Plasma Nesfatin-1 concentration and its correlation with HPA axis in depression model rats

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【**Abstract**】**Objective** To explore plasma Nesfatin-1 concentration and its correlation with hypothalamic pituitary adrenal (HPA) axis in depression model rats. **Methods** Twelve SD rats were randomly divided into normal control group (NCG) and depression model group (DMG). DMG received 5 consecutive weeks of 7 different chronic unpredictable mild stress (CUMS). The behavior of rats was evaluated by an open field test, sucrose preference test, and forced swimming test (FST). The concentration of plasma corticosterone and Nesfatin-1 were measured with an enzyme-linked immuno-sorbent assay (ELISA). **Results** Compared with NCG, DMG had a lower weight gain, lower index of sucrose preference and spent longer time being immobile in FST while all of these differences were statistically significant ($P<0.05$). The plasma concentrations of corticosterone and Nesfatin-1 were significantly higher in DMG than in theNCG ($P<0.05$). The plasma concentration of Nesfatin-1in DMG was positively correlated with the plasma corticosterone concentration. **Conclusion** CUMS could induce depression-like behavior in rats and increase the plasma concentration of Nesfatin-1 and corticosterone, while the HPA axis hyperactivity induced by CUMS may be associated with the increased concentration of Nesfatin-1.

【**Key words**】Depression; Nesfatin-1; Hypothalamic pituitary adrenal axis

Introduction

Depression with its high prevalence and great risk of suicide has brought about tremendous impacts to patients and their families. The global burden of disease study conducted by WHO pointed out that depression is expected to become the second largest source of burden of disease in 2020 after coronary heart disease. A growing number of evidence indicates that stress and hyperactivity of the HPA axis might be an influential factor in depression. However, the mechanism of how HPA axis is activated has not yet been fully illustrated.

In recent years, studies found that a significant abnormality of leptin in its secretion and metabolism, an appetite suppressor that has neuroendocrine functions appears in the pathogenesis of depression. After antidepressant treatment, leptin concentration in patients with depression will also alter [1]. Melanocyte stimulating hormone (MSH), another appetite suppressor is also involved in the pathogenesis of depression [2]. Research on the neuroendocrine mechanism in the pathogenesis of depression has gained great attention. In 2006, a research group called Oh-I from Gunma University in Japan found a new appetite suppressor Nesfatin-1 [3]. It is widely distributed in the central and peripheral nervous system and travels freely through the blood brain barrier which suggests that its effect may not be limited to the regulation of food intake. Several studies have shown that Nesfatin-1 plays an important role in the emotion regulation and affective disorders. [4, 5] while there are still many unsolved questions including through which neural pathways the abnormality of Nesfatin-1 affects the mood and its association with stress-related hyperactivity of HPA axis. This study aims to further study the alternation of plasma Nesfatin-1 concentration in depression model rats induced by CUMS and its correlation with HPA axis, in order to provide supports for future research on the mechanism of depression.

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Methods

Animals

15 male SPF SD rats [SCXK(Hunan), 2011–2011], were fed in a separately closed environment in Renmin Hospital of Wuhan University with 12–hour day time and 12–hour night time. Before the experiment, all rats were fed to adapt for one week. During the experiment, the rats were fed with tap water ad libitum under conditions of room temperature (23 ± 2)℃, humidity (40% to 50%) and controlled illumination (12: 12 h light/dark cycle, lights on/off: 8: 00/20: 00). All procedures were approved by the ethics committee in Renmin Hospital of Wuhan University. This research commenced in October 2015 and concluded in December 2015.

Instruments and chemicals

The main instruments and chemicals in this experiment were as follows: Animal movement track record analysis system (Ethovision3.0, Netherlands);

Rat plasma Nesfatin–1 and corticosterone Elisa kit (CEA242Ra, CEA540Ge produced by Wuhan USCN Business Co., Ltd.)

ELISA instrument (PerkinElmer Company).

Animal grouping

Three stray rats were screened out on the basis of the first–time open field test and weight measurement. The remaining 12 rats were then divided by random numbers into two groups of six, namely normal control group (NCG) and depression model group (DMG). Depression model group received five consecutive weeks of CUMS while normal control group was fed under normal conditions.

