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Original Article

Potential antidepressant role of low-dose naltrexone in a rat model of chronic unpredictable mild stress

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Abstract: Objectives: We aimed to investigate antidepressant effect of low-dose naltrexone (LDN) and to explore its potential mechanism of action using a rat model induced by chronic unpredictable mild stress (CUMS). Methods: The CUMS-induced rat model was established, and the antidepressant effect of LDN (0.1 mg/kg/day, given by intraperitoneal injection for 5 weeks) was investigated using sucrose preference test, open field test, forced swimming test, and novelty-induced hyponeophagia test. Moreover, the molecular biological changes including beta-endorphin (BEP), interleukin (IL)-1β, 5-hydroxy-tryptamine (5-HT), corticosterone, monoamine neurotransmitter and brain derived neurotrophic factor (BDNF) were also identified. Results: Behavioral changes were observed following 5 weeks of CUMS, including decreased sucrose preference, reduced locomotor activity, increased immobility time and elevated latency of feeding. However, after LDN treatment, these behavioral changes were significantly improved. In addition, LDN could inversed reduced BEP level in hypophysis, 5-HT level and BDNF expression level in hippocampus caused by CUMS. Moreover, LDN also could inversed increased levels of IL-1β and corticosterone induced by CUMS. Conclusion: The results suggest that LDN may have therapeutic actions on depression-like behavior induced by CUMS in rats by decreasing IL-1β in spleen and hippocampus tissue and plasma corticosterone levels and upregulating the BEP level in hypophysis tissue and plasma and the 5-HT, BDNF level in hippocampus tissue.

Keywords: Antidepressant effects, low-dose naltrexone, high-dose naltrexone, chronic unpredictable mild stress rat model of depression

Introduction

Depression is one of the most common psychiatric problems affecting more than 350 million people worldwide [1], and more than half among them would experience episodes [2]. Depression causes significant distress or impairment in physical healthy, mental health and quality of life [3]. Various therapies have been considered available for treating depression, but antidepressant is usually recognized as the first-line treatment for depression.

Recently, many synthetic chemical antidepressants were introduced, such as monoamine oxidase inhibitors, tricyclic antidepressants and selective serotonin reuptake inhibitors [4, 5]. Although these chemical antidepressants have been proved to have therapeutic responses, there are still a variety of side effects, such as psychomotor impairment and dependence liability [6, 7]. Naltrexone is clinically used to treat heroin and alcohol dependence [8, 9], which could block the pleasurable effects associated with alcohol drinking or heroin use [10]. Previous evidences showed that low-dose of naltrexone (LDN) might have a role in amelioration of psychiatric problems such as autism and depression [11]. For example, intermediate levels of LDN (0.25 mg/kg given every other day) have been found to benefit a subset of autistic children [12]. Kennedy et al. demonstrated that endogenous opioid neurotransmission on μ-opioid receptors was altered in major depressive disorder patients [13]. Moreover, it has been reported that naltrexone showed lower percent inhibition of delta-opioid receptors and greater variability in delta-opioid receptors blockade [14]. In addition, LDN at a dose of 0.1 mg/kg performed an important role in interfacing of the upregulated opioids and receptors [11, 16]. Therefore, the potential role of LDN in improv-

Table 1. Schematic representation of the experimental procedure and behavioral test

Time (day)	Experimental arrangement
1-3	Animal adaptation
4-6	Baseline sucrose preference test
7-8	Baseline open field test
9-10	Baseline forced swimming test
11-13	Baseline novelty-induced hyponeophagia (NIH) test
14	Cage tilt (45°C, 24 h)
15	Inversion of the light/dark cycle
16	Continuous overnight flash mode
17	Damp sawdust (200 ml of tap water spilled onto 100 g pad, 24 h)
18	6 h behavior restraint in a tube (diameter: 8 cm, length: 20 cm)
19	Fasting for 24 h, and water deprivation for 24 h
20	High temperature of 45°C for 5 min
21	Nip trail for 1 min
22-49	Repeat three cycles in a random order as day 14-21
50-52	All of experimental rats were rest
53-64	Repeated evaluation of the rat behavioral indicators in the above 4-13 days
65	Decapitation

ing depression is needed for further exploration.

Nowadays, the chronic unpredictable mild stress (CUMS) animal model has been proved as an effective animal model of depression for investigating antidepressants [17-19]. In this study, we established a CUMS rat model, and administrated LDN (0.1 mg/kg) and high-dose naltrexone (HDN) to rats to explore the potential antidepressant role of naltrexone by using behavioral tests. Moreover, the potential mechanism of action of LDN was also evaluated through detecting some molecular biological indexes including interleukin (IL)-1β and plasma corticosterone.

Materials and methods

Animal and drugs

Thirty-two female Sprague-Dawley (SD) rats weighing 150-170 g were obtained from the Laboratory Animal Center (approved by the ethical committee of the Second Affiliated Hospital of Harbin Medical University, China). Rats were housed at 22-25°C with 55±2% relative humidity, and exposed to a 12:12 h light-dark cycle with lights on at 07:00 am, free access to food and water. All procedures were in accordance with the "Principles of Laboratory Animal Care" and the PR China legislation for the use and care of laboratory animals. Moreover, the suffering of animals was minimized as much as possible.

Naltrexone was obtained from Sigma-Aldrich (St Louis, MO), and was dissolved in normal saline before experiment.

