ORIGINAL ARTICLE

Protective effects of VMY-2-95 on corticosterone-induced injuries in mice and cellular models

Ziru Yu, Dewen Kong, Yu Liang, Xiaoyue Zhao, Guanhua Du*

State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Beijing Key Laboratory of Drug Target and Screening Research, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Received 9 August 2020; received in revised form 29 September 2020; accepted 21 October 2020

KEY WORDS
\(\alpha_4\beta_2\) nAChR antagonist; VMY-2-95; Cholinergic–adrenergic theory; Depression; Corticosterone; SH-SY5Y cells; PKA-CREB-BDNF signaling pathway

Abstract Nicotinic \(\alpha_4\beta_2\) receptor antagonists have drawn increasing attention in the development of new antidepressants. In this study, we aimed to investigate the protective effect of VMY-2-95, the new selective antagonist of \(\alpha_4\beta_2\) nicotinic acetylcholine receptor (nAChR) on corticosterone (CORT) injured mice and cellular models. Fluoxetine was applied as a positive control, and the effects of VMY-2-95 were investigated with three different doses or concentrations (1, 3, 10 mg/kg in mice, and 0.003, 0.03, 0.1 \(\mu\)mol/L in cells). As a result, VMY-2-95 showed significant antidepressant-like effects in the CORT injured mice by improving neuromorphic function, promoting hippocampal nerve proliferation, and regulating the contents of monoamine transmitters. Meanwhile, VMY-2-95 exhibited protective effects on cell viability, cell oxidant, cell apoptosis, and mitochondrial energy metabolism on corticosterone-impaired SH-SY5Y cells. Also, the PKA–CREB–BDNF signaling pathway was up-regulated by VMY-2-95 both in vitro and in vivo, and pathway blockers were also combined with VMY-2-95 to verify the effects furtherly. Therefore, we preliminarily proved that VMY-2-95 had protective effects in depressed mice and against injuries induced by corticosterone. This work indicated that the application of VMY-2-95 is a potential pharmacological solution for depression. This study also supported the development of \(\alpha_4\beta_2\) nAChR antagonists towards neuropsychiatric dysfunctions.

© 2021 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

*Corresponding author.
E-mail address: dugh@imm.ac.cn (Guanhua Du).

Peer review under responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2021.03.002

2211-3835 © 2021 Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
1. Introduction

Depression is a public health threat because it is one primary reason for a person to suicide, and is one of the leading causes of disability around the world. Approximately 800,000 people die of suicide each year due to depressive disorders, and such disorders cause a considerable long-term burden to the general economy and public health system. Although cumulative studies have elucidated the pathophysiology of depression, the antidepressants are still limited to targeting the monoaminergic systems. In clinical studies, current antidepressants are found to be effective in only two-third of depression cases, and they typically need to take a long period to attenuate the depressive symptoms in patients.

The cholinergic–adrenergic theory of depression was first proposed in 1972. It has drawn more and more attention. The hyperactivation of the cholinergic system breaks the homeostasis between cholinergic and noradrenergic systems, leading to the inactivation of choline receptors and triggering depressive symptoms. Receptors are the material basis for the action of the acetylcholine system. α4β2 nicotinic acetylcholine receptor (nAChR) and α7 nAChR are the two antidepressant drug targets mostly discussed in recent years. Most α4β2 nAChR antagonists and a part of α4β2 nAChR agonists were found to exhibit antidepressant effects in both clinical and preclinical studies. Varenicline, a smoking cessation drug targeting α4β2 nAChR, was observed to ease the emotion in depressed patients. In animal studies, sazetidine-A and A-85380, the α4β2 nAChR ligands, showed antidepressant-like effects in tail suspension and forced swimming tests. Thus, it is necessary to develop new rapid-acting antidepressants with fewer side-effects and better efficacy, and α4β2 nAChR might be a potential therapeutic target on antidepressants. However, the effects of α4β2 nAChR in the central nervous system are still not apparent, and the research on efficacy and mechanism of α4β2 nAChR ligands is not detailed.

Stress is considered as the most common and essential factor that leads to depression, and stress paradigms have long been used to mimic critical symptoms of depression. Repeated corticosterone (CORT) treatment induces depression-like behaviors that marked by significant changes in behavioral traits, neurochemistry, and brain morphology. The hypothalamic–pituitary–adrenal (HPA) axis activated in response to stress in depressed patients. A high concentration of blood glucocorticoids (GCs) is reported due to the dysfunction in the feedback mechanism, and then the elevated GCs cause further dysfunction of the HPA axis. Another result is the reduced expression of the brain-derived neurotrophic factor (BDNF), and BDNF is a critical regulator of synaptic plasticity. Therefore, CORT-induced stress-based mouse model and cell injury model are suitable for evaluating the efficacy and mechanisms of potential antidepressants.

VMY-2-95 (Fig. 1), a nAChRs ligand with picomolar affinity and high selectivity for α4β2 receptor, has drawn our attention. We prepared the compound of VMY-2-95·2HCl to get better solubility. Research showed that VMY-2-95·2HCl was soluble in water and chemically stable, and VMY-2-95·2HCl had right drug-like properties and can penetrate the blood–brain barrier with oral administration. The discovery and development of VMY-2-95 may provide new ligand for evaluation in models of depression. In this study, the effects of selective nicotinic α4β2 receptor antagonist VMY-2-95 and the mechanism after CORT treatment are also discussed.

