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Research Report

Chronic unpredictable stress induces a reversible change of PER2 rhythm in the suprachiasmatic nucleus

Wen-Gao Jiang, Su-Xia Li, Shuang-Jiang Zhou, Yan Sun, Jie Shi, Lin Lu*

National Institute on Drug Dependence, Peking University, 38, Xue Yuan Road, Beijing 100191, China

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ABSTRACT

Many clinical studies have shown that circadian rhythm abnormalities are strongly associated with major depression. The master clock of the circadian system in mammals is located in the suprachiasmatic nucleus (SCN) within the anterior hypothalamus, where Per1 and Per2 are essential core components of circadian rhythm oscillation. Chronic unpredictable stress (CUS) is a reliable animal model of depression with good face, predictive, and constructive validity. In the present study, we investigated the effects of CUS on the circadian expression of PER1 and PER2 in the SCN. We found that CUS led to depressive-like behavior and reduced the amplitude of PER2 oscillation in the SCN, which were blocked by 3 weeks of desipramine (DMI) treatment. 2 weeks after termination of CUS, the decreased peak of PER2 expression returned to control levels, whereas depressive-like behavior remained unchanged. Our findings suggest that the dampened amplitude of PER2 expression in the SCN may participate in the development of depressive-like behavior induced by CUS but is unlikely involved in the long-lasting effects of CUS on depressive-like behavior.

1. Introduction

Numerous clinical studies have shown that circadian rhythm abnormalities are strongly associated with major depression. Many patients with major depression experience sleep disturbances, diurnal mood changes, and body temperature and hormone secretion dysregulation (McClung, 2007). Antidepressants and mood stabilizers have effects on circadian-related mechanisms (Duncan, 1996). Circadian manipulations, such as total sleep deprivation and bright light therapy, can reverse depressive symptoms within hours, whereas conventional antidepressants usually have a delayed onset of action in the order of weeks (Bao et al., 2008; Bunney and Potkin, 2008). Although accumulating clinical data support the hypothesis that circadian rhythm may play a role in depression, the underlying chronobiological mechanisms are still poorly understood.

Circadian abnormalities observed in major depression subjects and animal models are diverse and not unique to specific rhythms, indicating a more central origin (Turek, 2007). The suprachiasmatic nucleus (SCN) acts as a master pacemaker of the mammalian circadian rhythm system, coordinating physical and behavioral rhythms throughout the body for synchronization with environmental signals (Refinetti, 2006). A postmortem study of arginine vasopressin (AVP) expression in the SCN found less AVP mRNA and higher AVP immunoreactivity in the SCN in major depression patients (Zhou et al., 2001). Lesions of the SCN in rats reduced

* *Corresponding author*. Fax: +86 10 62032624.
E-mail address: jiangwg@bjmu.edu.cn (W.-G. Jiang).

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immobility in the forced swim test (Tataroglu et al., 2004). Light and electron microscopic studies found direct and indirect projections of the SCN to stress-related areas (Buijs et al., 1993; Luo and Aston-Jones, 2009; Sylvester et al., 2002). Agomelatine, a novel antidepressant that exerts antidepressant effects in several animal models of depression, targets melatonin receptors and serotonin-2C receptors, both of which have been identified in the SCN and found to be involved in the synchronization of biological rhythms (Racagni et al., 2007).

Per1 and Per2 are important components of the primary molecular clock in the SCN (Alvarez and Sehgal, 2002) and are involved in not only light responses but also the resetting of the circadian rhythm (Albrecht et al., 2001). Per1 or Per2 mutant mice exhibit abnormal rhythm of activity and body temperature in constant dark or light (Steinlechner et al., 2002). Per2$^{-/-}$ mice do not have a glucocorticoid rhythm or diurnal feeding rhythm (Yang et al., 2009b). Per2 mutant mice show less monoamine oxidase A (MAOA) mRNA expression and MAO activity in the mesolimbic dopaminergic system (Hampp et al., 2008). Although some evidence has revealed the role of clock genes in mood regulation (Hampp and Albrecht, 2008; Mukherjee et al., 2010), molecular changes in the SCN have not been elucidated in a chronic unpredictable stress (CUS) animal model.

Chronic unpredictable stress is a reliable animal model of depression with good face, predictive, and constructive validity (Willner, 1997). Different types of stressors, such as restraint, rotation, forced swim, and food or water deprivation, are applied to rats in a variable sequence for 3 or 4 weeks to mimic the development of depression induced by life stress in humans (Table 1). The present study investigated whether the CUS procedure influences PER1 and PER2 rhythm in the SCN and their long-lasting effects.