Chronic unpredictable mild stress rat model of depression

On the basis of the baseline sucrose preference index, the rats were randomly divided into four matched groups (8 rats/group): (1) Control group (CON); (2) NS-CUMS group: CUMS condition with normal saline; (3) LDN-CUMS group: CUMS condition with low-dose naltrexone treatment (0.1 mg/kg); (4) HDN-CUMS group: CUMS condition with high-dose naltrexone (10 mg/kg).

The rats, except the CON group, were individually housed and repeatedly exposed to a set of chronic unpredictable mild stressors in a random order every day for 5 weeks: cage tilt at 45°C for 24 h; inversion of the light/dark cycle; continuous overnight flash mode; damp sawdust for 24 h (200 mL of tap water spilled onto 100 g pad); behavior restraint in a tube for 6 h (diameter: 8 cm, length: 20 cm); fasting for 24 h; water deprivation for 24 h; high temperature at 45°C for 5 min; nip trail for 1 min [20, 21]. The rats in CON group were housed in the quiet and separate home with conventional breeding. All rats were administered different concentrations of naltrexone or normal saline via intraperitoneal injection at 6:00 pm-7:00 pm during the 5-week period of CUMS (day 14 to day 49).

After establishing models, animals were reoriented to the specific pathogen-free animal house for 3 days. Behavior test was handled on the fourth day (in **Table 1**).

Behavioral tests

Rats were housed in the testing chamber 30 min before each test. All tests were performed between 7:00 and 12:00 am.

Sucrose preference test

The sucrose preference test was performed as employed previously [21, 22]. Rats were cultivated to adapt to sucrose solution (1%, w/v) 2 days before the test. During adaptation period, 2 bottles of sucrose solution were placed in each cage for 24 h, and then one bottle of sucrose solution was substituted with tap-water for 24 h. After the adaptation period, the rats was expropriated of food and water for 24 h, and then rats were given free access to a bottle of 1% sucrose solution and a bottle of tap-water for 1 h in the sucrose preference test. At the end of the test, the bottles containing the remaining sucrose solution and tap-water were weighed, and the sucrose preference index was calculated as follows: sucrose preference index (%) = sucrose consumption/(sucrose consumption + tap-water consumption).

Open field test

The open field test was performed according to previous studies [22, 23]. Briefly, the test was performed in a gray square box (100 cm × 100 cm × 40 cm), the floor of which was divided into 25 equal squares, and was illuminated by a 100 W lamp above the center of the open field. Each rat was placed in the center of open field and observed for 5 min. The numbers of the locomotion, rearing and grooming were recorded using a video camera located 155-165 cm above the open field. Additionally, the arena was cleaned with 70% alcohol after each test.

Novelty-induced hyponeophagia (NIH) test

Hyponeophagia was defined as feeding inhibition produced by exposing to novelty, and the NIH test was performed according to previous studies [20, 24]. After fasting for 48 h, rats were placed into a new cage, in which there were thirty pieces of rat food particles in the cage center. The latency of feeding defined as time elapsed until rats began to eat was manually recorded by competent observers.

Forced swimming test

Forced swimming test was carried out on rats as described previously with minor modifications [25]. Briefly, each rat was placed in aplastic drum (40 cm tall, 25 cm in diameter) filled to 21.5±0.5 cm with 24±0.5°C water. Rats were forced to swim in the drum for 5 min, and each rat's behavior was recorded using a video camera above the drum in a dim light. Immobility time referred to the time spent by rat floating without floundering in the water except for small movement necessary to keep its head above the water. In addition, the drum would be cleaned after each test.

Preparation of blood and tissue samples

Eight rats from each group were deeply anesthetized with chloral hydrate (10%, 0.3 mL/100 g, i.p.), and the samples of blood and tissue were collected between 8:00 and 10:00 am. The collected blood samples were centrifuged immediately at 1000 × g for 15 min at 4°C to separate blood plasma.

In addition, the hypothalamus, hypophysis, hippocampus and spleen of rats were dissected onto a frosted glass plate on top of crushed ice. Tissue samples were snap-frozen in liquid nitrogen and stored at -80°C until use.

Measurement of beta-endorphin (BEP) in plasma and brain tissue

The BEP levels in plasma (containing 75% EDTA and aprotinin), hypothalamus and hypophysis were quantified by measuring the radioactivity of precipitate using a gamma-counter (GC-2010, USTC Chuangxin Co., Ltd, ZONKIA Branch) according to the instructions of commercially available radioimmunoassay kits (BNIBT, Beijing, China).

Measurement of interleukin (IL)-1β level

The spleen and hippocampus were homogenized in 5 μL/mg ice-cold phosphate-buffered saline (PBS) (0.02 mol/L, pH = 7.0-7.2) and centrifuged at 1500 × g for 15 min at 4°C, then the supernatant was collected. The levels of IL-1β in spleen and hippocampus tissue were measured according to the instructions of enzyme-linked immunosorbent assay kits (ELISA, BlueGene Biotech, China).