2. Materials and methods

2.1. Drugs and reagents

Compound VMY-2-95·2HCl (purity>99%) was synthesized by the Medical Center for Drug Discovery of Georgetown University (Washington DC, USA), and the structure is shown in Fig. 1. Fluoxetine was purchased from Eli Lilly and Co., USA. CORT was purchased from Aladdin, China. H89 and J147 were purchased from Targetmolecule Corp., USA.

Sodium nitroprusside and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma—Aldrich Chemical Co., USA. Dulbecco’s modified Eagle’s medium and fetal bovine serum were purchased from PAN Seratech Co., Germany. All other reagents were of commercially available analytical grade. Antibodies, including PKA/P-PKA and CREB/P-CREB, were purchased from Cell Signaling Technology, USA. GAPDH was from Proteintech, USA; Ki67, dopamine 2 receptor (D2R), c-FOS and BDNF were from Abcam, UK; β2 nAChR was from Alomone Labs, Israel; 5-hydroxytryptamine 1 receptor (5-HT1AR) was from Novus, USA; and anti-rabbit IgG (H+L) was purchased from Proteintech, USA.

Lactate dehydrogenase (LDH) Cytotoxicity Assay Kit, Total Glutathione Peroxidase (GPx) Assay Kit with NAPDH, Lipid Peroxidation Malondialdehyde (MDA) Assay Kit, Cu/Zn-superoxide dismutase (SOD) and Mn-SOD Assay Kit, Total Antioxidant Capacity (T-AOC) Assay Kit with a rapid ABTS method, Total Nitric Oxide (NO) Assay Kit and Annexin V-FITC Apoptosis Detection Kit were purchased from Beyotime Biotechnology, China. Mitochondrial Complex I Detection Kit was purchased from Jiang Lai Biotechnology, China. ELISA kits were purchased from Beijing Dongge Biotechnology Co., China.

2.2. Animals and grouping

C57BL/6 male mice (6–8 weeks old, weighing 18–22 g) were purchased from Laboratory Animal Centre of Beijing Hua-Fu-Kang Bioscience Co., Ltd. [Beijing, China; Animal certification number was SCXK (Jing) 2014-0004] at the beginning of the experiments. All animals were housed in a room with a controlled temperature of 23 ± 2 °C and humidity of 55 ± 5% with a regular 12 h light–dark cycle and allowed to have water and food ad libitum. Each experimental group consisted of 5–6 animals and all animals were adapted to the laboratory conditions for three days. All animal care and experimental procedures regarding the animals were approved by the Ethnic Committees of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.

A total of 72 C57BL/6 mice were randomly divided into 6 groups: control, model, fluoxetine (3 mg/kg), VMY-2-95 low dose (1 mg/kg), VMY-2-95 middle dose (3 mg/kg) and VMY-2-95 high dose (10 mg/kg). The structure of VMY-2-95·2HCl is shown in Fig. 1.
dose (10 mg/kg) groups. Each group except the control group was intraperitoneally injected with 20 mg/kg CORT daily, and intragastrically administrated of fluoxetine and VMY-2-95 at 1 μL/10 g daily after CORT injection from Day 21 to Day 28. The control group mice were injected or administrated with volume-equal saline.

2.3. Behavioral tests

All the behavioral tests were carried out between 08:30 and 17:30 and matched between the groups. The observers were blind to the treatment. On Day 28, tail suspension test (TST) and forced swimming test (FST) were detected, and on Day 29, sucrose preference test (SPT) and open-field test (OFT) were conducted. See specific steps in Supporting Information.

2.4. Sacrifice and sample preparation

On Day 30, mice were anesthetized with ether, and blood samples were collected by retro orbital puncture. Then the eyeball blood was coagulated for 30 min at room temperature, centrifuged to separate plasma, and stored at −20 °C. Among them, three mice of every group transcervically perfused with PBS, followed by 4% formaldehyde fixation and the whole brain samples were post-fixed in 4% paraformaldehyde to perform immunohistochemistry analyses, and the hippocampus of the rest mice brains were preserved after blood collection and stored at −80 °C for Western blotting.

2.5. Neuronal morphology testing

Histologic staining was performed using cresyl violet Nissl stain to study the number, structural integrity of neurons, and the cell fullness in the hippocampus of mice.

2.6. Assay of neurotransmitter contents

The content of dopamine (DA), 5-hydroxytryptamine (5-HT), acetylcholine (ACH), norepinephrine (NE), CORT, adreno-corticotrophic hormone (ACTH), and corticotropin releasing hormone (CRH) in the plasma supernatant was estimated on a microplate reader. Cell viability and cytotoxicity/mortality were calculated according Eqs. (1) and (2):

\[
\text{Cell viability (\%) = } \left( \frac{A_{\text{experiment}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \right) \times 100
\]

\[
\text{Cytotoxicity or mortality (\%) = } \left( \frac{A_{\text{experiment}} - A_{\text{blank}}}{A_{\text{absorbance of cell maximum enzyme activity}}} \right) \times 100
\]

