Original Research

Histone acetylation and expression of mono-aminergic transmitters synthetases involved in CUS-induced depressive rats

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Abstract

Histone acetylation has been linked to depression, the etiology of which involves many factors such as genetics, environments, and epigenetics. The aim of the present study was to investigate whether it was associated with epigenetic histone modification and gene expression of enzymes responsible for the biosynthesis of norepinephrine and serotonin in rat depression model induced by chronic unpredictable stress (CUS). Eight-week-old male Sprague-Dawley rats were exposed to CUS over 28 days. It was shown that the CUS-induced rats displayed remarked anxiety- and depression-like behavior with weakened locomotor activity in open field test and prolonged immobility in forced swimming test. Western blot revealed that CUS led to significant decrease in acetylation of H3 at Lysine 9 (K9) and H4 at Lysine 12 (K12) with obviously increasing histone deacetylases 5 (HDAC5) expression in hippocampus of CUS-induced rats. Meanwhile, there was an obviously decreased expression of tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) both at protein and mRNA levels. Administration of sodium valproate (VPA), a histone deacetylase 5 (HDAC5) inhibitor, not only significantly relieved the anxiety- and depression-like behaviors of CUS-induced rats but also clearly blunted decrease of H3(K9) and H4(K12) acetylation and expression of TH and TPH, and prevented increase of HDAC5 expression. The results indicate that there exists possible interrelation between TH and TPH gene expression and epigenetic histone acetylation in CUS-induced depressive rats, which at least partly contributes to the etiology of depression.

Keywords: Histone acetylation, histone deacetylase, tyrosine hydroxylase, tryptophan hydroxylase, chronic unpredictable stress

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Introduction

Depression is a growing worldwide problem, the etiology of which is still to be elucidated with controversies all along. This psychiatric disorder, varying in symptoms and response to antidepressants, has brought major social and economic consequences. One poorly understood in the characteristics of antidepressants is there exists an obviously delayed therapeutic effect for the patients after the drug administration.[1] This phenomenon is associated with slow adaptation in the relevant neurons that underlies the beneficial effect of the drug. The mechanism of the adaptation has not been clear as yet, but it is considered that an enduring change of chromatin state may be involved. Recently, it was reported that manifested neurobiologic changes in response to chronic stressors comprised histone modification in the hippocampus that was a brain region related to major depression because of its crucial role in regulation of motivation, emotion, and stress response.[2–4] In general, the modification of histone acetylation promotes gene transcription by either loosening the DNA-histone interaction and allowing the transcriptional machineries to bind the DNA or directly serving as recognition sites for factors promoting transcription.[5] Histone acetylation, especially targeting at the N-terminal lysine residues in histone 3 (H3) and histone 4 (H4), had aroused concerns in depression for acetylation modification partly involves transcription of some genes, such as brain-derived neurotrophic factor (BDNF) and serotonin transporter.[6,7] In addition, owing to the fact that histone acetyltransferases (HATs) and histone deacetylases (HDACs) are two key enzyme families in the process of histone acetylation, HDACs family and its inhibitors had attracted much attention in studies aiming at therapeutic targets of depression.[8,9] In fact, among the huge members of HDACs family, HDAC5

was considered a potential target of antidepressant, and its down-regulation was reported to be connected with therapeutic effect of some drugs on depression in animal model.[2] Furthermore, HDAC5 was found to play a crucial role in controlling behavioral adaptation to chronic emotional stimuli[10] and sodium butyrate, an HDAC inhibitor, was found to alleviate depressive symptoms in rodents.[11] However, the data of histone acetylation almost came from the depressive mice model induced by social defeat stress.

It is well known that tyrosine hydroxylase (TH) and tryptophan hydroxylase (TPH) are rate-limiting enzymes for the biosynthesis of mono-aminergic transmitters (such as norepinephrine, dopamine, and serotonin). Some clinical researches showed that there existed obvious alterations of TH and TPH immunoreactivity in locus coeruleus and dorsal raphe nucleus from depressive suicide victims.[12,13] Depressive animal models induced by chronic stress showed down-regulation of TH expression in locus coeruleus and reduction of TPH activity in hippocampus at the stage of pronounced depression.[14,15] Although physicians have attached importance to both transporter inhibitors of norepinephrine and serotonin in clinical treatment of depression as yet, new antidepressants with different pharmacological mechanism are eagerly awaited, considering that some major depression patients showed refractory response to many antidepressive treatments.[16] TH and TPH may be potential candidate targets in investigation of antidepressants. A report from Fu displayed the prospect of TH as a candidate, which was evidenced that supplementing TH alleviated the depressive symptoms of mice in forced swim test and tail suspension test.[17] However, whether histone acetylation modification affects the expression of TH and TPH in stress-induced depression animal model has not been reported.