Table 2. Baseline values of the tests conducted from day 4 to 13

Indexes	CON		NS-CUMS		LDN-CUMS		HDN-CUMS		F	P
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD		
Locomotor activity	8	108.63±16.52	8	111.63±21.62	8	107.38±22.97	8	109.88±18.92	0.065	0.978
Rearing	8	34.88±6.92	8	35.25±7.48	8	31.50±7.09	8	28.75±4.53	1.725	0.185
Grooming	8	3.50±2.07	8	3.75±1.91	8	3.88±2.10	8	4.63±2.07	0.452	0.718
Rearing and grooming	8	38.38±7.19	8	39.00±7.37	8	35.38±8.53	8	33.38±5.07	1.084	0.372
Sucrose preference index	8	0.80±0.08	8	0.81±0.08	8	0.80±0.10	8	0.82±0.07	0.082	0.969
Immobility time	8	155.63±11.30	8	160.75±21.20	8	165.75±18.53	8	159.38±22.57	0.392	0.759
Latency of feeding	8	131.88±49.66	8	135.00±38.55	8	139.50±42.18	8	133.63±32.35	0.050	0.985

SD: Standard Deviation; N: Number of cases. CON: control group. NS-CUMS: Normal saline-chronic unpredictable mild stress group. LDN-CUMS: Low-dose naltrexone-chronic unpredictable mild stress group. HDN-CUMS: High-dose naltrexone-chronic unpredictable mild stress group.

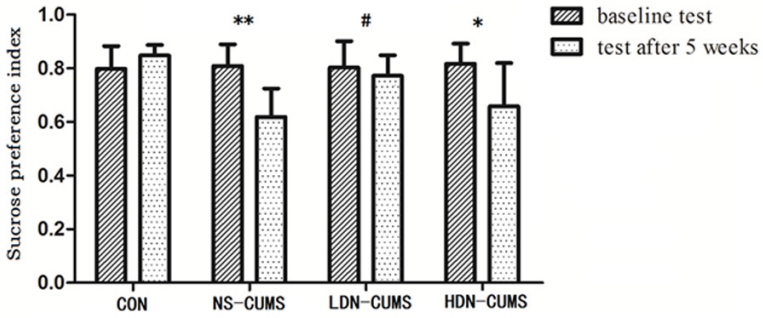

Figure 1. Effects of low-dose naltrexone on sucrose preference index. $^{*}P<0.05$, $^{**}P<0.01$ vs. CON group; $^{\#}P<0.05$ vs. NS-CUMS group.

Measurement of corticosterone in plasma

The levels of corticosterone in plasma were measured using high performance liquid chromatography (HPLC) method as reported previously [26]. Before measurement, the plasma was extracted with 5 mL ethyl acetate, and vortex-mixed for 30 seconds. Then, the extract was washed with 1 mL of 0.1 M sodium hydroxide and 1 mL of water, transferred to clean tubes, and evaporated to dryness. Finally, the residue was re-dissolved in mobile phase (acetonitrile-water-acetic acid-TEA, 22:78:0.1:0.03, v/v) and injected into a HPLC (ShimadzuLC20A, Japan) equipped with a 25 cm C18 column, and an ultraviolet detector at 254 nm.

Measurement of monoamine neurotransmitter

The levels of 5-hydroxy-tryptamine (5-HT), norepinephrine (NE) and dopamine (DA) in rat's unilateral hippocampus tissue were detected as described previously [27]. Briefly, the brain tissue were weighed, homogenized in ice-cold 0.1 Nperchloric acid (0.5 mL/100 mg tissue) containing 10^{-7} M ascorbic acid and centrifuged at 5200 × g for 30 min at 4°C before detection. The 200 μL supernatant obtained through a 0.22 μm filter was reacted with the benzylamine derivatization reagent solution for 2 min at 24°C, and followed with the diphenyl ether derivatization reagent solution for 20 min at 50°C. Finally, 20 μL sample was injected into a HPLC with a fluorescence detection operated at an excitation wavelength of 345 nm and an emission wavelength of 480 nm.

Immunohistochemistry analysis of brain derived neurotrophic factor (BDNF)

Another 3 rats in each group were sacrificed and perfused intracardially with heparinized PBS followed by 0.1 mol/L PBS (pH = 7.4) containing 4% paraformaldehyde. The coronal hippocampus was dissected, and then cut into 5 μm thick hippocampus sections after fixing with a solution containing 4% paraformaldehyde for 24 h and embedding in paraffin. After dewaxed, rehydrated and blocked with 5% bovine serum albumin for 30 min, the sections were incubated with rabbit anti-BDNF antibody (1:50 dilution, BOSTER, Wuhan, China) overnight at 4°C, and subsequently reacted with the biotinylated secondary antibody for 30 min at room temperature. Diaminobenzidine was used as a chromogen, and the hippocampal CA3 region of each brain section was observed and pictured with microscope (Nikon H600L, Japan). The expression of BDNF proteins was quantified and statically analysed followed the reference reported [28]. The staining density was measured by detecting the average gray value and integrated optical density (IOD) using the Image-Pro Plus 6.0.