2.9. Cell viability and LDH cytotoxicity assay

The cells were collected when they had grown to 80%—90% confluence, then the cells were treated with the different concentrations of CORT for 24 h or preincubated with VMY-2-95 at gradient concentration (0.003, 0.03 and 0.1 μmol/L) for 2 h before the CORT exposure. The control group cells were added with volume-equal medium without CORT, and the model group had no preincubation with VMY-2-95. Then the supernatant was aspirated for LDH assay, and MTT assay (0.5 g/L) was conducted to 96-well plate for 4 h at 37 °C. The LDH was measured at 490 nm and cell viability at 570 nm using a microplate reader. Cell viability and cytotoxicity/mortality were calculated according Eqs. (1) and (2):

\[
\text{Cell viability (\%) = } \left( \frac{A_{\text{experiment}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \right) \times 100
\]

\[
\text{Cytotoxicity or mortality (\%) = } \left( \frac{A_{\text{experiment}} - A_{\text{blank}}}{A_{\text{absorbance of cell maximum enzyme activity}}} \right) \times 100
\]

2.10. Assay of extracellular NO

The content of NO was measured following the instructions of the kit using the Griess reaction. The absorbance was measured at 540 nm.

2.11. Assay of GPs, MDA, SOD, T-AOC and adenosine triphosphate (ATP) in SH-SY5Y cells

After incubating the cells with drug for a corresponding period of time, the contents of GPs, MDA, SOD as antioxidant indexes and the T-AOC were tested, and the contents of ATP and the activity of mitochondrial complex I were also tested on a microplate reader according to the guidelines of assay kits.

2.12. Assay of cytochrome c (Cyt-c) contents

The contents of Cyt-c in the cell culture fluid were estimated on a microplate reader of which the absorbance was read at 450 nm according to the guidelines of the ELISA kit as per the methodology provided by the manufacturer.

2.13. Flow cytometry detection of apoptosis

The cells were trypsinized and resuspended by centrifugation to make cell suspension. Annexin V-FITC binding fluid, Annexin V-FITC, and propidium iodide staining solution were added in order and then tested in BD FACS Aria II flow cytometry within 1 h.

2.14. Western blotting

The lysate was added into mice hippocampus tissue and SH-SY5Y cells, and then supernatant after centrifugation was collected, and the protein concentration was determined using bicinchoninic acid protein assay kits. After mixed with 5× loading buffer and heated at 100 °C for 15 min, protein extracts were separated by a sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto a polyvinylidene fluoride membrane. The blots were blocked using 5% bovine serum albumin in Tris buffered saline with TBST buffer for 1.5 h and then were incubated at 4 °C overnight with respective primary antibodies diluted in 1% bovine serum albumin for anti-P-PKA.
antibody (1:1000), anti-P-CREB (1:1000), anti-BDNF (1:1000) or GAPDH (internal control, 1:1000). Subsequently, membranes were washed using TBST for 4 × 5 min and were incubated with secondary antibody conjugated to horseradish peroxidase (1:5000) for 1 h at room temperature. Then protein membranes were washed in the same way, and finally, bands were detected by enhanced chemiluminescence Western blotting detection reagents. Membranes marking P-PKA and P-CREB were re-incubated with primary antibodies for PKA (1:1000) and CREB (1:1000) after stripping for 15 min, and the next steps were as mentioned above.

2.15. Statistical analysis

All data were analyzed using the SPSS 22.0 software package and expressed as mean ± standard error means (SEM) at the animal level.

Figure 2  Behavior improving effects of VMY-2-95 in mice induced by corticosterone (CORT). (A) Changes in body weight, (B) immobility time in the tail suspension test (TST), (C) immobility time in the forced swimming test (FST), (D) changes in percent of sucrose preference, (E1) motion track in the open-field test (OFT), (E2) percent of central grid crossing number in the OFT, (E3) percent of central grid crossing time in the OFT. Data are presented as mean ± SEM, n = 12. **P < 0.01, ***P < 0.001 versus control group in A; and *P < 0.05, **P < 0.01, ***P < 0.001 versus control group, and *P < 0.05, **P < 0.01, ***P < 0.001 versus model group in B–E.
Figure 3  The effects of VMY-2-95 on neuron morphology and proliferation. (A) Nissl denaturation staining in the hippocampus (400×, scale bar indicates 100 μm), (B1) immunohistochemistry pictures of Ki67 in different groups of mice (400×, scale bar indicates 100 μm), (B2) the mean optical density of Ki67 in hippocampus, (C1) immunohistochemistry pictures of c-FOS in different groups of mice (400×, scale bar indicates 100 μm), (C2) the mean optical density of c-FOS in hippocampus, (D) the effect of different concentrations of corticosterone (CORT) on the viability of cells, (F) the effect of different concentrations of VMY-2-95 on the viability of cells, (E) the effect of VMY-2-95 on the cell morphology (200×, scale bar indicates 100 μm), (G) the protect effect of VMY-2-95 on the cell viability, and (H) the protect effect of VMY-2-95 on the cytotoxicity. Data are presented as mean ± SEM, n = 3 in mice; and data are represented as the mean ± SD, n = 3 in SH-SY5Y cells. *P < 0.05, **P < 0.01, ***P < 0.001 versus control cells in D and E; and *P < 0.05, **P < 0.01, ***P < 0.001 versus control group, and *P < 0.05, **P < 0.01, ***P < 0.001 versus model group in the others.
behavior tests and mean ± standard deviation (SD) at the tissue and cell level. The figure was drawn by Origin 2018, Adobe Photoshop CC 2018, and Adobe Illustrator 2018 software. Results in vitro were analyzed by T-test and one-way ANOVA followed by Tukey’s post hoc test. Probability (P) values less than 0.05 were considered statistically significant.

