperaction of HPA axis, and thereby high blood concentration of glucocorticoids which will decrease the brain-derived neurotrophic factor (BDNF) level^[2,4]. Based on related reports and our previous studies, stress-induced accumulation of glutamic acid and its *N*-methylaspartate (NMDA) mediated the actions of glucocorticoids^[7]. Moreover, three kinds of antidepressants all can protect PC12 cells from the corticosterone-induced lesion^[8], and FLU or DMI up-regulates the hippocampal neurogenesis in chronically stressed mice^[9]. But effect of MOC on the neurogenesis is not well demonstrated. In order to explore the possible common action mechanisms for antidepressants, we observed the effects of MOC on the cell proliferation in NMDA-treated PC12 cells and S-phase progenitor cells in subgranule zone of hippocampus in chronically stressed mice.

MATERIALS AND METHODS

Animals Male mice (18±2 g) of the Kunming strain were provided by the Animal Center of Academy of Military Medical Sciences, Beijing (Grade II).

Drugs and reagents MOC (white powder with purity >98 %) was provided by the Chemical Synthesis Lab of our institute; Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were from Gibco BRL (USA). NMDA was from ACROS ORGANICS (USA). BrdU, mouse BrdU monoclonal antibody, BDNF antibody, and the immunohistochemistry detection kits were purchased from Boster Biological Technology Ltd (Wuhan, China).

PC12 cell culture and detection of cell cycle with flow cytometry The PC12 cell line was kindly presented by Dr You WAN in Peking University. The cells were seeded in 12-well plates (Costar, USA) at a density of 2×10^8 cells/L and cultured in the medium consisted of 90 % DMEM, 5 % heat-inactivated horse serum, 5 % fetal bovine serum, benzylpenicillin 200 kU/L, and streptomycin 100 mg/L in a humidified incubator (Napco, USA) with 5 % CO₂ for 24 h. In drug-treated groups, the cells were then incubated with NMDA 600 μmol/L in the presence of MOC 2 and 10 μmol/L for another 3 d. The culture medium was renewed every

1-2 d. For flow cytometry detection, the cells were detached and washed with PBS twice, then resuspended in 1 mL cold 70 % ethanol and stored at -20 °C for 24 h. After being washed with PBS, cells were resuspended in 200 μL PBS containing RNase A 200 mg/L and incubated at 37 °C for 30 min, then centrifuged at 1000×*g* for 10 min. The cells were stained with 300 μL PBS containing propidium iodide 10 mg/L at 4 °C for 30 min (avoiding light). By using FACS Calibur flow cytometry (USA), 2×10^4 cells were counted and percentage of the S-phase cells accumulation was calculated.

Chronic stress procedure in mice and preparation of the brain freezing sections Mice were randomly divided into three groups according to their body weights as normal control, chronic stress control, and stress+MOC (40 mg/kg, ip) group respectively. MOC was injected daily 30 min before each stressor and the control group received only water injections daily. The chronic stress regimen was based on Li *et al*^[10]. Stressors were administered alternately one per day over a period 24 d between 8:30 AM to 10:30 AM. The following seven stressors were used: cold swim (10 °C) for 6 min; overhang for 30 min; foot shock (1 mA, 1-s duration, average 1 shock/min) for 30 min; water deprivation for 24 h; tail pinch (1cm apart from the end of the tail) for 1 min; food deprivation for 24 h; and high speed horizontal shaking for 45 min. For BrdU labeling, mice were administered BrdU (100 mg/kg, ip, twice per d for 2 d) 4 d after the last drug and stressor treatment. Twenty-four hours after the last BrdU injection, mice were killed and transcardially perfused (cold saline for 5 min following by 4 % cold paraformaldehyde for 15 min). After perfusion, all brains were post-fixed overnight in paraformaldehyde at 4 °C and stored at 4 °C in 30 % sucrose. Serial sections of the brains were cut (30 μm sections) through the entire hippocampus on a freezing microtome, and sections were stored at -20 °C.