Increasing evidences supported that chronic stressors could promote the derangement of oxidative and anti-oxidative balance in both depressive patients and animal models and antidepressants were able to rectify this derangement.[18–21] It was considered that oxidative stress aberration in oxidative and nitrosative stress pathways underpinned depression and was a key component of the illness with inflammatory process.[22]

Sodium valproate (VPA) is a common antiepileptic drug with mood-stablizing effect and HDAC inhibitive activity.[23] In this study VPA was used as a tool to investigate if there existed possible interrelation between histone acetylation modification and expression of TH and TPH gene in CUS-induced depression in rats. The experimental results may provide a new train of thought for exploring the etiology and pharmacotherapy of depression.

Materials and methods

Animals

Eighty male Sprague-Dawley rats (180–200 g, 8-week-old) were used in these experiments. All the animals were ordered from animal experimental center of Chongqing Medical University. Rats were housed in groups of 4/cage (25°C ± 2°C, 60–70% humidity, 12-h light/dark cycle) with food and water available *ad libitum*. All experiments involving animals were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and approved by the Animal Care and Use Committee of Chongqing Medical University. Rats were allowed to habituate the colony room for seven days before experiment.

Experimental design

Eighty rats were randomly divided into four groups: control group, chronic unpredictable stress (CUS) group, VPA group, and VPA plus CUS (VPA + CUS) group. VPA (Wuhan Shengtianyu Technology Co. Ltd, Wuhan, China) was given to rats of VPA and VPA + CUS groups by intragastric gavage (300 mg/kg daily for 28 days). The drug was dissolved in physiologic saline(0.9% w/v). Same volume of solvent as ''a placebo'' was given to control and CUS rats.

CUS paradigm

Rats were exposed to a series of unpredictable stressors described before,[24] with slight modifications: 1-min trail nip, 5-min cold swim at 4°C, 24-h cage tilting (45°), 24-h wet bedding, 24-h food deprivation, 24-h water deprivation, 5-min thermal environment (47°C, in a 30 cm (L) × 12 cm (W) × 10 cm (H) metal box), 2-h noise, overnight illumination, and alteration of light-dark cycle (2-h light/dark cycle). Rats were individually exposed to the stressors once a day over 28 days. Same stressor was never performed successesively. Rats of control and VPA group were not subjected to any stressors. On the day 29 at 8.00 am, animals were killed by rapid decapitation. Brain was removed, and hippocampi were dissected and frozen immediately at −80°C until analyses.

Open-field test

The open-field test (OFT) was performed according to the method described by Grundmann et al.,[25] with some modifications. Briefly, a wooden box (100 cm × 100 cm × 40 cm) with black inner surface and 4 cm × 4 cm square grids in bottom was used and a quiet environment with dim light was provided. Rats were individually placed at the center of box bottom and allowed to freely explore for 5 min. The locomotive activity of rat was recorded by two experienced observers. Ambulation score (number of squares crossed), rearing score (number of hindlimb standing with forelimb off the ground), and grooming number were collected. The box was cleaned thoroughly before the next test.

Forced swim test

Forced swim test (FST) used by Makino et al.[26] was conducted with some modifications. Rats were individually forced to swim for 6 min in a vertical transparent acrylic cylinder (40 cm height × 30 cm diameter) with 30 cm water (at 20 ± 1°C) in depth. Entire procedure was videotaped to observe the process of rat-forced swim. The time that rat took to display more than 2.0 s stationary pose was recorded as immobility time which represented despair

behavior of the rat and the total duration of immobility during the last 4 min was measured.

