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Hippocampal astrocyte atrophy in a mouse depression model induced by corticosterone is reversed by fluoxetine instead of benzodiazepine diazepam

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ABSTRACT

Astrocytes have become promising new agents against major depressive disorders (MDD) primarily due to the crucial role they play in the pathogenesis of such disorders. However, a simple and reliable animal model that can be used to screen for astrocyte-targeting antidepressants has not yet been developed. In this study, we utilized a repeated corticosterone (CORT) injection paradigm to develop a mouse depression model wherein we examined the occurrence of alterations in hippocampal astrocyte population by using two astrocytic markers, namely, glial fibrillary acidic protein (GFAP) and S100β. Moreover, we determined the effects of fluoxetine and diazepam on CORT-induced astrocytic alterations to assess the predictive validity. Results showed that repeated CORT injections showed no effects on the number of $GFAP^+$ and $S100\beta^+$ astrocytes, but they decreased the protrusion length of $GFAP^+$ astrocytes and GFAP protein expression in the hippocampus. Furthermore, repeated CORT injections produced a sustained increase of S100β protein levels in the entire hippocampus of male mice. CORT-induced hippocampal astrocyte disruption was antagonized by chronic fluoxetine treatment. By contrast, the anxiolytic drug diazepam was ineffective in the same experimental setting. All these findings suggest that the repeated CORT injection paradigm produces the astrocytic alterations similar to those in MDD and can serve as a useful mouse model to screen antidepressants meant to target astrocytes. These observations can also help in further discussing the underlying mechanisms of CORT-induced astrocytic alterations.

1. Introduction

The monoamine and neurotrophin hypotheses of depression comprise the major paradigms that explain the pathophysiology of major depressive disorders (MDD). The former believes that depression is linked to disturbances in serotonergic and noradrenergic neurotransmissions (Avissar and Schreiber, 2002). Meanwhile, the latter assumes that reduced neurogenesis plays an important role in the depression etiopathogenesis (Gass and Riva, 2007; Serafini et al., 2014). In these two theories, neuronal dysfunction is the common mechanism underlying the depressive disorder.

However, the neurobiology of the astrocytes associated with depression has recently received increasing attention because astrocytes are recognized as active neuron partners and nutrient supplies and are actively involved in signalization processes (Hines and Haydon, 2014; Páv et al., 2008; Rial et al., 2016; Xiao and Hu, 2014). In addition, postmortem studies have reported alterations in the number, morphology, and protein expression of astroglia in MDD. For example, the use of antibodies against astrocyte-specific markers, such as glial fibrillary acidic protein (GFAP) and S100β, a calcium-binding protein, has consistently yielded dramatic reductions in the number of astrocytes in the prefrontal cortex (Miguel-Hidalgo et al., 2000), hippocampus (Cobb et al., 2016; Gos et al., 2013), and amygdala (Altshuler et al., 2010). These reductions are paralleled by astrocyte hypotrophy, that is, shrinkage of GFAP-positive astrocytic size and protrusion length (Davis et al., 2002; Miguel-Hidalgo et al., 2010). Additionally, the reduction of GFAP protein and mRNA levels was demonstrated in the frontal cortex (Johnston-Wilson et al., 2000; Nagy et al., 2015; Si et al., 2004), amygdala (Altshuler et al., 2010; Kekesi et al., 2012), locus coeruleus (Chandley et al., 2013), and cerebellum (Fatemi et al., 2004) of subjects with mood disorders.

On the basis of clinical findings, animal models have been commonly developed to further study the underlying etiology and pathophysiology of diseases and screen for therapeutic or preventive drugs that target the cellular or molecular aspects of pathogenesis. Given that stressful life events are major causes of depression and may also be responsible for the severity and recurrence of this illness, repeated or chronic stress exposure has become one of the first methods for

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developing ethological models of depression (Rupniak, 2003; McArthur and Borsini, 2006). However, the effects of stress restraints on emotional behavior are variable, leading to increased experimental variability (Beck and Luine, 2002; Gregus et al., 2005; Yin et al., 2016). To avoid this problem, we used exogenous corticosterone (CORT) administration in our previous studies as a means to upgrade this kind of ethological model of depression (Zhao et al., 2008). The rodents submitted to chronic CORT administration showed multiple anxiety- and depressive-like changes in behavior and neurochemistry similar to those observed in MDD (Ago et al., 2013; David et al., 2009; Iijima et al., 2010; Lee et al., 2013). This neuroendocrine model of depression is highly reproducible and easy to set up and has been used to evaluate the efficacy of potential antidepressants (Lee et al., 2013; Mei et al., 2014) or electroconvulsive therapy (O'Donovan et al., 2014), explore the action mechanism of antidepressants (de Sousa et al., 2015; Oliveira et al., 2017), and improve our knowledge about the MDD pathophysiology (Dwivedi et al., 2015).

Given that astrocytes are currently being discussed as possible therapeutic agents against MDD (Wang et al., 2017a, 2017b), developing a simple and reliable animal model for screening astrocyte-targeting drugs is needed. Consequently, in this study, we investigated the effects of chronic CORT injection on the number, morphology, and protein expression of astroglia by using stereological techniques and Western blot analysis. We also determined whether the repeated CORT injection paradigm produced the alterations in astrocyte morphology similar to those in MDD. Additionally, we observed the effects of fluoxetine (one of the commonly prescribed selective serotonin reuptake inhibitors) and diazepam (one of the most widely used benzodiazepines) on hippocampal astrocyte alterations in morphology and protein expression induced by CORT to assess the predictive validity of this neuroendocrine model of depression.