Immunohistochemistry determination The operating procedure was mainly performed according to the specification of immunohistochemistry kit with some variations. Briefly, after the DNA denaturation and several PBS rinses, sections were then incubated for 30 min in 2 mol/L HCl and then 10 min in 0.1 mol/L borate sodium (pH 8.5). After washing with PBS, sections were incubated for 30 min in methanol containing 0.5 % H₂O₂ to eliminate endogenous peroxidases. After blocking with normal goat serum for 60 min at 37 °C, sections were incubated with anti-mouse BrdU (1:400)

or BDNF (1:400) overnight at 4 °C. Sections were then incubated for 1 h with secondary antibody (biotinylated goat anti-mouse) followed by amplification with an streptavidin-biotin complex, and sections were visualized with DAB for about 45 min. Finally, BrdU-positive cells or BDNF level was observed and pictures were taken with microscope (Olympus BX50, Japan).

Statistics Values were given as mean±SD. Comparisons of groups were made with one-way ANOVA analysis.

RESULTS

Effect of MOC on the percentage of S-phase in NMDA-treated PC12 cells Treatment with NMDA 600 μmol/L for 3 d significantly reduced the percentage of S-phase in PC12 cells, while the percentage of other cell cycle phases did not change notably, indicating that high concentration of NMDA inhibit the proliferation of PC12 cells. In the presence of MOC 2 and 10 μmol/L, however, the percentage of S-phase increased, indicating that MOC up-regulated the proliferation in NMDA-treated PC12 cells (Fig 1).

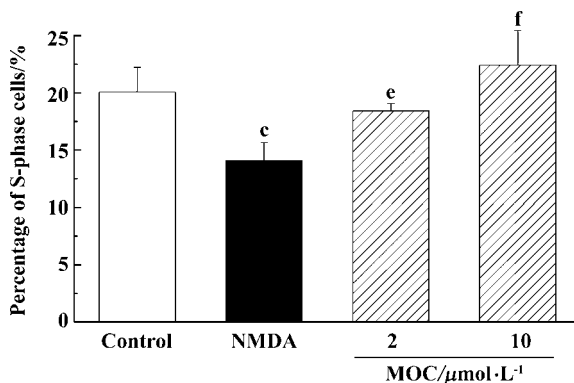


Fig 1. Effect of antidepressants on the percentage of S-phase in NMDA-treated PC12 cells. *n*=5-7. Mean±SD. **P*<0.01 vs control. °*P*<0.05, †*P*<0.01 vs NMDA-treated group. Cells were exposed to NMDA 600 μmol/L in the absence or presence of MOC for 3 d and the percentage of S-phase cells was measured by flow cytometry.

Effect of MOC on the proliferation of hippocampal progenitor cells in chronically stressed mice BrdU is a label for proliferating cells, which can incorporate into DNA in S-phase cells stably. In chronically stressed mice, the BrdU-positive cells (shown as brown granules) in subgranule zone of hippocampus were reduced, or even disappeared compared with the nor-

mal control. While chronic treatment with MOC 40 mg/kg (ip) could reverse these changes and increase the number of BrdU-positive cells in subgranule zone significantly (Fig 2, 3), indicating that MOC up-regulated the proliferation of hippocampal progenitor cells