Western blot

Histones from hippocampus were extracted using total histone extraction kit (Epigentek Group Inc., Brooklyn, NY, USA). Nuclear and cytoplasmic proteins were extracted using nuclear and cytoplasmic protein extraction kit (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China). The proteins were stored at −80°C until use. Protein concentration was determined by bicinchoninic acid (BCA) kit (Beyotime Institute of Biotechnology). Then protein samples (50 μg/lane) were separated on a gel (12% for histones, 10% for nuclear and cytoplasmic proteins) by SDS-PAGE electrophoresis and transferred onto polyvinylidene fluoride membranes. For histone acetylation of H3, histone fractions were probed using the following antibodies: anti-acetyl lysine9 (K9) H3, anti-acetyl lysine14 (K14) H3; and blots for modified histone antibody were stripped and re-probed for total H3 (1:1000 dilution, Millipore, Billerica, MA, USA). For histone acetylation of H4, histone fractions were probed with anti-acetyl lysine12 (K12) H4; blots for modified histone antibody were stripped and re-probed for total H4 (1:1000 dilution, Millipore, Billerica, MA, USA). H3 (K9, K14) and H4 (K12) modifications were normallized to total H3 and H4 protein expression, respectively. For nuclear protein, blot was probed using anti-HDAC5 antibody (1:1000 dilution, Millipore, Billerica, MA, USA) and Lamin B served as a loading control. For cytoplasmic proteins, blots were probed with anti-TH antibody, anti-TPH antibody (1:1000 dilution, Millipore, Billerica, MA, USA) and β-actin served as a loading control. After incubation with primary antibody, membranes were washed and incubated with peroxidase-conjugated goat antirabbit or rabbit antisheep secondary antibodies (1:3000 dilution, Beijing Zhongshan Goldbridge Biotechnology, Beijing, China) for 1 h at room temperature. Proteins were visualized with ECL (Pierce Biotechnologies, Rockford, IL, USA) and exposed on imaging system (Bio Rad, Hercules, CA, USA). Band signals were acquired in the linear range of the scanner and analyzed using Quantity One software.

Real-time RT-PCR

RNA was extracted from the hippocampus using Total RNA Kit I (Omega, Stamford, CT, USA) according to the manufacturer's manual. Residual genomic DNA was digested with RNase-free DNase set (Qiagen, Hilden, Germany); 1 μg of total RNA was used for cDNA synthesis with PrimeScript™ RT reagent Kit (TaKaRa Biotech, Dalian, Liaoning, China) after assessing RNA quality and quantity with spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Real-time PCR was performed using the SYBR Premix Ex Taq II (TaKaRa Biotech) on Real-Time PCR detection system (Bio Rad). β-Actin served as the loading control. Amplification specificity was verified with melting curve and cycle thresholds (Ct) of amplification were expressed as $2^{-\Delta\Delta Ct}$ value. The primer sequences were designed as follows: 5′ AGAGGAC AGCATCCCACAGC 3′ (forward) and 5′ ATCACG GGCGG-ACAGTAGA 3′ (reverse) for TH; 5′ TTTGTAGC CAACATTCCTCA 3′ (forward) and 5′ ACTAT-TGAA AGTAGAAACCACCTC 3′ (reverse) for TPH; 5′ CGTAAA GACCTCTATGCCAACA 3′ (forward) and 5′ TAGGAGC CAGGGCAGTAATC 3′ (reverse) for β-actin.

Determination of SOD activity and MDA content in hippocampus

Isolated hippocampus was weighed. Then the hippocampus was homogenized by electric tissue homogenizer (IKA Werke GmbH & Co., Staufen, Germany) in cold saline, and centrifuged at 5000*g* for 10 min at 4°C in centrifuge (Thermo Fisher Scientific, Rockford, IL, USA). Supernatant was collected for determination of SOD activity and MDA content according to SOD assay kit and MDA assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Statistical analysis

Data were presented as mean ± SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by group comparisons by the Bonferroni test with GraphPad 5. Significance was defined as $P < 0.05$.

Results

Behavioral changes in OFT and FST

CUS rats displayed a significant decrease of both ambulation score ($P < 0.001$; Figure 1(a)) and rearing score ($P < 0.01$; Figure 1(b)) in OFT, compared with control. Administration of VPA showed a trend to preventing the decrease of both ambulation and rearing score caused by CUS ($P > 0.05$, VPA + CUS group vs CUS group). Administration of VPA did not obviously affect locomotive activity of normal rats ($P > 0.05$, VPA group vs control group). However, FST showed that the immobility time from CUS rats was significantly prolonged in comparison with control ($P < 0.01$, Figure 1(d)). Administration of VPA obviously inhibited the increase of immobility time ($P < 0.01$, VPA + CUS group vs CUS group). Administration of VPA did not obviously affect immobility time of normal rats ($P > 0.05$, VPA group vs control group). These results indicate that CUS induces rats to display anxiety-depression-like behaviors and VPA can obviously reverse despair behavior of CUS rats.