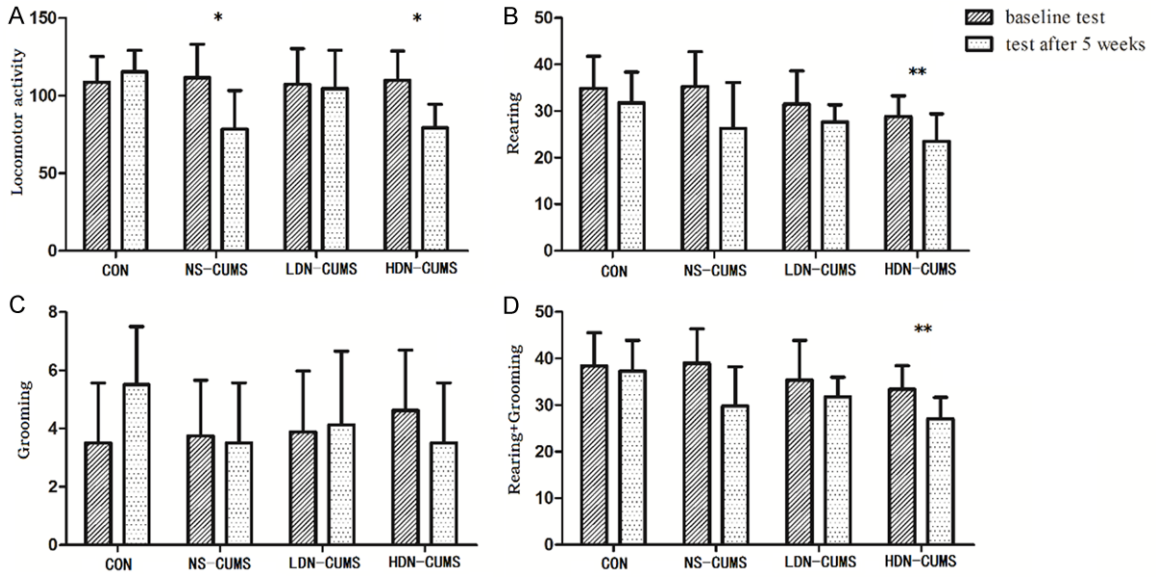

Figure 2. Effects of low-dose naltrexone on chronic unpredictable mild stress-induced rat model on locomotor activity, rearing, grooming, and the total number of rearing and grooming. (A) Effects of low-dose naltrexone on locomotor activity, (B) rearing, (C) grooming, (D) and the total number of rearing and grooming in the open field test. $^{*}P<0.05$, $^{**}P<0.01$ vs. CON group.

Statistical analyses

The experimental data were analyzed by SPSS software (version 13.01S; Beijing Stats Data Mining Co. Ltd., Beijing, China). All the data were reported as means ± SD (standard deviations). The differences about behavioral data among groups were analyzed by repeated measurement ANOVA (analysis of variance) and biochemical data were analyzed by one-way ANOVA (Furthermore, homogeneity of variance assumptions was checked using Levene test. If $P<0.1$, we defined homogeneity of variance is satisfied. Then, Tukey's HSD (Honestly Significant Difference) test would be used to assess differences among groups. Otherwise, Dunnett's T3 would be carried out to analyze differences among groups.

Results

Overall values comparisons for analyzing LDN anti-depression effects

Baseline values of the tests conducted from day 4 to 13 were shown in **Table 2**. The differences among groups were analyzed using ANOVA, and no significant difference were found for all included indexed in the tests among 4 groups. Therefore, it is available for next step study.

Sucrose preference index increased in LDN-CUMS rats

As shown in **Figure 1**, rats in NS-CUMS and HDN-CUMS groups showed significantly lower sucrose preference index than that in CON group ($P<0.01$; $P<0.05$). Meanwhile, LDN treatment significantly increased the sucrose preference index compared with the NS-CUMS group ($P<0.05$).

Unsignificant difference found between LDN-CUMS and CON rats in the open field test

The activity of locomotion, rearing and grooming were observed in the open field test (**Figure 2**). Compared with CON group, locomotor activity was significantly decreased in NS-CUMS and HDN-CUMS groups ($P<0.05$). Both rearing and total number of rearing and grooming were significantly reduced after HDN treatment compared with CON group ($P<0.01$). Moreover, no significant differences on performance in the open field test were found between LDN-CUMS and CON group.

Unsignificant difference found between LDN-CUMS and CON rats in immobility time and latency of feeding test

As shown in **Figure 3A**, the immobility time in NS-CUMS and HDN-CUMS groups were signifi-

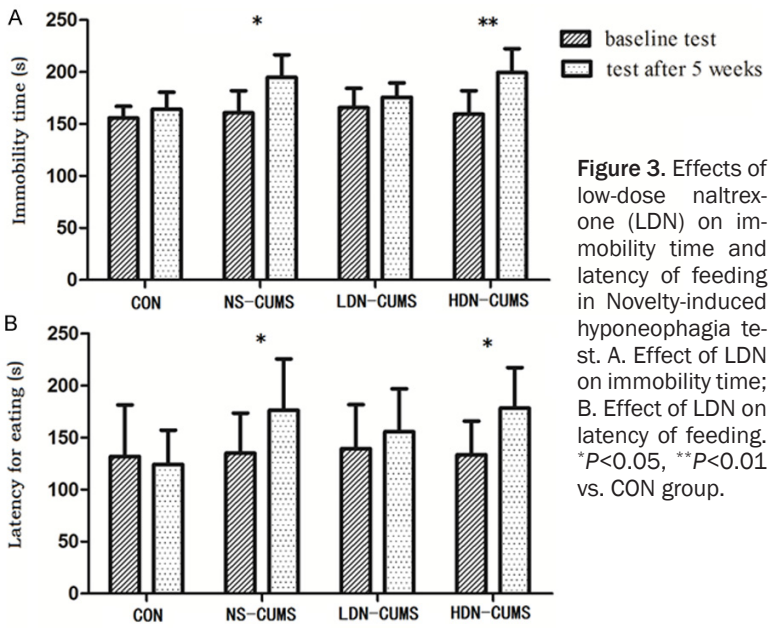

Figure 3. Effects of low-dose naltrexone (LDN) on immobility time and latency of feeding in Novelty-induced hyponeophagia test. A. Effect of LDN on immobility time; B. Effect of LDN on latency of feeding. $^{*}P<0.05$, $^{**}P<0.01$ vs. CON group.

cantly longer than that in CON group ($P<0.05$; $P<0.01$). Compared with CON group, latency of feeding was significantly increased in NS-CUMS group and HDN-CUMS group ($P<0.05$) (**Figure 3B**). In addition, no significant differences were found both in forcing swimming and novel feed tests between LDN-CUMS group and CON group.