3. Results

3.1. VMY-2-95 modified the depression-like and anxiety-like behaviors of mice with CORT treatment

After CORT injection, the mice showed significant weight drop. While VMY-2-95 and fluoxetine were observed to increase the mice body weight after one week of treatment (Fig. 2A). Mice received CORT injection also showed distinct depression-like behaviors in the TST, FST, and SPT. VMY-2-95 significantly decreased the immobility time in TST (Fig. 2B) and FST (Fig. 2C), and increased the percent of sucrose preference (Fig. 2D) in SPT. Moreover, the antidepressant-like effects of VMY-2-95 at 3 and 10 mg/kg dose were similar in FST, SPT, and were better than those of fluoxetine. In the open field test, the effect of VMY-2-95 and fluoxetine treatment can be visually reflected by the mice’s motion track (Fig. 2E). After 4 weeks of CORT injection, mice of the model group showed significantly reduced total crossing number, central grid crossing number percent and time compared with the control mice. VMY-2-95 at three different doses all significantly reversed these changes reflecting avoidance behaviors of mice, and the effect of fluoxetine was similar as that of low-dose VMY-2-95 (Fig. 2E2–E4).

3.2. VMY-2-95 protected the morphology and vitality of neurons against the injury induces by CORT

As shown in Fig. 3A, the neurons in the hippocampus were sparse in the CORT model group, the number of cell layers significantly reduced, and cell shrinkage and fragmentation occurred. VMY-2-95 administration conferred excellent protection in tissue morphology. The expression of KIf67, an indicator for detecting cell proliferation activity in mice, was significantly decreased, and the expression of c-FOS was also significantly decreased induced by the stress response. VMY-2-95 significantly alleviated these changes (Fig. 3B and C). As shown in Fig. 3D, we chose different concentrations at 50, 100, 200, 400, 800, 1000, 1200, and 1600 μmol/L of CORT to detect the cell viability. The CORT was found to inhibit the proliferation of SH-SY5Y cells with a significant concentration-dependent manner. Furthermore, we chose 800 μmol/L CORT, of which the inhabitation was around 50% to carry out the follow-up injury study. Then the effects of different concentrations at 0.1, 1, 3, 10, 30, and 100 μmol/L of VMY-2-95 on the cell viability were detected, which were toxic to cells only when concentrations were above 30 μmol/L (Fig. 3E). As shown in Fig. 3F and G, 0.1 μmol/L VMY-2-95 showed significant protection on the cell viability and morphology against injury induced by CORT, and consistent with this, VMY-2-95 significantly decreased the LDH content of CORT-injury SH-SY5Y cells (Fig. 3H).

3.3. VMY-2-95 regulated transmitter contents and receptor expression

As shown in Fig. 4A and B, the ACh content in the mice injected with CORT significantly increased compared with the control group, and the NE content significantly decreased. Fluoxetine and VMY-2-95 increased the content of NE, but fluoxetine had no effect on the content of ACh, and VMY-2-95 made the content of ACh higher than that of the model group. As shown in Fig. 4D, the 5-HT content of the mice injected with CORT significantly decreased compared with that of the control group, and VMY-2-95 alleviated the change. The mean optical density of DA receptors and 5-HT receptors in the DG region of the hippocampus was also detected, and we found that the expression of D2R and 5-HT1AR were both significantly decreased. VMY-2-95 increased the expression of 5-HT1AR, but fluoxetine did not affect the changes of both D2R and 5-HT1AR (Fig. 4E and F). Around the HPA axis, we tested the contents of related transmitters in mice. Results show that CRH, ACTH and CORT of the model mice significantly increased, and VMY-2-95 effectively reduced the excessive release of these three transmitters (Fig. 4G).

3.4. VMY-2-95 exerted neuroprotective effects on SH-SY5Y cells through different mechanisms

As shown in Fig. 5A–E, VMY-2-95 significantly inhibited the increased NO and MDA content induced by CORT, and increased GPs and SOD content with a concentration-dependent manner, thus significantly increased the total antioxidant capacity of SH-SY5Y cells. As shown in Fig. 5F, flow cytometry showed that CORT caused significant cell apoptosis, and VMY-2-95 significantly increased cell viability and inhibited the apoptosis with a good dose–effect relationship. Meanwhile, CORT caused a significant increase in the content of Cyt-c, and VMY-2-95 significantly decreased this change in SH-SY5Y cells (Fig. 5G). Furthermore, as shown in Fig. 5H and I, VMY-2-95 significantly increased the relative value of intracellular ATP level and the activity of mitochondrial complex I on SH-SY5Y cells, which provided reliable protection for mitochondrial energy metabolism.

3.5. VMY-2-95 had effects on the PKA–CREB–BDNF signaling pathway

As shown in Fig. 6A and B, both the PKA and CREB phosphorylation levels in the hippocampus of mice injected with CORT were significantly decreased compared with those of the control group, and the expression levels of BDNF was also significantly decreased (Fig. 6C and D). Fluoxetine and VMY-2-95 ameliorated these changes, and VMY-2-95 was found to significantly increase the expression of P-PKA, P-CREB, and BDNF. Consistent with the mice experiment results, pretreatment with VMY-2-95 significantly ameliorated this down-regulation of P-PKA, P-CREB and BDNF expression in SH-SY5Y cells (Fig. 6E–G). We retested the protective effect of VMY-2-95 when combined with PKA inhibitor H89 and BDNF inhibitor J147. Results show that compound VMY-2-95 almost lost its protection on SH-SY5Y cells when the PKA-BDNF pathway blocked. Both the cell viability and NO release content had no more significant difference compared with those of the model group (Fig. 6H and I).