Modifications of histone acetylation in hippocampus

It was shown in Figure 2(a)–(d) that there existed a significantly decreasing acetylation of H3 (K9) and H4 (K12) and a significantly increasing HDAC5 protein expression in CUS rats, compared with control ($P < 0.05$). Administration of VPA only obviously prevented decrease of H4 (K12) acetylation and increase of HDAC5 protein expression ($P < 0.05$, VPA + CUS group vs CUS group, Figure 2(c) and (d)). Administration of VPA neither obviously affect acetylation of H3 (K9 and K14) or HDAC5 protein expression of normal rats. It is certain that CUS clearly decreases histone

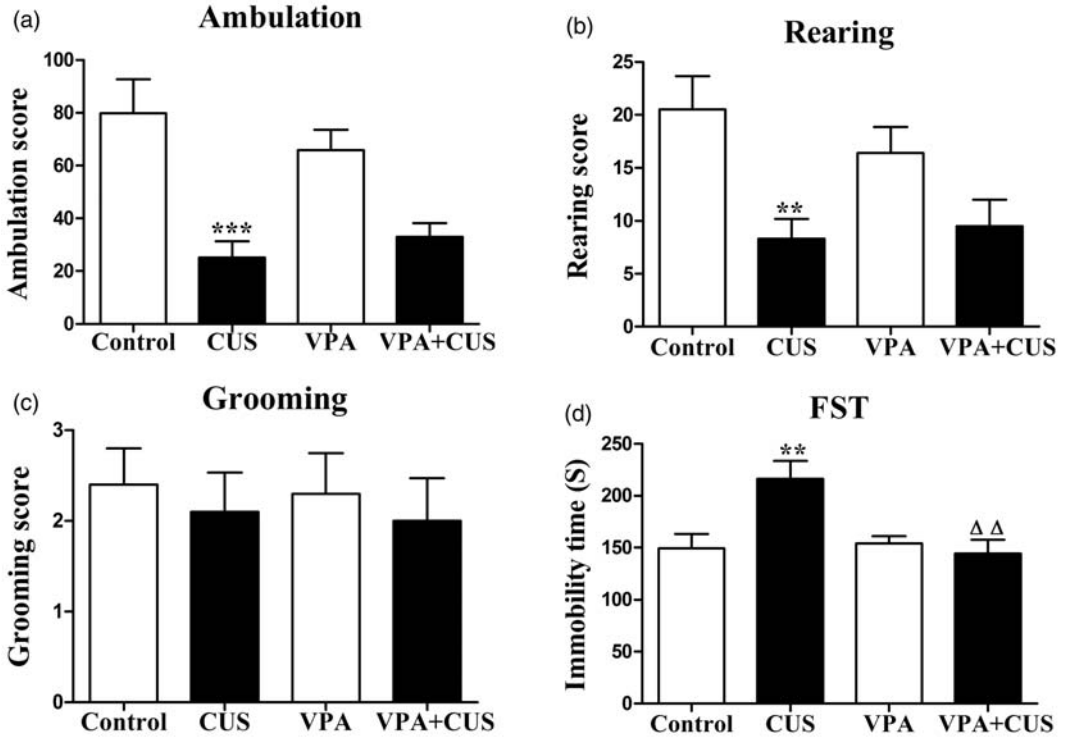

Figure 1 CUS-induced behavioral changes and interfering effects of VPA. (a), (b), and (c) represent ambulation score, rearing score, and grooming score of rats in open field test, respectively. (d) Immobility time of rats in the forced swim test. CUS: chronic unpredictable stress; VPA: sodium valproate. Data of each group were expressed as mean ± SEM; $^{**}P < 0.01$, $^{***}P < 0.001$, compared with control; $\Delta\Delta P < 0.01$, compared with CUS ($n = 10$ rats per group)