BEP and 5-HT up-expression

As shown in **Figure 4A**, compared with CON group, the BEP concentrations in hypophysis and plasma were significantly decreased in HDN-CUMS group ($P<0.05$). In addition, compared with NS-CUMS group, hypophysial BEP concentrations were significantly increased in LDN-CUMS group ($P<0.05$). As for BEP concentration in hypothalamus, no significant differences were found among the four groups.

As shown in **Figure 4B**, the levels of 5-HT in hippocampus tissue were significantly lower in both NS-CUMS group and HDN-CUMS group ($P<0.01$) than those in CON group. Additionally, no significant differences were found among the four groups with regard to DA and NE levels in hippocampus tissue.

IL-1β down-expression in the spleen and hippocampus

As shown in **Figure 5A**, the IL-1β levels both in spleen and hippocampus were significantly increased in NS-CUMS group ($P<0.01$) than those in CON group. As shown in **Figure 5B**, the levels of plasma corticosterone were significantly higher in NS-CUMS and HDN-CUMS groups than that in CON group ($P<0.05$). Compared with NS-CUMS group, plasma corticosterone level in LDN-CUMS group were significantly lower ($P<0.05$) than that in NS-CUMS group ($P<0.05$).

BDNF up-expression in rat hippocampus

As shown in **Figure 6**, compared with CON group, the expression of BDNF was significantly reduced in NS-CUMS and HDN-CUMS groups ($P<0.05$). The BDNF expression levels of LDN-CUMS group were elevated compared with NS-CUMS group ($P<0.05$). Furthermore, the expression of BDNF was significantly decreased in HDN-CUMS group compared with LDN-CUMS group ($P<0.05$).

Discussion

Depression is a serious public mental disease [29]. Naltrexone has been widely used in treatment of alcohol dependence and heroin dependence [30, 31]. However, the research focusing on antidepressant effects of LDN has rarely been reported. In this study, we established a CUMS-induced rat model, and studied the antidepressant effects of LDN using 4 groups' rats which showed similar baseline value during behavioral tests before the CUMS procedures. We found that LDN would have a therapeutic effect on depression behavior in the rat model by improving sucrose preference, decreasing locomotor activity, reducing immobility time and holding back prolongation of latency of feeding. Further data showed that antidepressant effects might be mediated by decreasing plasma corticosterone level, up-regulating BEP level in hypophysis, 5-HT level in hippocampus, and BDNF expression level, and inhibiting the increase of IL-1β caused by CUMS.

The CUMS-induced rat model is considered as an effective animal model of depression