4. Discussion

Depression, the most prevalent psychiatric disorder, is characterized by increased negative affect (i.e., depressed mood) and reduced positive affect (i.e., anhedonia). The development of antidepressants with new targets needs to be studied as soon as
In this area, choline receptor antagonists show great potential. In this area, choline receptor antagonists show great potential. Neuronal $\alpha_4\beta_2$ nAChR is a receptor involved in the role of neurotransmitters regulation and release, and this ionic channel participates in the biological process of memory, learning, and attention. The $\alpha_4\beta_2$ nAChR subtype is a remarkable therapeutic target since this is one of the most abundant receptors in the central nervous system. Many selective $\alpha_4\beta_2$ nAChR partial agonists such as sazetidine-A and nAChR antagonist such as mecamylamine can regulate depression-like behaviors, and researches showed that VMY-2-95 as a selective $\alpha_4\beta_2$ nAChR antagonist could be developed for an antidepressant.

Stress in our daily life severely results in the dysregulation of the HPA axis and then implicates in the development of depression. It has demonstrated that CORT-mediated hormonal disorders are the direct cause of depressed patients, and exogenous CORT administration could develop mouse depression model mimicking HPA-axis induced depression-like state, leading to depression-like indexes such as reduced sucrose intake and increased immobility time in TST and FST. We established the depression model by a daily injection of 20 mg/kg CORT for 21 consecutive days as reported. In our experiments, VMY-2-95 showed significant antidepressant-like effects in SPT, TST, FST, OFT with an excellent dose-effect relationship, and the effects were more pronounced compared with fluoxetine. As the transmitters play an essential effect on neural activity, and significant changes occurred in the signal pathway involving both pathology and pharmacology, hence, we investigated the mechanism of

Figure 4 The effects of VMY-2-95 on contents of transmitter and expression of the receptors. The contents of acetylcholine (ACh, A) and norepinephrine (NE, B) in the plasma supernatant of mice, and the contents of dopamine (DA, C) and 5-hydroxytryptamine (5-HT, D), and the mean optical density of dopamine 2 receptor (D2R, E1) in dentate gyrus region of hippocampus, (E2) immunohistochemistry pictures of D2R in different groups of mice (400x, scale bar indicates 100 μm), (F1) the mean optical density of 5-hydroxytryptamine 1 receptor (5-HT1AR) in dentate gyrus region of hippocampus, (F2) immunohistochemistry pictures of 5-HT1AR in different groups of mice (400x, scale bar indicates 100 μm), contents of corticotropin releasing hormone (CRH, G1), adreno-cortico-tropic-hormone (ACTH, G2), and corticosterone (CORT, G3). Data are presented as mean ± SEM, n = 3 in E and F, and n = 10–12 in others. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus control group, and $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ versus model group.
VMY-2-95 in terms of transmitters and pathway changes. Further, we investigated the manifestations of anti-oxidant, anti-apoptosis and mitochondrial energy metabolism at the cellular level. Thereby the mechanisms of the antidepressant potential of VMY-2-95 were advanced.

CORT can cross the blood-brain barrier, and corticosteroid receptors enrich in the hippocampus. Therefore, certain hippocampal functions are susceptible to disruption by stress. It is reported that GCs and antidepressants modulated neurogenesis in opposing directions. Chronic CORT exposure affected the proliferation of progenitor cells in the dentate gyrus of the hippocampus, and hippocampal neurogenesis is required for treatment in the depressed model induced by CORT. Here, we showed that chronic CORT treatment damaged hippocampus morphology, reduced the expression of Ki67 and c-FOS, while VMY-2-95 effectively improved hippocampal neurogenesis in depressed mice. Consistent with this, VMY-2-95 protected the cell viability of SH-SY5Y cells well against injury induced by CORT. VMY-2-95 had significant protective effects at both tissue morphology and cell levels.

Many first-line antidepressants are 5-HT and norepinephrine reuptake inhibitors. It is reported that neurotransmitter and metabolite disorder was involved in the process of depression, such as 5-HT, DA, monoamine oxidase, NE. We showed that VMY-2-95 could significantly recover the level of 5-HT and the corresponding receptor, but the effect of VMY-2-95 on regulating the content of DA was not detected. Consistent with the cholinergic system theory of depression, we found the significantly increased ACh content and decreased NE content in model mice. However, the VMY-2-95 only alleviated the change of NE but further increased the content of ACh instead, and we thought it was due to the properties of the antagonist of nAChRs that VMY-2-95 increased the content of ACh directly. It is worth mentioning that the action of VMY-2-95 inhibited the entire over-activated cholinergic system.

Extensive clinical and animal research evidence has shown that chronic stress leads to over-activation of the HPA axis and concomitant elevation of GCs production. Furthermore, the remarkable roles of the HPA axis and BDNF playing in the actions of antidepressants have also been demonstrated. BDNF is a neurogenesis marker involved in cell differentiation, neuronal survival, migration, and synaptic plasticity, and the PKA-CREB-BDNF signaling pathway is the classic signaling pathway studied in depression research. In our experiments, VMY-2-95 was found to regulate the HPA axis function by decreasing the content of GCs.
CRH, ACTH and CORT. Meanwhile, VMY-2-95 was found to regulate the PKA–CREB–BDNF signaling pathway both in CORT injured mice and SH-SY5Y cells damaged by CORT.