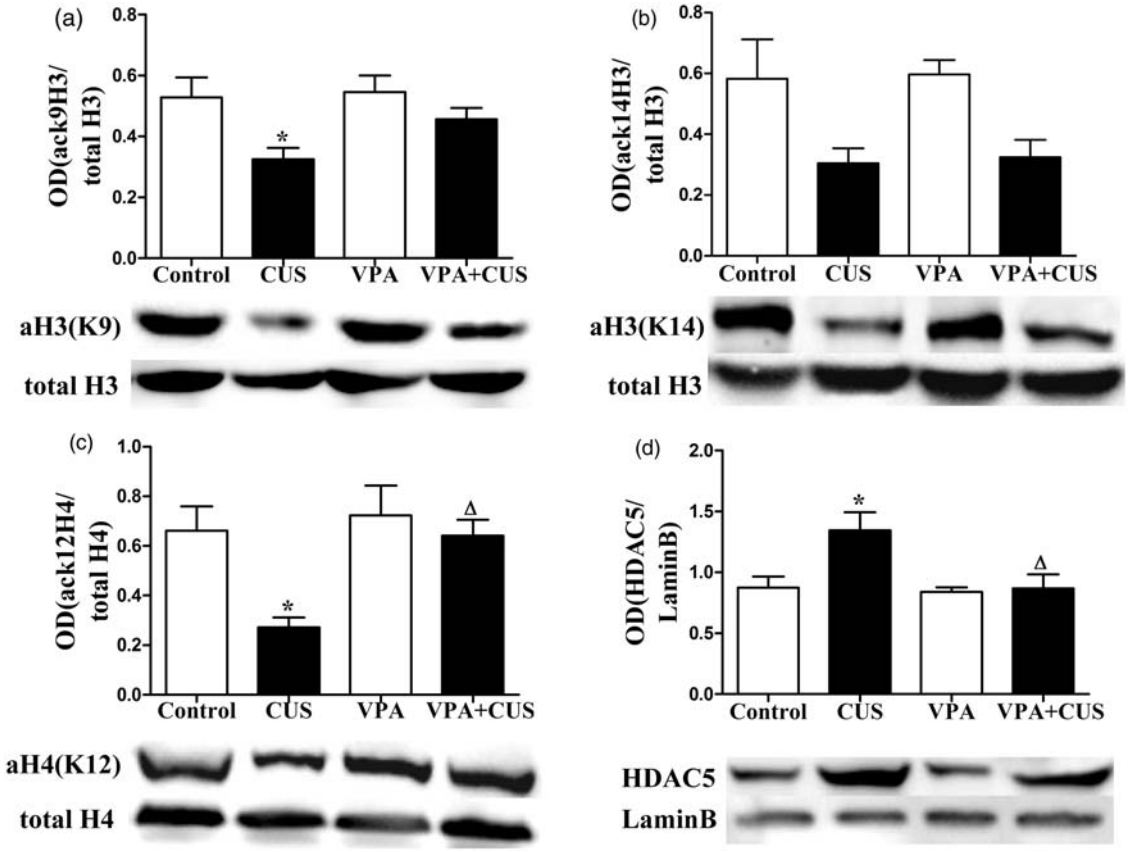

Figure 2 CUS-induced changes of histone acetylation and HDAC5 protein expression and interfering effects of VPA in hippocampus. (a), (b), and (c) represent protein expression from acetylation of H3 (K9), H3 (K14), and H4 (K12), respectively. (d) HDAC5 protein expression. CUS: chronic unpredictable stress; HDAC: histone deacetylase; VPA: sodium valproate. Data of each group were shown as mean ± SEM; $^{*}P < 0.05$, compared with control; $\Delta P < 0.05$, compared with CUS ($n = 5$ rats per group)