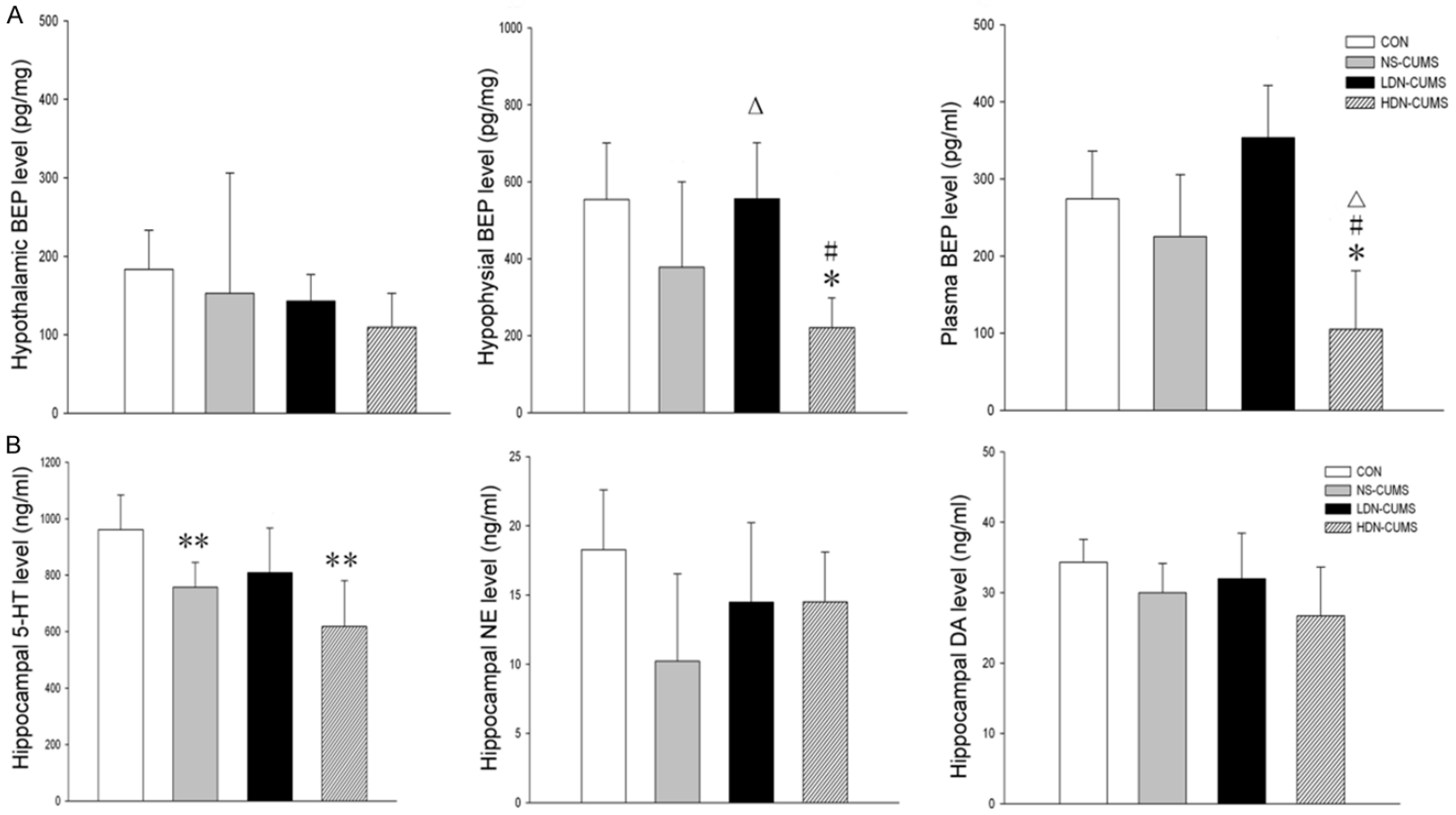

Figure 4. Effects of low-dose naltrexone on β-Endorphin (BEP) concentrations and levels of 5-hydroxytrypytamine(5-HT), norepinephrine (NE) and dopamine (DA). A. BEP in hypothalamus brain tissue, hypophysis brain tissue and plasma; B. 5-HT, NE and DA in hippocampal brain tissue. **P*<0.05, ***P*<0.01 vs. CON group; $^{\Delta}P$<0.05 vs. NS-CUMS group; $^{\#}P$<0.05 vs. LDN-CUMS group.

