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Tianeptine reverses stress-induced asymmetrical hippocampal volume and *N*-acetylaspartate loss in rats: An in vivo study

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ABSTRACT

Stress-induced hippocampal volume loss and decrease in *N*-acetylaspartate (NAA) level have been reported to be associated with impaired neural plasticity and neuronal damage in adults. Accordingly, reversing structural and metabolite damage in the hippocampus may be a desirable goal for antidepressant therapy. The present study investigated the effects of tianeptine on chronic stress-induced hippocampal volume loss and metabolite alterations in vivo in 24 Sprague-Dawley rats. Rats were subjected to a consecutive 28-day forced swimming test stress. Tianeptine (50 mg/kg) or saline was administered intragastrically 4 h after swimming each day. Spontaneous behaviors, serum corticosterone concentration, hippocampal volume and NAA level were evaluated after stress. Chronic tianeptine treatment counteracted the chronic stress-induced suppression of spontaneous behaviors, elevated serum corticosterone concentration, reduced hippocampal volume and decreased NAA level. Moreover, we found asymmetrical right–left hippocampal volume loss in stressed rats, with the left hippocampus more sensitive to chronic stress than the right hippocampus. In addition, stressed rats showed a decreased level of hippocampal metabolites, without significant loss of hippocampal volume. These findings provide experimental evidence for impaired structural plasticity of the brain being an important feature of depressive illness and suggest that prophylactic tianeptine treatments could reverse structural changes in brain. The structural and neurochemical alterations in the hippocampus may be valuable indexes for evaluating the prophylactic and curative effect of antidepressant treatments in depressive and stress-related disorders.

1. Introduction

The impact of stress on the body has been increasingly recognized. Stress disorders, depression and psychophysiological disorders are the most common life-threatening illnesses and are critical to public health (Murray and Lopez, 1997).

The hippocampus is a major brain structure needed for learning and memory; it is also one of the most-studied brain regions involved in depression and stress-related disorders (McEwen, 1999, 2000). Both *in vivo* and *in vitro* studies have explored hippocampal impairment induced by stress in humans and animals. Recently, some brain imaging studies in humans revealed that the hippocampus undergoes selective volume reduction in stress-related neuropsychiatric disorders such as recurrent depressive illness (Sheline et al., 1996; Bremner et al., 2000) and major depression (Lee et al., 2002). Moreover, the hippocampal volume of patients with post-traumatic stress disorder (PTSD) is decreased unilaterally or bilaterally (Bremner, 1999; Gilbertson et al., 2002). Compelling animal studies showed that stress is associated with changes in hippocampal structure and function (Luo et al., 2005; Murakami et al., 2005; Czeh et al., 2006).

N-acetylasparate (NAA) is generally accepted as a marker of neuronal integrity and viability, reflecting neuronal density or metabolism (Urenjak et al., 1993) that can be detected by proton magnetic resonance spectroscopy (^{1}H-MRS). Previous studies have found NAA levels decreased in the hippocampus of veterans with

Abbreviations: ^{1}H-MRS, Proton Magnetic Resonance Spectroscopy; ANOVA, Analysis of Variance; Cho, Choline Moieties; Cr, Creatine; DG, Dentate Gyrus; ELISA, Enzyme Linked Immunosorbent Assay; FLASH, Fast Low Angle Shot; FST, Forced Swimming Test; HPLC, High Performance Liquid Chromatography; MRI, Magnetic Resonance Imagine; NAA, N-acetylaspartate; OFT, Open Field Test; PRESS, Point-resolved Spectroscopy; PTSD, Post-traumatic Stress Disorder; SAX, Strong Anion Exchange; T1WI, T1-weighted Image; T2WI, T2-weighted Image; TE, Echo Time; TR, Repetition Time; VOI, Volume of Interest.

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PTSD by MRI and ^{1}H-MRS (Schuff et al., 1997) and in the medial temporal lobe of such veterans by ^{1}H-MRS (Freeman et al., 1998). Thus, stress-induced alterations in hippocampal structure and metabolite levels can be detected by MRI and ^{1}H-MRS *in vivo*.

Tianeptine is a clinically effective antidepressant with structural similarities to tricyclic antidepressants and is thought to enhance serotonin uptake *ex vivo* (Wagstaff et al., 2001). The neurobiological properties of tianeptine involve the interplay between numerous neurotransmitter systems that are involved in regulating structural and functional plasticity in the hippocampus (McEwen and Olie, 2005). In animal studies, tianeptine could reduce apoptosis in the dentate gyrus (Lucassen et al., 2004) and was found to be cytoprotective against the effects of proinflammatory cytokines in the cortex and white matter in mice (Chen et al., 2000; Son et al., 2003; Kim et al., 2004). Therefore, tianeptine has multiple neuroprotective activities in stress animal models.

MRI and ^{1}H-MRS could be a feasible method for evaluating the neuroprotective effect of antidepressants on brain structure and metabolites. In this study, we assessed the prophylactic effects of tianeptine by evaluating spontaneous behaviors, serum corticosterone concentration, hippocampal volume and hippocampal metabolite concentration *in vivo* and *in vitro* in rats under chronic forced swimming test (FST) stress.

