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Up-regulation of GluR1 in paraventricular nucleus and greater expressions of synapse related proteins in the hypothalamus of chronic unpredictable stress-induced depressive rats

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ABSTRACT

Converging evidence indicates that abnormal glutamatergic and synaptic systems may be associated with the pathophysiology of depression. Over-activation of corticotropin-releasing hormone (CRH) neurons in the hypothalamic paraventricular nucleus (PVN) plays a key role in the hypothalamic-pituitary-adrenal (HPA) axis hyperactivity and is a prominent feature in depression. In this study, we examined the effect of chronic unpredictable stress (CUS) in rats on the expression of AMPA subunit GluR1 protein in the hypothalamic PVN as well as on the expression of GluR1 and several synapse-related proteins in the hypothalamus. We found that CUS in rats induced CRH over-expression accompanied by significant GluR1 up-regulation in the hypothalamic PVN and GluR1 co-localized with CRH in the PVN area. The expression of GluR1 and three synapse-related proteins (PSD95, SYP and MAP2) in the hypothalamus were also increased at both mRNA and protein levels in CUS rats. Correlation analysis showed that the levels of the above proteins are directly correlated with depression-like behaviors of rats in sucrose preference test, open field test and forced swim test. These findings suggest that the augmented glutamatergic and synaptic signaling might be a potential mechanism underlying CRH over-activation in the hypothalamic PVN and contribute to CUS-induced depression-like behaviors in rats.

1. Introduction

With high lifetime prevalence and heavy economic burden, depression is one of the most prominent psychiatric disorders and the leading cause of disability in modern society [1]. Hyperactivity of hypothalamus-pituitary-adrenal (HPA) axis has long been closely associated with a major part of the depressive symptomatology [2,3]. Stressful life events and genetic- and epigenetic-risk factors for depression have been linked to hyper-activated HPA axis in adulthood. When patients or animal models of depression are treated or when patients show spontaneous remission, the HPA axis function returns to normal [4]. The hypothalamus is the initial part of the HPA axis and has been one of the most important brain structures implicated in the etiology of mood disorders [5]. As the central driving force, the corticotropin-releasing hormone (CRH)-producing neurons in the hypothalamic paraventricular nucleus (PVN) play a key role in determining the HPA axis state and are closely involved in stress response and the pathogenesis of depression [6]. Over-activation of the hypothalamic CRH neurons has been repeatedly observed in depressive patients as well as animal models by accumulating studies [7,8], while the exact cellular and molecular mechanisms underlying CRH neurons hyperactivity are yet to be fully characterized.

Recent research into depression has focused on the involvement of long-term intra-cellular processes, leading to abnormal neuronal plasticity in brains of depressed patients [9]. Disruption of synaptic function and glutamatergic transmission is evidenced to contribute to the pathophysiology of depression [10]. Human post-mortem samples analysis revealed significant dysregulation of synapse and glutamate related genes in the hippocampus and medial temporal lobe of patients with bipolar disorder (BD) or major depression (MD) [11,12]. Chronic social isolation-reared mice from early life exhibited aggressive and depressive-like behaviors, accompanied by increased expression of AMPA receptor subunits in the prefrontal cortex as well as in the amygdala region [11,13]. However, whether there is hypothalamic plasticity under depressive conditions and its potential involvement in the pathogenesis of depression remains unclear.

Chronic unpredictable stress (CUS) is considered to be a valid and useful experimental model of depression [14,15]. Rats or mice exposed

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to CUS develop behavioral and neurobiological changes that can be used to model comparable aspects of human depression. Elucidating the molecular mechanisms of CUS-induced depression-like behavior may help us understand the pathogenesis of depression. Herein, we established a rat model of depression using the CUS paradigm and detected the depression-like behaviors. HPA axis state was assessed by measuring serum corticosterone (CORT) level and CRH expression in the hypothalamic PVN. We detected the expression of AMPA receptor subunit 1 (GluR1) in hypothalamic PVN and its co-localization with CRH neurons, and further measured mRNA and protein levels of GluR1 and three important synapse-related proteins including postsynaptic density protein 95 (PSD95), synaptophysin (SYP) and microtubule associated protein 2 (MAP2) in the hypothalamus. We also made correlation analysis between GluR1, PSD95, SYP, MAP2 protein levels and depression-like behaviors in sucrose preference test (SPT), open field test (OFT) and forced swim test (FST).