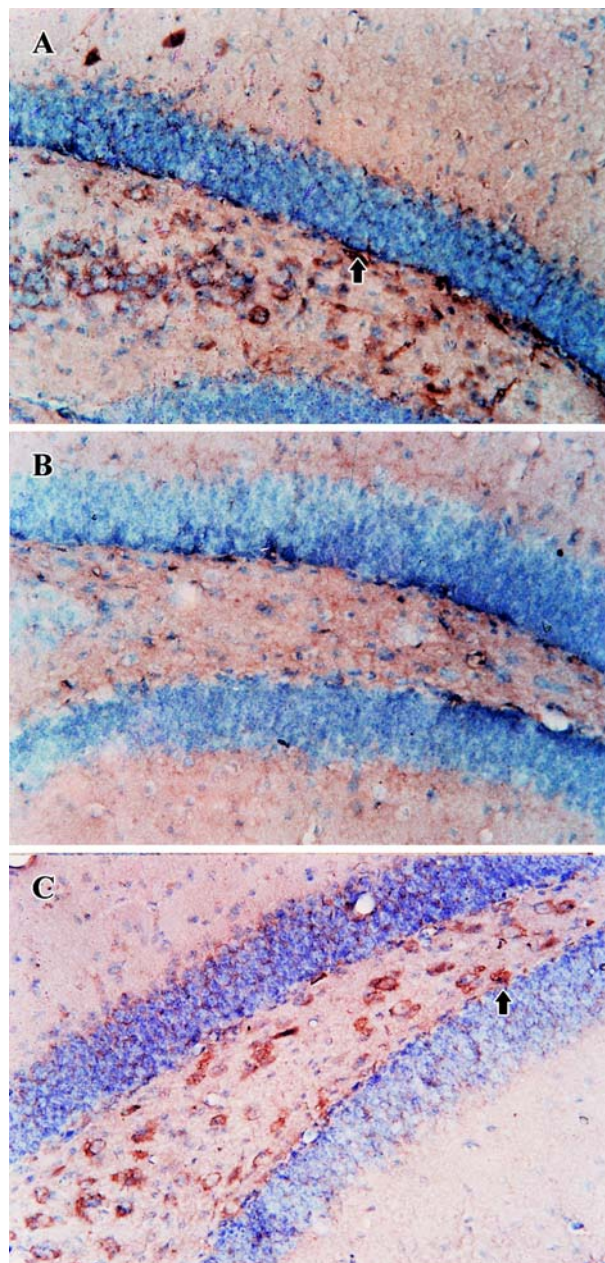


Fig 2. Effect of MOC on the proliferation of hippocampal progenitor cells in chronically stressed mice. A: normal control; B: stress control; C: stress+MOC 40 mg/kg. Chronically stressed mice received injections of BrdU 4 d after the last stressor or drug treatment and were killed 24 h after the last BrdU injection. The BrdU-positive cells in dentate gyrus were detected by immunohistochemistry and shown as brown granules (see arrowheads). ×450.

in chronically stressed mice.

Effect of MOC on the BDNF level in hippocampus of the chronically stressed mice BDNF is a key factor involved in hippocampal neurogenesis. Compared with normal control, BDNF level in the hippocampal subfields including subgranule zone decreased in stressed mice, which was shown as reduction of the brown granules. Chronic treatment with MOC 40 mg/kg (ip) could reverse these changes (Fig 4), indicating that BDNF increase is one of the mechanisms for MOC's action in chronically stressed mice.

DISCUSSION

It has been well demonstrated that hippocampal volume in patients with major depression is reduced compared to healthy controls^[2]. Now, we think that it may be a result of stress-related neurotoxicity and neurogenesis decrease in hippocampus.

Our previous results also showed that three kinds of classical antidepressants all protected PC12 cells, as well as primarily cultured hippocampus neurons from the lesion induced by corticosterone, while antipsychotic drugs, chlorpromazine or diazepam, had no such effect, which suggested that the cytoprotective effect was a common action mechanism for antidepressants^[8]. Studies on several developing new drugs in our lab all supported this hypothesis^[11-13].

Beside of the cytotoxicity, we also found that high concentration of corticosterone elevated the glutamic acid content in PC12 cells at the same time. Moreover, NMDA receptor antagonist, MK801, inhibited the corticosterone-induced lesion in PC12, while the agonist glycyl aggravated it (unpublished data). Combined with corresponding reports^[7], we hypothesize that endogenous glutamic acid release and its NMDA receptors mediate the corticosterone-induced lesion in hippocampal neurons. The "excitotoxic hypothesis" proposes that excessive stimulation of NMDA receptors, such as excessive stimulation or release of glutamic acid, can result in cell death. In fact, NMDA receptors are found in high density in the cerebral cortex, hippocampus, striatum, and amygdala^[14]. In this study, MOC increase the proliferation in NMDA-treated PC12 cells, suggesting that this may be one of the mechanisms for cytoprotection of MOC.