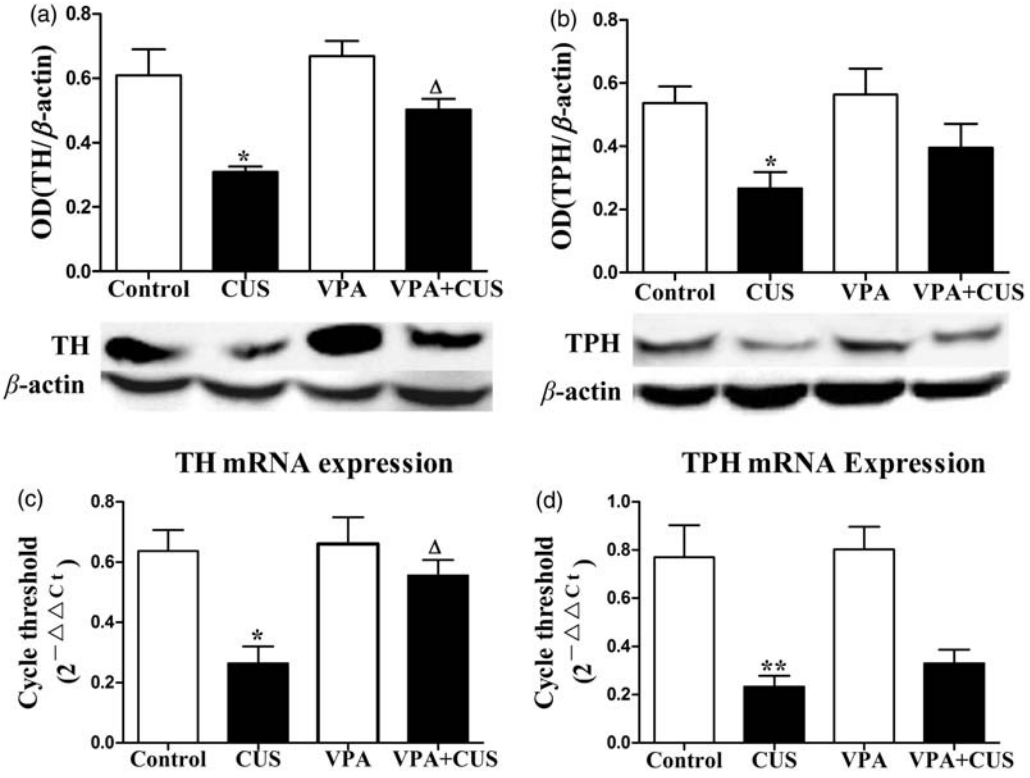

Figure 3 CUS-induced expression of TH and TPH protein and mRNA in hippocampus and interfering effects of VPA. (a) and (b), protein expression of TH and TPH. (c) and (d) mRNA expression of TH and TPH. CUS: chronic unpredictable stress; VPA: sodium valproate; TH: tyrosine hydroxylase; TPH: tryptophan hydroxylase. Data of each group were shown as mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control; $\Delta P < 0.05$, compared with CUS ($n = 5$ rats per group)

acetylation modificantion and elevates HDAC5 protein expression in CUS-induced depressive rats; VPA can obviously reverse the increase of HDAC5 protein expression and partly inhibits decrease of histone acetylation.

TH and TPH expression in the hippocampus

Figure 3(a)–(d) showed a significantly decreasing protein and mRNA expression of TH and TPH in hippocampus of CUS rats, compared with control ($P < 0.05$; $P < 0.01$). Administration of VPA clearly inhibited decrease of TH protein and mRNA expression($P < 0.05$, VPA + CUS group vs CUS group), but only partly reversed the decrease of expression from TPH protein and mRNA ($P > 0.05$, VPA + CUS group vs corresponding CUS group). Administration of VPA did not affect expression of both TH and TPH expression of normal rats.

Alteration in SOD activity and MDA content

Figure 4(a) and (b) showed that there were a remarkably increasing MDA level ($P < 0.01$) and a decreasing SOD activity ($P < 0.05$) in hippocampus of CUS rats compared with control. Administration of VPA obviously prevented CUS-induced increase of MDA level and decrease of SOD activity ($P < 0.05$, VPA + CUS group vs corresponding CUS group). Administration of VPA did not affect MDA level and SOD activity in hippocampus of normal rats.

Discussion

It has been evidenced that early life events may promote depression in some individuals or its resiliency in others.[16] Although it is generally accepted that interaction of genetic predispositions with environmental factors brought depression, genetic material is considered insensitive to environmental conditions and some genetic sequence is existing in the organism throughout life, which means that environmental stress-induced non-genetic changes of body may be associated with depression.[27] Based on the evidences from rodent models, it was suggested that epigenetic histone modifications were implicated in behavioral phenotypes related to depression.[28] It was reported that HDACs were one of two key enzyme families in the process of histone acetylation modification and high HDACs expression led to histone hypoacetylation, whereas HDACs inhibitors elevated histone acetylation and allevated despair behavior in rodent depressive model.[8,9] Indeed, a clinical research showed that an antidepressant, paroxetine could make high HDAC5 mRNA expression of peripheral leukocytes decreased to normal level with improvement of depressive symptoms in the major depression.[29] Tsankova et al.[30] reported that chronic electro-convulsive shock therapy increased H3 (K14) acetylation in the hippocampus, which was linked to improvement of depressive symptom. Hollis et al.[31] found that the level of histone acetylation at H3 (K14) was related to locomotive activity