2. Methods

2.1. Experimental animals

Male C57BL/6N mice weighing 18–22 g each were purchased from the Laboratory Animal Center of Nanjing Medical University (Nanjing, China). These mice were housed on a 12 h light/dark cycle with ad libitum access to food and water at a constant temperature of 25 °C ± 1 °C. The animals were housed in groups of five per cage and allowed to habituate for one week. All animal procedures were approved by the Institutional Animal Care and Use Committee of Nanjing University of Chinese Medicine and carried out in accordance with the Guidelines of Accommodation and Care for Animals formulated by the Chinese Convention for the protection of vertebrate animals used for experimental and other scientific purposes. The minimum number of animals required to obtain consistent data was used.

2.2. Animal model

The animals were divided into two groups, namely, control and CORT. The mice in the CORT group were injected subcutaneously with CORT (20 mg/kg, suspended in physiological saline containing 0.1% dimethyl sulfoxide and 0.1% Tween-80; Sigma) once a day at random times during light phase (Zhao et al., 2008). A similar corticosterone injection paradigm has been reported to cause a persistent elevation of plasma corticosterone levels (i.e., lasting for 24 h), with peak levels occurring within 4 h of the injection (Sousa et al., 1998a). The control mice were injected only with the vehicle.

2.3. Experimental procedures

2.3.1. Experiment 1: time course of behavior and astrocyte response to chronic CORT injection

Mice were randomly assigned to six experimental groups (n = 10/group). Three groups composed the CORT groups, whereas the three other groups composed the control groups. Behavioral assessments of the mice (10 controls and 10 CORT) were conducted during the light period after being injected for 1, 3, and 5 weeks (see Supplementary Fig. S1). A minimum of 1 h after behavioral tests, blood samples (up to 200 μL per animal) were collected into heparinized tubes from the lateral saphenous vein of the mice. Plasma was separated by centrifugation at 3000 rpm and stored at − 20 °C until the assay of CORT concentrations. The mice were then sacrificed for Western blot analysis (five controls and five CORT) and morphological analysis (five controls and five CORT).

2.3.2. Experiment 2: therapeutic effects of fluoxetine on CORT-induced behavioral and hippocampal astrocyte changes

As depicted in Supplementary Fig. S1, animals were divided into four experimental groups: *Control group* (n = 10), *Control + Fluoxetine group* (n = 10), *CORT* (n = 10), and *CORT + Fluoxetine* (n = 10). The experiment consisted of two phases and lasted for five weeks. The first experimental phase lasted for three weeks. During which, the mice of the *CORT* and *CORT + Fluoxetine* groups were submitted to daily CORT injections. The second experimental phase consisted of fluoxetine treatment for two weeks while animals maintained daily CORT injection. Mice of the *Control + Fluoxetine* and *CORT + Fluoxetine* groups received fluoxetine hydrochloride (10 mg/kg, dissolved in physiological saline; Suzhou Pharmaceutical Co., Ltd., China) orally in the morning. The administered dose was based on previous reports that fluoxetine could effectively produce behavioral effects in the corticosterone-induced mouse depression model (Chen et al., 2014; Pytka et al., 2017). Animals of the *Control* and *CORT* groups were administrated orally with the vehicle. Five weeks later, mice were subjected to behavioral testing followed by terminal blood sample collection. Then, five animals per group were randomly chosen for Western blot analysis. The remainder of the animals was used for morphological analysis.

2.3.3. Experiment 3: therapeutic effects of diazepam on CORT-induced behavioral and hippocampal astrocyte changes

As depicted in Supplementary Fig. S1, animals were divided into four experimental groups, as follows: *Control group* (n = 10), the *Control + Diazepam group* (n = 10), *CORT* (n = 10), and the *CORT + Diazepam* group (n = 10). Mice were treated as described in Experiment 2 but with diazepam hydrochloride (1 mg/kg, dissolved in physiological saline; Suzhou Pharmaceutical Co., Ltd., China). The administered dose was based on previous reports that diazepam could ameliorate depression-like behavior in the depression model induced by chronic stress (Sun et al., 2013; Zhao et al., 2012).

2.4. Behavioral tests

2.4.1. Elevated plus maze

Mouse behavior was assessed for 5 min in the elevated plus maze test. According to the description in Pellow et al.'s (1985) studies, the apparatus consisted of two open arms (30 cm × 10 cm) alternating at right angles with two arms enclosed by 25 cm high walls. Four arms delimited a central area (10 cm × 10 cm). The entire apparatus was placed on a frame with 76 cm height. The test began with the placement of the mouse at the center of the maze with its head facing a closed arm. The time spent and the visits to the open arms were recorded, and a four-paw criterion was used for the arm entries.

2.4.2. Forced swimming test and tail suspension test

The forced swimming test (FST) used in this study was similar to that described by Porsolt et al. (1977). After 2 h of elevated plus maze test, the mice were subjected to the FST. Briefly, mice were individually placed in 10 cm of ambient temperature water (25 °C ± 1 °C) placed in 2 L glass beakers and were allowed to swim for 5 min. The durations of immobility were recorded during the last 4 min of the test. Immobility