Depression rat model

DMG rats received seven different stimuli including food deprivation for 24 h, water deprivation for 24 h, 45° cage tilt for 24 h, damp environment (100 g bedding/100 ml water), 4℃ icy water swimming for 5 min, 40℃ hot water swimming for 5 min, tail clipping at 1 cm away from the rat tail root [6]. One stimulus was picked randomly every day and the same stimulus was not repeated continuously. Seven days as a period, the stimulation process lasted a total of five weeks. Five weeks later, two groups of rats received behavioral evaluation at the same time. When the behavioral differences between the two groups were proven to be statistically significant, depression model rats were confirmed to be successfully established.

Open field test

This test was conducted prior to the start of establishing two groups of model rats to evaluate their behaviors. The open field was 120 cm long, 90 cm wide, 35 cm high with four walls painted black. Rats were carefully put in the center of the open field by holding their rat tails.

Ethovision3.0 system was then applied to record and analyze their 5 min behavior in the field. Only when it was confirmed of no statistically significant difference of behavior between the two groups of rats, the establishment of model rats started. Two other behavior evaluations namely, sucrose preference test and forced swimming test were utilized after the establishment of model rats.

Sucrose preference test

Before the test, a 24 h 1% sucrose adaptability training was performed. After 24 h of being abstained from water, all model rats were prepared two weighed bottles of water (1 % sucrose solution and regular animal drinking water)[7]. 24 hours later, index of sucrose preference was calculated.

$$Index of\ sucrose\ preference = \frac{sucrose\ consumption}{(sucrose\ consumption + water\ consumption)} * 100\%$$

Forced swimming test

Rats were put in a cylindrical aquarium (60 cm high, 30 cm in diameter) containing 40 cm deep water (25 ± 2 ℃)[8].

They stayed in water for 6 minutes and in the last 4 minutes the amount of time spent being immobile was recorded (this refers to the state in which the rats stopped struggling in the water, or remained afloat with slight limb movements to keep their heads above water).

Specimen collection and ELISA

24 hours after behavior evaluations, rats were deeply anesthetized with 20% urethane (7 ml/kg, intraperitoneally). Blood samples were taken from hearts to EDTA tube, then centrifuged to gain the plasma specimen that were stored in a −80℃ refrigerator. Plasma Nesfatin−1 and corticosterone concentration was measured through ELISA under instructions provided by the guidebook.

Statistical analysis

Data were analyzed by SPSS 19.0 software. The Kolmogorov−Smirnov test was used to measure the normality of measurement data. Data that conformed to normality were displayed in the form of ($\bar{x}\pm s$). Independent sample t−test was applied when the variances of the two groups were equal and statistical differences are significant when $P<0.05$.

Results

Effects of CUMS on the behavior of rats

Before CUMS, no significant difference was shown between the two groups in weight, horizontal motion distance in the open field within 5 minutes, and number of times of rats standing up ($P>0.05$) (Table 1). After CUMS, the weight gain of DMG was significantly slower than that of NCG. Index of sucrose preference of DMG was lower than that of NCG and the amount of time when rats remained immobile in DMG was significantly longer than in NCG ($P<0.05$) (Table 2).

Table 1. Behavior evaluation of rats before CUMS ($\bar{x}\pm s$)

Groups	n	Weight (g)	Open field test	
			Standing up (times)	Horizontal motion (cm)
HCG	6	306.00 ± 13.52	9.83 ± 3.82	10119.83 ± 75.15
DMG	6	307.00 ± 11.23	7.83 ± 5.71	10154.00 ± 93.53
t		−0.139	0.714	−0.698
P		0.892	0.492	0.501

Table 2. Behavior evaluation of rats after CUMS ($\bar{x}\pm s$)

Groups	n	Weight gain (g)	Index of sucrose preference (%)	Immobile time in forced swimming (s)
HCG	6	121.00 ± 12.60	0.91 ± 0.05	65.83 ± 11.16
DMG	6	85.17 ± 28.31	0.68 ± 0.06	108.83 ± 18.90
t		2.830	7.997	−4.798
P		0.018	0.000	0.001

Effects of CUMS on rats plasma Nesfatin−1 and corticosterone concentration

After CUMS, plasma Nesfatin−1 and corticosterone concentrations of DMG were significantly higher than that of NCG ($P<0.05$). In Pearson correlation analysis, it was found that plasma Nesfatin−1 concentrations in DMG were positively correlated to its plasma corticosterone levels ($r=0.827$, $P=0.827$) (Table 3).