2. Methods

2.1. Animals

We used 24 adult male Sprague-Dawley rats (180 to 200 g) supplied by the Experimental Animal Center of Wuhan University. All rats were housed in cages (3 rats/cage) and had free access to food and water in a 12-h light/dark cycle, with lights on at 08:00 h, under constant temperature (25 °C) and a clean air conditioning system. All animals lived in the laboratory for at least 7 days before the experiment. Experiments were carried out in accordance with the US National Institutes of Health guidelines for the care and use of laboratory animals to minimize pain or discomfort.

Rats were randomly divided into four groups ($n=6$ in each group) for treatment: control, FST stress, FST stress + saline (1.5 ml) and FST stress + tianeptine (50 mg/kg body weight [Stablon; Servier, Courbevoie, France])(Czeh et al., 2001). All experimental rats underwent 28 consecutive days of the FST. Saline and tianeptine were given intragastrically 4 h each day after the test. The behavioral manipulations were carried out in a random order.

2.2. Forced swimming test

Rats underwent the FST in the morning (8:00-12:00 h) as described elsewhere (Broqua et al., 1992) with slight modification. Briefly, rats were individually placed into a plastic cylinder (height × diameter: 70 × 50 cm) filled with 25 °C water at a depth of 40 cm. Rats were removed after 15 min of swimming and placed under a heat lamp for 15 min before being returned to their cages. Water was changed between each test. At 24 h after the first exposure, rats were again placed into the swimming cylinder (under the same condition) for 15 min/d for 28 consecutive days.

2.3. Open field test (OFT)

The OFT was carried out 1 day before and the day after the FST session between 7:00 and 11:00 h. The open-field apparatus consists of a plastic box (100-cm square chamber, 50-cm high walls). The walls of the open field were painted matte black, and the bottom was divided into 25 equal squares (20 × 20 cm) by white-colored grids. The animals were tested in a quiet room. Four fluorescent lights, 3 m above the box, provided diffuse and even overhead illumination. Each rat was tested individually and once only each time; the spontaneous behaviors of rats on the floor were recorded for 5 min by two trained people who were unaware of the experimental condition. The number of squares crossed (Crossing, i.e., at least 3 paws in a quadrant), and grooming (Grooming, i.e., rubbing the body with paws or mouth and rubbing the head with paws), and rearing behavior (Rearing, i.e., animal standing upright on its hind legs) were counted. After each animal was tested, the apparatus was thoroughly cleaned with cotton pads moistened with 50% ethanol.

2.4. MRI and ^{1}H-MRS

2.4.1. Structural MRI

Following the day of the second OFT, rats underwent structural MRI to evaluate the hippocampal volume *in vivo*. MRI scanning involved a 4.7 T Bruker BIOSPEC-47/30 MRI imager (Bruker Medical, Ettlingen, Germany) at a constant room temperature (18 ± 2 °C). Rats were anesthetized with 6% chloral hydrate (0.5 ml/kg, intraperitoneally); an additional 0.3 ml/kg was administrated each hour to maintain anesthesia. A rubber tube filled with fluid 37 °C water was placed on the abdomen to maintain body temperature. The signal was received through a surface coil placed directly on the animal's head. A series of T2-weighted images (T2WI) were acquired with a spin-echo sequence and the following parameters: repetition time (TR) 3110 ms; echo time (TE) 20 ms, and 6 echoes, slice thickness 0.8 mm, with an interval of 1.6 mm. Another scan was performed to capture the 0.8 mm interval images. In total, 14 slices were captured for the whole hippocampus. After structure scanning, images containing the hippocampus were analyzed by use of ParaVision 2.1.1 (Bruker Medical, Ettlingen, Germany). Eight slices were used for hippocampal volume evaluation. The hippocampal formation was drawn manually by a trained person using the rat brain atlas (*The Rat Brain*, 5th edition, George Paxinos and Charles Watson) and without knowledge of the experiment. The hippocampal volume was calculated by multiplying the computed areas by the slice thickness.

To minimize measurement bias caused by individual variance, the hippocampal boundary of all slices was identified or delimited by *Shu*. Standardized hippocampal volumes were calculated as a ratio of hippocampus to brain volume of co-existing hippocampus (Fig. 1A).

2.4.2. Proton magnetic resonance spectroscopy (^{1}H-MRS)

Animals were measured in a prone position with their head firmly fixed between a specially made plastic holder and the surface coil. Following structural scanning, ^{1}H-MRS data were acquired to evaluate relative *in vivo* brain metabolite concentrations of NAA and choline (Cho). A point-resolved spectroscopy (PRESS) sequence was used to acquire localized proton spectra from bilateral hippocampi with a voxel size of 2.0 × 2.0 × 2.0 mm, TR 3500 ms, and TE 40 ms. The position of the volume of interest was carefully selected from multislice sagittal and coronal T1-weighted gradient-echo images [fast low angle shot (FLASH), TR/TE = 150/5 ms, 20° flip angle, 50 mm field-of-view, 256 × 256 data matrix, 1 mm sections] and centrally placed in the hippocampus. Ratios of NAA/Cr were measured by a professional without knowledge of the experimental condition (Fig. 1B).

2.5. Determination of serum corticosterone concentration

All rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and decapitated between 9:00 and 10:00 h on the day after the MRI scan. Samples of trunk blood were collected, centrifuged and stored at −20 °C. Serum corticosterone concentration was detected by a commercially available ELISA kit from R&D Systems (Minneapolis, MN, USA) following the manufacturer's instructions. All values were between the lowest and the highest concentration of the standard curve.