2. Results

2.1. *Chronic unpredictable stress-induced depressive-like behavior and decreases in peak PER2 expression in the SCN*

A total of 96 rats were assigned to four groups: control, DMI, CUS, and CUS+DMI (n=24/group). Depressive-like behavior was examined using the open field, sucrose preference test, and novelty-suppressed feeding test after the CUS procedure was finished. The rats were then prepared for immunohistochemistry (IHC) staining at 4 h intervals (n=4/time point). PER1 and PER2 expression in the SCN was visualized by IHC staining and analyzed with Image-Pro Plus version 6.0 software.

Chronic unpredictable stress significantly decreased the number of crossings ($F_{1,20}$=26.35, p<0.001) and rearings ($F_{1,20}$=4.62, p<0.05) in the open field test. Rats treated with CUS had a lower percentage of sucrose consumption ($F_{1,20}$=21.83, p<0.001) than controls. Latency in the novelty-suppressed feeding test was significantly higher in CUS rats compared with controls ($F_{1,20}$=19.223, p<0.001). 3 weeks of DMI administration prevented the effects of CUS on crossings and rearings and in the sucrose preference test and novelty-suppressed feeding test ($F_{1,20}$=10.71, p<0.01; $F_{1,20}$=6.35, p<0.05; $F_{1,20}$=4.17, p<0.05; $F_{1,20}$=27.03, p<0.001; respectively; Fig. 1).

Immunohistochemistry staining showed that PER2 expression in the SCN peaked at ZT9–ZT13 in all groups. Chronic unpredictable stress decreased peak PER2 expression in the SCN at ZT9 ($F_{1,8}$=10.08, p<0.01) and ZT13 ($F_{1,8}$=6.16, p<0.05; Fig. 2) but did not have an effect on PER1 expression in the SCN (Fig. 3). 3 weeks of DMI treatment prevented the inhibitory effects of CUS on PER2 expression in the SCN at ZT13 ($F_{1,8}$=35.28, p<0.001; Fig. 2).

2.2. *Depressive-like behavior endured for 2 weeks after termination of the CUS procedure, but the decreased amplitude of PER2 expression in the SCN recovered*

A total of 48 rats were used in this experiment. Twenty-four rats underwent the CUS procedure, and the other 24 rats were used as controls. At the end of the CUS procedure, six control rats and six CUS rats were subjected to the behavioral tests and sacrificed at ZT1 and ZT9, respectively, for PER2 IHC staining. The remaining rats were left free from stress for another 2 weeks. The behavioral tests were then conducted. The rats were sacrificed for PER2 IHC staining at ZT1 and ZT9, respectively.

2 weeks after termination of the CUS procedure, the behavioral tests revealed a significant decrease in the number of crossings ($F_{1,12}$=13.18, p<0.01; Fig. 4A), decrease in the number of rearings ($F_{1,12}$=20.90, p<0.01; Fig. 4B), decrease in sucrose preference ($F_{1,12}$=20.90, p<0.001, Fig. 4C), and increase in the latency to find food ($F_{1,12}$=23.73, p<0.001; Fig. 4D) in rats that had experienced CUS compared with controls. The Student–Newman–Keuls *post hoc* test revealed that depressive-like behavior, with regard to rearing, sucrose preference, and novelty-suppressed feeding, remained the same 2 weeks after the end of the CUS procedure. However, IHC staining analysis showed that 2 weeks after stress exposure, peak PER2

Table 1 – Chronic unpredictable stress procedure.

Week day	Week 1	Week 2	Week 3	Week 4
Monday	Restraint; rotation	Cold; hot	Forced swim; restraint	Reverse L/D cycle; crowding
Tuesday	Food deprivation; pairing	Wet sawdust; cage tilt	Wet sawdust; cage tilt	Restraint; rotation
Wednesday	Cold; hot	Forced swim; restraint	Reverse L/D cycle; crowding	Food deprivation; pairing
Thursday	Wet sawdust; cage tilt	Water deprivation; cage tilt	Restraint; rotation	Cold; hot
Friday	Forced swim; restraint	Reverse L/D cycle; crowding	Food deprivation; pairing	Water deprivation; cage tilt
Saturday	Water deprivation; cage tilt	Restraint; rotation	Cold; hot	Wet sawdust; cage tilt
Sunday	Reverse L/D cycle; crowding	Food deprivation; pairing	Water deprivation; cage tilt	Forced swim; restraint