Moreover, CORT causes nerve damage in many ways, such as the concomitant oxidative damage\(^5\), energy metabolism damage\(^5\), and cell apoptosis. We initially confirmed that VMY-2-95 significantly elevated the activity of antioxidants such as SOD and GPs, and reduced the levels of oxidative damage markers (NO and MDA) to increase the antioxidant capacity of SH-SY5Y cells. Also, VMY-2-95 effectively reduced apoptosis and decreased the content of Cyt-c. Besides, VMY-2-95 increased the level of ATP and the activity of mitochondrial complex I to rescue impaired mitochondrial function caused by CORT treatment. Thus, VMY-2-95 provided comprehensive protection of SH-SY5Y cells by various routes.

We have initially proved the antidepressant-like effect of the selective nicotinic \(\alpha_4\beta_2\) receptor antagonist VMY-2-95 both in vitro and in vivo, suggesting that \(\alpha_4\beta_2\) nAChR would be a new target for the development of antidepressants. Multiple targets and mechanisms involved in anti-depression and neuroprotection of VMY-2-95 should be further discussed.

5. Conclusions

In conclusion, our results reveal that VMY-2-95 reversed depression-like behaviors in chronic CORT-induced depression model in C57 mice by improving neuromorphic function, promoting hippocampal nerve proliferation, and regulating the contents of monoamine transmitters through the PKA–CREB–BDNF signaling pathway. Meanwhile, VMY-2-95 effectively protected

---

Figures 6

Effects of VMY-2-95 on the PKA–CREB–BDNF signaling pathways. The protein expressions of (A) P-PKA and PKA, (B) P-CREB and CREB, and (C) BDNF in hippocampus; (D1) immunohistochemistry pictures of BDNF in different groups of mice (400x, scale bar indicates 100 \(\mu\)m); (D2) the mean OD of BDNF in hippocampus; the protein expressions of (E) P-PKA and PKA, (F) P-CREB and CREB, and (G) BDNF in SH-SY5Y cells; (H) cell viability when VMY-2-95 combined with H89 or J147; and (I) NO content when VMY-2-95 combined with H89 or J147. Data are presented as mean ± SEM, \(n = 12\) in A–C; and data are presented as mean ± SD, \(n = 3\) in others. \(*P < 0.05, **P < 0.01, ***P < 0.001\) versus control group, and \(*P < 0.05, **P < 0.01, ***P < 0.001\) versus model group.
SH-SY5Y cells against injury induced by CORT through protecting the cell viability, improving antioxidant and anti-apoptosis capacity, improving mitochondrial energy metabolism. Our experiments suggested that VMY-2-95 is a promising lead compound as well as improving mitochondrial energy metabolism. Our experiments analyzed data; Ziru Yu wrote the paper. All authors reviewed the manuscript.

Conflicts of interest
The authors declare no conflict of interest.

Appendix A. Supporting information
Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2021.03.002.