In the hippocampus, progenitor cells are located in the subgranular zone where they proliferate and differentiate into new neurons. A recent analysis reports that there are approximately 9000 new cells per day in

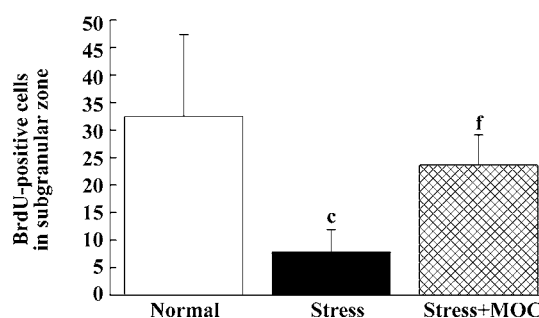


Fig 3. Effect of MOC on the number of BrdU-positive cells hippocampal subgranular zone in chronically stressed mice. *n*=6. Mean±SD. **P*<0.01 vs control. †*P*<0.01 vs stress group.

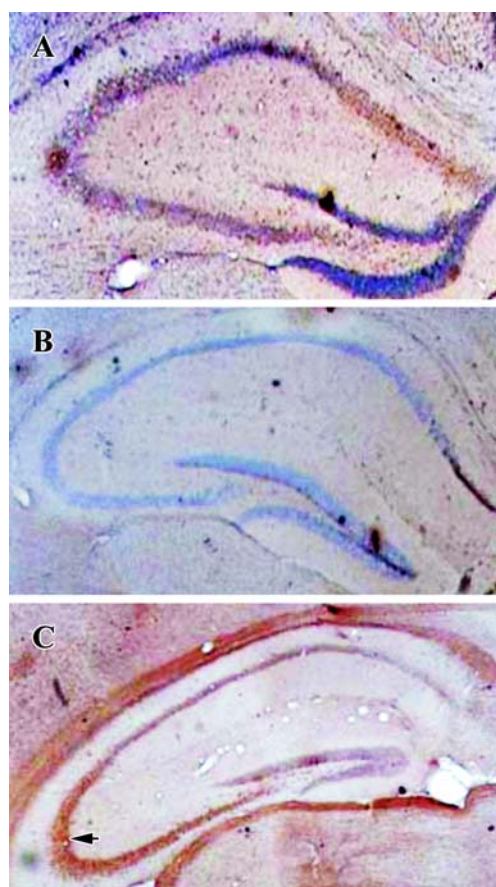


Fig 4. Effect of MOC on the BDNF level in hippocampus of chronically stressed mice. **A:** normal control; **B:** stress control; **C:** stress+MOC 40 mg/kg. The BDNF was detected by immunohistochemistry and shown as brown granules (see arrowheads). ×90.

adult rodent hippocampus^[15]. Approximately 50 % of these cells differentiate and express cellular markers of neurons. It is also estimated that the new granular neurons represents about 60 % of the afferents from the entorhinal cortex and 30 % of CA3 pyramidal cells re-

ceiving efferent projections from granule cells^[5]. In this study, MOC reversed the stress-induced decrease of progenitor cell proliferation in subgranular zone, indicating it can increase the hippocampal neurogenesis in stressed mice. Combined with our previous result^[9], we hypothesize that up-regulation of the stress-induced hippocampal neurogenesis decrease is one of the common mechanisms for antidepressants.

MOC is a monoamine oxidase inhibitor, thereby can increase the levels of brain monoamines [such as serotonin (5-HT), norepinephrine (NE)]. In this study, the dose of 40 mg/kg of MOC in mice is based the references^[16,17], which is also effective in animal behavior models. It has been well reported that 5-HT or NE up-regulates neurogenesis. Moreover, BDNF is also a key downstream factor that mediates neurogenesis^[5]. In this study, MOC reversed the stress-induced decrease of hippocampal of BDNF level in a same time course of the up-regulation of neurogenesis. Our previous studies also demonstrate that antidepressants increase NGF mRNA level in PC12 cells^[8]. So, increase of the 5-HT and/or NE, and downstream of BDNF level is at least part of the mechanisms for neurogenesis up-regulation of MOC.

In summary, all results extend more evidence for our “stress related neural lesion-neurogenesis impairment hypothesis”. Excessive death and neurogenesis down-regulation may lead to decrease of neuron number and transmission, which may be the key reason for depressive disorder. Antidepressants, which exert neuroprotection and neurogenesis up-regulation, could reverse the above unbalance in the brain. It is a new strategy for antidepressant screening and discovery.

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