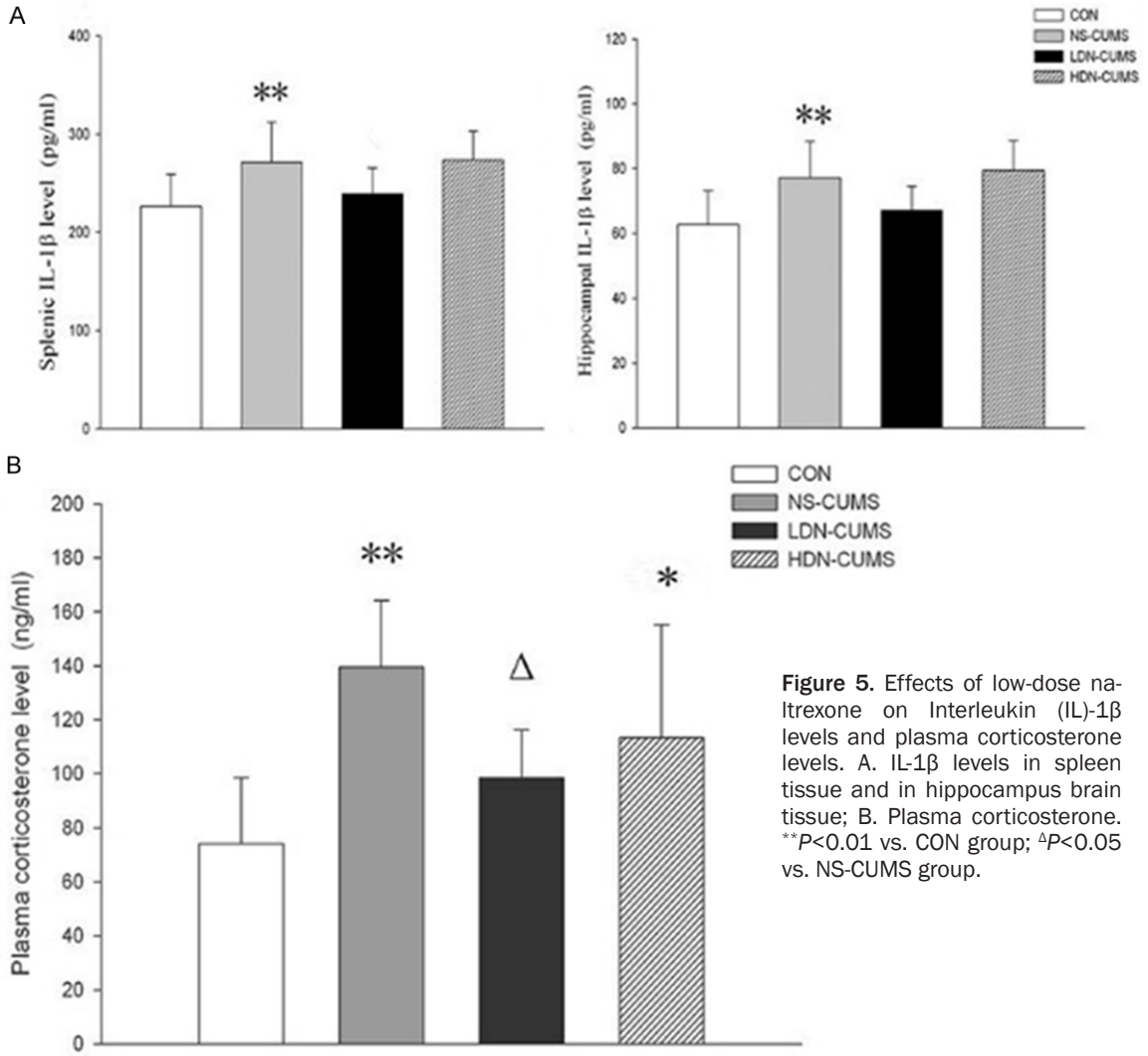

Figure 5. Effects of low-dose naltrexone on Interleukin (IL)-1β levels and plasma corticosterone levels. A. IL-1β levels in spleen tissue and in hippocampus brain tissue; B. Plasma corticosterone. $^{**}P<0.01$ vs. CON group; $^{\Delta}P<0.05$ vs. NS-CUMS group.

because the rat exposing to CUMS can exhibit a series of depression-like symptoms [18]. CUMS for 5-weeks could induce behavior changes such as anhedonia, (i.e., lessened intake of a delicious sweet solution) [21], psychomotor retardation (i.e., decreased locomotor activity) [32], refractory loss of interests (i.e., decreased rearing and grooming, which is driving by the instinct interests of rats to explore a novel environment) [17, 33], recurrent thoughts of death (i.e., dramatically increased immobility time) [34], and anxiety-like behavior (i.e., increased latency for feeding) [20, 24]. CUMS caused depression-like symptoms by inducing changes in behavior, including decreased sucrose preference, reduced locomotor activity and total number of rearing and grooming, increased immobility time and elevated latency of feeding, which indicated that a CUMS-induced rat model was successfully established in our research.

Recent studies have demonstrated that inflammatory mediators (i.e., IL-1β) may play a critical role in the pathophysiology of depression [35]. IL-1β is considered as an inflammatory biomarker for depression, and the peripheral levels of IL-1β are increased in depression patients [36, 37]. In the present study, CUMS markedly increased IL-1β levels in the spleen and hippocampus. Furthermore, LDN treatment significantly reversed the changes and reduced the IL-1β levels. The above experimental data suggested that LDN could inhibit the increase of IL-1β levels induced by CUMS.

Meanwhile, increasing evidences have showed that inflammation could lead to depression by