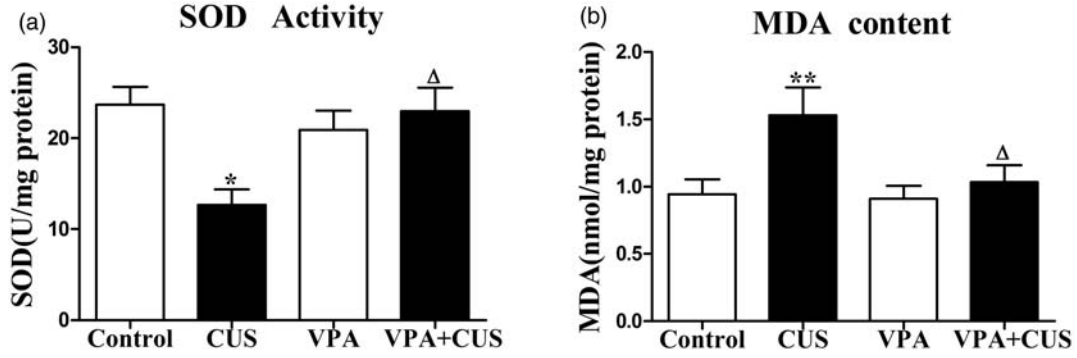

Figure 4 CUS-induced changes of SOD activity and MDA content in hipocampus and interfering effect of VPA. (a) SOD activity. (b) MDA content. CUS: chronic unpredictable stress; VPA: sodium valproate. Data of each group were shown as mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$, compared with control; $\Delta P < 0.05$, compared with CUS ($n = 8$ rats per group)

of rats: the higher the locomotive activity, the higher level of histone acetylation was.

In this experiment CUS-induced rats displayed a significantly decreasing acetylation at both H3 (K9) and H4 (K12) in the hippocampus with obvious anxiety-depression-like behaviors. These results were consistent with that reported by Tsankova et al.[2] using social defeat mouse model and by Ferland et al.[32] using chronic variable stress rat model. Our experimental results also showed that administration of VPA significantly prevented high HDAC5 expression and hypoacetylation of H4 (K12) caused by CUS with obviously improving despair behavior of rats. Owing to the fact that acetylation of H3 (K9) and H4 (K12) is associated with more open chromatin conformation and transcriptional activation,[33] it is suggested that hypoacetylation of histone following CUS may result in change of chromatin state and then affect the modulation of depression-linking gene expression.

TH and TPH are key enzymes responsible for monoanime transmitter biosynthesis, but what role the TH and TPH plays in depression is still to be identified. Zhao et al.[34] found that repeated injection of corticosterone induced depression-like behavior of mice with decrease of TH protein and mRNA levels in hippocampus. Sung et al.[35] reported that repeated separation from pups induced low TPH expression in dorsal raphe of maternal rats, but contrary result was also reported in depressive rats induced by chronic stress.[36] Our studies showed that CUS caused decrease of TH and TPH expression at both mRNA and protein levels in the hippocampus, and VPA administration generally inhibited the decrease. Considering that VPA is an HDAC inhibitor, which elevates histone acetylation level, it is reasonable to propose that the CUS may promote HDAC expression and decrease histone acetylation, which in turn down-regulates the expression of TH and TPH, and then leads to anxiety-depression-like behaviors of rats. That epigenetic histone acetylation modulates gene expression in response to environmental stimuli is widely accepted.[37]

In addition, clinical and laboratory research have evidenced that chronic stress induces oxidative stress, which leads to imbalance between oxidative stress and antioxidative system in the body.[38,39] Similar results were obtained in our study, which showed that there were decreased SOD activity and increased MDA level in the hippocampus from CUS-induced depressive rats. Oxidative stress relying on reactive oxygen species (ROS) can exert damage to not only proteins and lipids but also DNA and cause chromatin remodeling through interfering with histone acetylation and deacetylation.[40]

In conclusion, histone acetylation and modulation of TH and TPH expression in hippocampus closely connect with each other in the CUS-induced depressive model of rats, which may at least partly contribute to the etiology of depression.

Author contributions: The work presented here was carried out by all authors in collaboration. DL, H-MQ and Q-XZ designed the research scheme. DL, H-MQ, H-ZF, X-YH, L-JW, L-JQ carried out the experiments. DL, H-JX and X-HJ were involved in data collection and analyzed the data. DL wrote the manuscript. Q-XZ revised the manuscript and took overall responsibility.

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