Table 3. Plasma Nesfatin−1 and corticosterone concentration in rats after CUMS ($\bar{x}\pm s$)

Groups	n	Nesfatin−1 (ng/ml)	Corticosterone (pg/mL)
HCG	6	1.79 ± 0.87	132.71 ± 14.31
DMG	6	3.50 ± 1.23	166.53 ± 5.34
t		−2.778	−5.424
P		0.020	0.000

Discussion

With a rising prevalence of depression, antide-pressants have been more and more widely applied which have been proven to be effective for most patients. However, there are still about 30% to 50% of patients with depression to whom the antidepressant treatments are ineffective or of low efficacy, which is suggestive of other mechanisms to explain the pathogenesis of depression. Negative life events, especially chronic stress have certain correlations with depression. Stress and hyperactivity of HPA axis are likely to be important causing factors of depression. Effective antidepressant treatment can restore the over-activated HPA axis to its normal level. A significant bearing between the activity of HPA axis after treatment and depression relapses may exist. In this study, compared with rats in normal control group, rats after CUMS had lower index of sucrose preference, restrained yet significantly lower weight gain, and longer time of remaining immobile in forced swimming test. This result showed that rats under CUMS suffered lack of pleasure and de-spair in behavior, ultimately causing depression-like symptoms in rats. Meanwhile, plasma corticosterone concentra-tions were found to be higher in DMG than in NCG which illustrates that CUMS can lead to hyperactivity of the HPA axis. This may be an important part in the mechanism of causing depression-like symptoms in rats. The mechanism through which stress activates HPA axis, however, has not yet been fully illustrated.

In this study, we found that plasma Nesfatin-1 concentration in DMG was significantly higher than in the control group and the concentration of plasma Nesfatin-1 was positively correlated with corticosterone concentration. This result agrees with the findings in the clinical study by Ari and colleagues who found that serum Nesfatin-1 concentration of patients with depression was higher than that of healthy controls and it was positively correlated with Hamilton depres-sion rating scale (HAMD) scores [4], which suggests that there is a certain correlation between depression-like symptoms in depression model rats and altered levels of plasma Nesfatin-1 as well as corticosterone caused by chronic stress. The elevation of corticosterone concentration means the possible activation of HPA axis. It is the elevation of Nesfatin-1 led by stress that causes the rise of corticosterone concentration or the elevation of corticosterone concentration led by stress that causes the rise of Nesfatin-1 level? This relationship between the elevation of Nesfatin-1 in depression model rats and the activation of HPA axis has still not been clearly understood .

Animal experiments showed that injection of Nesfatin-1 in the lateral ventricle can activate HPA axis and lead to anxiety and fear. Goebel and colleagues found that most of the stress responses can activate Nesfatin-1 neurons in the raphe nucleus and locus coeruleus[9]. Yoshida and colleagues claimed that Nesfatin-1 first activates serotonin (5-HT) neurons in the raphe nucleus and norepinephrine neurons in the locus coeruleus, and then activate corticotrophin re-leasing factor (CRF) neurons in the paraventricular nucleus, so as to activate the HPA axis [10], which suggests a possi-ble elevation of Nesfatin-1 concentration in the process of chronic stress factors activating the HPA axis. In other words, chronic stress can lead to the altered concentration of plasma Nesfatin-1 that may be involved in the neuroendocrine regulation mechanism of depression through activating HPA axis.

Nesfatin-1 is very likely to become a new target for treating depression caused by chronic stress and the elevation of both plasma Nesfatin-1 and corticosterone concentrations is probably a biological marker of depression caused by chron-ic stress. Nonetheless, further research on these hypotheses is required to better understand the underlying mechanism.

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