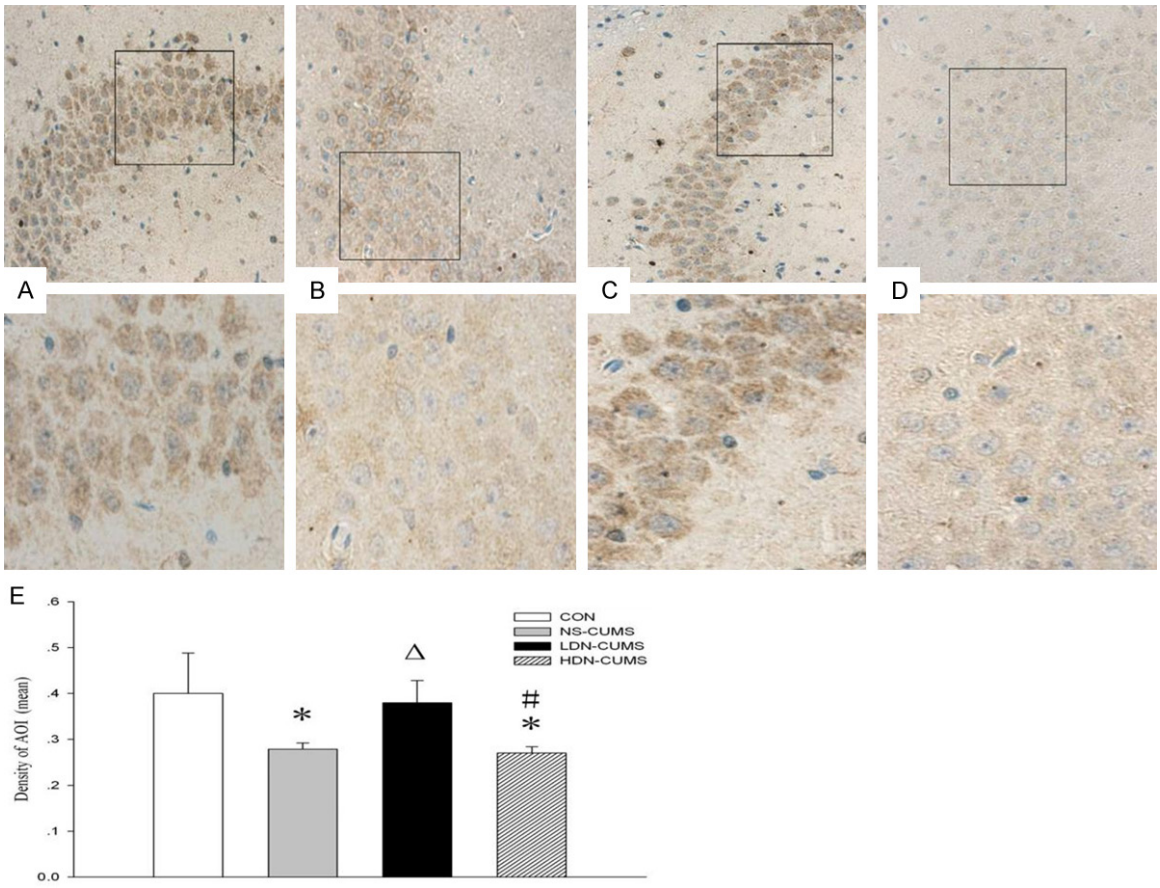

Figure 6. Effect of low-dose naltrexone on expression of brain derived neurotrophic factor (BDNF) in hippocampus brain tissue of CUMS rat. A. CON group; B. NS-CUMS group; C. LDN-CUMS; D. HDN-CUMS group. BDNF levels were determined by immunohistochemical assay and the cells in brown were BDNF positive. The images above were magnified 400 times, and the images below were magnified 1000 times. E. Comparison of the mean density of AOI (area of interesting) of BDNF. Immunohistochemical analysis of BDNF proteins was performed on sections of rat brain of CON group, NS-CMS group, LDN-CUMS and HDN-CUMS group. $^{*}P<0.05$ vs. CON group; $^{\Delta}P<0.05$ vs. NS-CUMS group; $^{\#}P<0.05$ vs. LDN-CUMS group.

affecting the hypothalamic-pituitary-adrenal (HPA) axis activation, modulating neurotransmitters, and alternating of neuroplasticity [38]. It has been found that animals with exogenous administration of IL-1β into brain could exhibit depressive-like symptoms, and this could be attenuated by pretreatment with IL-1β receptor antagonist [39, 40]. Recent researches have shown that IL-1β could activate HPA axis, which is accompanied with increase of plasma levels of ACTH and corticosterone [41, 42]. Moreover, it has been reported that mice with deletion of the IL-1 receptor type I did not increase CUMS-induced plasma corticosterone level, and the obstruction of endogenous corticosterone release eliminated CUMS-induced depression [43]. In addition, previous data suggested that naltrexone could participate in regulating chronic physical or psychological stress through modifying corticosterone plasma levels [44]. In our study, we found that LDN administration significantly reversed the increase of IL-1β levels and plasma corticosterone level induced by CUMS. Thus, the potential mechanism of LDN as an antidepressant in the CUMS-induced model may be related to the decreased plasma corticosterone and IL-1β levels in CUMS model.

BEP, a neuromodulator and neurotransmitter in the central nervous system, has been demonstrated to inhibit the generation of stress hormones, produces analgesia and a feeling of cheerfulness [43]. Accumulating evidences suggest that a low level of central BEP is related with the course of stress-related psychodisturbance, depression and posttraumatic

stress disorder [45, 46]. Moreover, previous study has put forward that naltrexone could elevate BEP expression levels in autistic children [47]. Our results inferred that BEP expression levels might be regulated after the LDN treatment on depression. In addition, several studies showed that monoamine neurotransmitters (NE, DA and 5-HT) are also involved in the pathogenesis of depression and play important roles in mediating the effects of antidepressant [48]. Moreover, IL-1 may reduce 5-HT level by lowering the availability of its precursor tryptophan [49]. In our study, IL-1β levels decreased and 5-HT was significantly increased in CUMS rats after LDN treatment. Thus, we suggested that up-regulation of 5-HT levels might be related with inversed IL-1β levels.

Ample evidence indicates that IL-1β could regulate hippocampal neurogenesis via the inhibition of the expression level of BDNF, which is involved in synaptic plasticity and related with cognitive dysfunction by CUMS induced-depression [43, 50], and up-regulation of BDNF expression plays a critical role in antidepressant treatment [51]. In line with the previous reports, our results indicated that rats exposed to CUMS exhibited a reduced expression of BDNF proteins in the hippocampus [52, 38]. After LDN treatment, BDNF levels in CUMS-induced rats were similar with that in healthy control group. These results indicated that the mechanism of the antidepressant effect of LDN may be also related to the increased BDNF expression in the hippocampus.

However, several limitations in this present study should be reminded. Firstly, although the experiment evidence has demonstrated the effect of naltrexone on up-regulation of BEP was dose-dependent, we did not explore the best dose of naltrexone on regulating BEP levels. Further experiment is needed to explore the relationship between naltrexone and BEP levels before clinical use. Secondly, positive control group was not established in this study. Although change of neurotransmitter concentration has been observed in previous study on traditionally antidepressants, including amitriptyline and desipramine [15, 53], intuitive parameters to evaluate LDN antidepressant effect is still needed. In addition, neurobiological results on unstressed rats about how naltrexone works will be provided in our further study.

Conclusions

In conclusion, it appears that LDN has a therapeutic effect on depression behavior in CUMS-induced rat model, and mechanisms underlying the antidepressant-like effects of LDN might be associated with the decrease in plasma corticosterone levels, up-regulation of BEP in hypophysis, 5-HT level in hippocampus, and BDNF expression by inhibiting the increase of IL-1β induced by CUMS. However, the exact target of the antidepressant effect of LDN remains unknown, and our group will try to resolve the above limitations in future studies.

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Disclosure of conflict of interest

None.

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