References
1. GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 2017;390:1211–59.
2. World Health Organization (WHO). Depression. World Health Organization. Published [February 2017]. Updated [30 January 2020]. Accessed [25 June, 2019]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/depression.
3. Chang T, Fava M. The future of psychopharmacology of depression. J Clin Psychiatry 2010;71:971–5.
4. Gould TD, Zarate Jr CA, Thompson SM. Molecular pharmacology and neurobiology of rapid-acting antidepressants. Annu Rev Pharmacol Toxicol 2019;59:213–36.
5. Janowsky DS, el-Yousef MK, Davis JM, Sekerke HJ. A cholinergic-adrenergic hypothesis of mania and depression. Lancet 1972;2:632–5.
6. Yu LF, Zhang HK, Caldaroni BJ, Eaton JB, Lukas RJ, Kozikowski AP. Recent developments in novel antidepressants targeting α4β2 nicotinic acetylcholine receptors. J Med Chem 2014;57:8204–23.
7. Kalkman HO, Feuerbach D. Modulatory effects of α7 nAChRs on the immune system and its relevance for CNS disorders. Cell Mol Life Sci 2016;73:2511–30.
8. Yu Ziru, Du Guanhua. The research advance on the α4β2 acetylcholine receptor and depression. Acta Pharmac Sin 2018;53:1583–90.
9. Jurado-Coronel JC, Avila-Rodriguez M, Capani F, Gonzalez J, Moran VE1, Barreto GE. Targeting the nicotinic acetylcholine receptors (nAChRs) in astrocytes as a potential therapeutic target in Parkinson’s disease. Curr Pharm Des 2016;22:1305–11.
10. Aboul-Fotouh S. Behavioral effects of nicotinic antagonist mecamylamine in a rat model of depression: prefrontal cortex level of BDNF protein and monoaminergic neurotransmitters. Psychopharmacology 2015;232:1095–105.
11. Wu J, Cippitelli A, Zhang Y, Debevec G, Schoch J, Ozawa A, et al. Highly selective and potent α4β2 nAChR antagonist inhibits nicotine self-administration and reinstatement in rats. J Med Chem 2017;60:1092–104.
12. Kausch O. Treatment of depression in a former smoker with varenicline? A case report and discussion. J Neuropsychiatry Clin Neurosci 2014;26:172–5.
13. Saricicek A, Esterlis I, Maloney KH, Mineur YS, Ruf BM, Muralidharan A, et al. Persistent β2α4nicotinic acetylcholine receptor dysfunction in major depressive disorder. Am J Psychiatry 2014;169:551–9.
14. Andreasen JT, Nielsen EØ, Christensen JK, Olsen GM, Peters D, Mitra NR, et al. Subtype-selective nicotinic acetylcholine receptor agonists enhance the responsiveness to citalopram and reboxetine in the mouse forced swim test. J Psychopharmacol 2011;25:1347–56.
15. Blum AP, Van Arnem EB, German LA, Lester HA, Dougherty DA. Binding interactions with the complementary subunit of nicotinic receptors. J Biol Chem 2013;288:6991–7.
16. Yu LF, Tuckmantel W, Eaton JB, Caldaroni B, Fedolak A, Hanania T, et al. Identification of novel α4β2 nicotinic acetylcholine receptor (nAChR) agonists based on an isoxazol ether scaffold that demonstrate antidepressant-like activity. J Med Chem 2012;55:812–23.
17. Zhang HK, Eaton JB, Yu LF, Nys M, Mazzolari A, van Elk R, et al. Insights into the structural determinants required for high-affinity binding of chiral cyclopropane-containing ligands to α4β2-nicotinic acetylcholine receptors: an integrated approach to behaviorally active nicotinic ligands. J Med Chem 2012;55:8028–37.
18. Zhang H, Tuckmantel W, Eaton JB, Yuan PW, Yu LF, Bajaj KM, et al. Chemistry and behavioral studies identify chiral cyclopropanes as selective α4β2 nicotinic acetylcholine receptor partial agonists exhibiting an antidepressant profile. J Med Chem 2012;55:717–24.
19. Liu J, Yu LF, Eaton JB, Caldaroni B, Cavino K, Ruiz C, et al. Discovery of isoxazole analogues of zanetidin-A as selective α4β2 nicotinic acetylcholine receptor partial agonists for the treatment of depression. J Med Chem 2011;54:7280–8.
20. Yu LF, Eaton JB, Fedolak A, Zhang HK, Hanania T, Brunner D, et al. Discovery of highly potent and selective α4β2 nicotinic acetylcholine receptor (nAChR) partial agonists containing an isoxazolopyridine ether scaffold that demonstrate antidepressant-like activity. J Med Chem 2012;55:9998–10009.
21. Buckley MJ, Surowy C, Meyer M, Curzon P. Mechanism of action of A-85380 in an animal model of depression. Prog Neuro-Psychopharmacol Biol Psychiatry 2004;28:723–30.
22. Gupta D, Radhakrishnan M, Kurhe Y. Effect of a novel 5-HT 3 receptor antagonist 4i, in corticosterone-induced depression-like behavior and oxidative stress in mice. Steroids 2015;96:95–102.
23. Ali SH, Madhana RM, K V A, Kasala ER, Bodduluri LN, Pitta S, et al. Resveratrol ameliorates depressive-like behavior in repeated corticosterone-induced depression in mice. Steroids 2015;101:37–42.
24. Xie X, Shen Q, Ma L, Chen Y, Zhao B, Fu Z. Chronic corticosterone-induced depression mediates premature aging in rats. J Affect Disord 2018;229:254–61.
25. Keller J, Gomez R, Williams G, Lemble A, Lazzaroni L, Murphy GM Jr, et al. HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. Mol Psychiatry 2017;22:527–36.
26. Caviedes A, Lafourcade C, Soto C, Wynenek U. BDNF/NF-eB signaling in the neurobiology of depression. Curr Pharm Des 2017;23:3154–63.
27. Ma H, Wang W, Xu S, Wang L, Wang X. Potassium 2-(1-hydroxypentyl)-benzoate improves depressive-like behaviors in rat model. Acta Pharmac Sin B 2018;8:881–8.
28. Yenugonda VM, Xiao Y, Levin ED, Rezvani AH, Tran T, Al-Muhtasib N, et al. Design, synthesis and discovery of picomolar...
selective α4β2 nicotinic acetylcholine receptor ligands. J Med Chem 2013;56:8404–21.