2. Materials and methods

2.1. Animals

Forty male Sprague-Dawley rats weighting 140–160 g were provided from the Experimental Animal Center of Anhui Medical University. All rats were housed in plastic cages under standardized conditions of temperature and humidity with a 12 h light-dark cycle. The study and all protocols got the permission from the Ethic Committee and the Animal Experimental Committee of Anhui Medical University, which were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

2.2. CUS procedure

The CUS procedure was carried out in accordance with the references [16] with minor modifications. After 7-day acclimatization, rats were randomly divided into control and CUS group (20 rats per group). Rats in the control group were housed together (5 rats per cage) with food and water ad libitum, while CUS rats were housed in individual cages and exposed to the following stressors for 21 consecutive days: 1) 30 min of warm swim (30 °C); 2) 5 min of cold swim (8 °C); 3) 1 min of tail pinch (1 cm from the tip of the tail); 4) 24 h of food and water deprivation; 5) 24 h of cage tilting (30°); 6) 24 h of social crowding (25 rats per cage); 7) 24 h of wet bedding (200 mL water per cage). The stressors were randomly performed once daily between 09:00 h and 12:00 h (except 24 h of stressor) and each stressor represented 3 times within 21 days.

2.3. Sucrose preference test, open field test and forced swim test

SPT was applied to measure anhedonia and performed similar to the reference [16]. All rats were trained to consume sucrose solution for 24 h: two bottles of 2% sucrose solution were placed in each cage. After 12 h of food and water deprivation, rats were given free access to two bottles (100 mL 2% sucrose solution and 100 mL pure water) for 4 h. Consumption weight of sucrose solution and pure water were recorded and the sucrose preference (%) was calculated as the percentage of sucrose solution consumption from the total liquid consumption.

OFT was applied to analyze spontaneous exploratory activity and curiosity of animals to a novel environment [17]. A four-sided black wooden box was used as the open field apparatus. The floor was divided into 16 equal squares divided by white lines and the four squares in center were defined as the center zone. The rats were placed individually into the center zone and freely explored the unfamiliar area for 5 min. The numbers of total crossing squares, rearing and grooming were recorded during this period. In order to avoid the mutual influence, the apparatus was cleaned and dried after occupancy by each rat.

FST is a highly reliable test and has strong validity for evaluating depressive-like behavioral status [18]. The rats were placed individually in a cylinder (60 cm in height × 25 cm in diameter) filled with 30 cm of water (maintained at 24–25 °C). The FST paradigm includes 2 sections: an initial 15-min pretest followed 24 h later by a 5-min test. The test was monitored by a video surveillance system for later scoring. Rats were considered to be immobile when they did not make any active movements. The total immobility time was recorded during the 5-min test.

2.4. Blood and tissue preparation

24 h after the behavioral test, rats were deeply anesthetized with chloral hydrate and decapitated. Trunk blood and whole brains were collected immediately. Blood samples were collected, chilled on ice, centrifuged at 4 °C and the serum was stored at − 20 °C. For morphology experiment, the whole brains were fixed in 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4 °C for 24 h, blocked and embedded in paraffin. For mRNA and western blot analysis, the hypothalamus were dissected and collected according to the literature [19] before being quickly frozen in liquid nitrogen and preserved at − 80 °C.

2.5. Serum CORT measurement

The CORT serum levels were measured by a commercially available ELISA kit according to the manufacturer's instructions (RapidBio Lab, California, USA). The samples and standards were all run in duplicates and the data were then averaged.

2.6. Immunohistochemistry of CRH and GluR1

Serial coronal sections (3 μm) of the hypothalamus were cut using a Leica microtome (Leica RM 2135) and collected at 150-μm intervals. The paraffin sections were hydrated, rinsed and microwave-treated in 0.05 M citrate-buffered saline. The sections were treated with 3% H_2O_2 for 10 min at room temperature and incubated with 5% goat serum for 15 min at 37 °C. Subsequently, sections were incubated at 4 °C overnight with rabbit polyclonal anti-CRH (ab8901, Abcam, Cambridge, UK) or mouse monoclonal anti-GluR1 (ab174785, Abcam). Then the sections were incubated with biotinylated secondary antibodies and avidin-biotin horseradish peroxidase complex for 30 min at 37 °C. Sections were visualized with diaminobenzidine for 10 min, dehydrated, hyalinized in xylene and mounted with neutral gum. The sections were examined and photographed using Nikon 80i light microscope equipped with a Canon digital camera. For each animal, sections containing the PVN were identified using a rat brain stereotaxic atlas [20]. The number of immunoreactive cells within the PVN was counted bilaterally at 400 × magnification by an investigator blind to the experimental treatment and the total number of immunoreactive cells throughout the PVN for each animal was semiquantatively estimated as described previously [21,22]. Briefly, an estimate of immunoreactive cellular density for each section was calculated, i.e., the number of immunoreactive cells within the PVN divided by the volume (PVN area multiply with the section's thickness 3 μm). Then the average cellular density of all sections was obtained for each animal. The total number of the immunoreactive cells was calculated by multiplying the average cellular density with the total volume of the PVN. The total volume of the PVN delineated by immunoreactive neurons was determined as described by Bao et al. [21] and calculated according to the Cavalieri principle [23].

2.7. Double-labeling immunofluorescence of GluR1 and CRH

The sections were incubated for 1 h in 5% normal goat serum, and incubated overnight at 4 °C with the intermixture of primary antibody