29. Kong H, Song JK, Yenugonda VM, Zhang L, Shuo T, Cheenha AK, et al. Preclinical studies of the potent and selective nicotinic α4β2 receptor ligand VMY-2-95. Mol Pharm 2015;12:393–402.
30. Han J, Wang DS, Liu SB, Zhao MG. Cytisine, a partial agonist of α4β2 nicotinic acetylcholine receptors, reduced unpredictable chronic mild stress-induced depression-like behaviors. Biomol Ther (Seoul) 2016;24:291–7.
31. Mineur YS, Mose TN, Blakeman S, Picciotto MR. Hippocampal α2 nicotinic ACh receptors contribute to modulation of depression-like behaviour in C57BL/6J mice. Br J Pharmacol 2018;175:1903–14.
32. Laikowski MM, Reisdorfer F, Moura S. NAcHR α4β2 subtype and their relation with nicotine addiction, cognition, depression and hyperactivity disorder. Curr Med Chem 2019;26:3792–811.
33. Adermark L, Söderpalm B, Burkhardt M. Brain region specific modulation of ethanol-induced depression of GABAergic neurons in the brain reward system by the nicotine receptor antagonist mecamylamine. Alcohol 2014;48:455–61.
34. Gupta D, Radhakrishnan M, Kurhe Y. Effect of a novel 5-HT3 receptor antagonist 4i, in corticosterone-induced depression-like behavior and oxidative stress in mice. Steroids 2015;96:95–102.
35. Mao QQ, Huang Z, Zhong XM, Xian YF, Ip SP. Piperine reverses the effects of corticosterone on behavior and hippocampal BDNF expression in mice. Neurochem Int 2014;74:36–41.
36. Pitta S, Augustine BB, Kasala ER, Sulakhiya K, Ravindranath V, Lahkar M. Honokiol reverses depression-like behavior and decrease in brain BDNF levels induced by chronic corticosterone injections in mice. Pharmacogn J 2013;5:211–5.
37. Lee B, Sur B, Shim I, Lee H, Hahn DH. Angelica gigas ameliorates depression-like symptoms in rats following chronic corticosterone administration. BMC Complement Altern Med 2015;15:210.
38. Zhang L, Zhang J, Sun H, Liu H, Yang Y, Yao Z. Exposure to enriched environment restores the mRNA expression of mineralocorticoid and glucocorticoid receptors in the hippocampus and ameliorates depression-like symptoms in chronically stressed rats. Curr Neurovasc Res 2011;8:286–93.
39. Ma L, Shen Q, Yang S, Xie X, Xiao Q, Yu C, et al. Effect of chronic corticosterone-induced depression on circadian rhythms and age-related phenotypes in mice. Acta Biochim Biophys Sin (Shanghai) 2018;50:1236–46.
40. Luassen PJ, Oomen CA, Naninck EF, Fitzsimons CP, van Dam AM, Czeh B, et al. Regulation of adult neurogenesis and plasticity by (early) stress, glucocorticoids, and inflammation. Cold Spring Harb Perspect Biol 2015;7:a021303.
41. Murata K, Fujita N, Takahashi R, Inui A. Ninjinjyoite improves behavioral abnormalities and hippocampal neurogenesis in the corticosterone model of depression. Front Pharmacol 2018;9:1216.
42. Latendresse G, Elmore C, Deneris A. Selective serotonin reuptake inhibitors as first-line antidepressant therapy for perinatal depression. J Midwifery Womens Health 2017;62:317–28.
43. Koenig AM, Thase ME. First-line pharmacotherapies for depression—what is the best choice?. Pol Arch Med Wewn 2009;119:478–86.
44. Huang SJ, Zhang XH, Wang YY, Pan JH, Cui HM, Fang SP, et al. Effects of kaixin jieyu decoction on behavior, monoamine neurotransmitter levels, and serotonin receptor subtype expression in the brain of a rat depression model. Chin J Integr Med 2014;20:280–5.
45. Tan T, Wang YQ, Wang J, Wang ZW, Wang H, Cao HQ, et al. Targeting peptide-decorated biomimetic lipoproteins improve deep penetration and cancer cells accessibility in solid tumor. Acta Pharm Sin B 2020;10:529–45.
46. Chen JL, Shi BY, Xiang H, Hou WJ, Qin XM, Tian JS, et al. 1H NMR-based metabolic profiling of liver in chronic unpredictable mild stress rats with genipin treatment. J Pharm Biomed Anal 2015;115:150–8.
47. Higley MJ, Picciotto MR. Neumodulation by acetylcholine: examples from schizophrenia and depression. Curr Opin Neurobiol 2014;29:88–95.
48. Mineur YS, Obayemi A, Wigstrønd MB, Fote GM, Calaro CA, Li AM, et al. Cholinergic signaling in the hippocampus regulates social stress resilience and anxiety- and depression-like behavior. Proc Natl Acad Sci U S A 2013;110:3573–8.
49. Pariante CM. Why are depressed patients inflamed? A reflection on 20 years of research on depression, glucocorticoid resistance and inflammation. Eur Neuropsychopharmacol 2017;27:554–9.
50. Kunugi H, Hori H, Adachi N, Numakawa T. Interface between hypothalamic–pituitary–adrenal axis and brain-derived neurotrophic factor in depression. Psychiatry Clin Neurosci 2010;64:447–59.
51. Han YX, Tao C, Gao XR, Wang LL, Jiang FH, Wang C, et al. BDNF-related imbalance of copine 6 and synaptic plasticity markers couples with depression-like behavior and immune activation in CUMS rats. Front Neurosci 2018;12:731.
52. Chen Q, Luo Y, Kuang S, Yang Y, Tian X, Ma J, et al. Cyclooxygenase-2 signalling pathway in the cortex is involved in the pathophysiological mechanisms in the rat model of depression. Sci Rep 2017;7:488.
53. Gite S, Ross RP, Kirke D, Guihéneuf F, Aussant J, Stengel JB, et al. Nutraceuticals to promote neuronal plasticity in response to corticosterone-induced stress in human neuroblastoma cells. Nutr Neurosci 2019;22:551–68.
54. Martín-Montáñez E, Millon C, Borañé F, García-Guirado F, Pedraza C, Lara E, et al. IGF-II promotes neuroprotection and neuroplasticity recovery in a long-lasting model of oxidative damage induced by glucocorticoids. Redox Biol 2017;13:69–81.
55. Xu B, Lang L, Li S, Yuan J, Wang J, Yang H, et al. Corticosterone excess-mediated mitochondrial damage induces hippocampal neuronal autophagy in mice following cold exposure. Animals (Basel) 2019;9:6682.