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L-tyrosine improves neuroendocrine function in a mouse model of chronic stress

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Abstract

Adult BALB/c mice, individually housed, were stimulated with nine different stressors, arranged randomly, for 4 continuous weeks to generate an animal model of chronic stress. In chronically stressed mice, spontaneous locomotor activity was significantly decreased, escape latency in the Morris water maze test was prolonged, serum levels of total thyrotropin and total triiodothyronine were significantly decreased, and dopamine and norepinephrine content in the pallium, hippocampus and hypothalamus were significantly reduced. All of these changes were suppressed, to varying degrees, by L-tyrosine supplementation. These findings indicate that the neuroendocrine network plays an important role in chronic stress, and that L-tyrosine supplementation has therapeutic effects.

Key Words

chronic unpredictable stress; neuroendocrine network; total thyrotropin; total triiodothyronine; dopamine; norepinephrine; L-tyrosine; neural regeneration

Abbreviations

CUS, chronic and unpredictable stress group; TT4, total triiodothyronine; TT3, total thyrotropin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone

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INTRODUCTION

Stress is the body's non-specific response to both internal and external stimuli. Persistent or excessive stress, surpassing the individual's capacity to adapt and cope, disturbs normal physiological function and behavior, even leading to physical and psychiatric diseases[1-3]. Statistics show that the stress response can cause histological, physiological and biochemical changes[4-6]. As the pace of social life increases and competition intensifies, people generally are in a state of chronic stress that can give rise to a series of familial and social problems. Therefore, it is an urgent task for modern medicine to study relevant mechanism of stress, and to investigate methods to alleviate its impact on human health. Tyrosine, an aromatic amino acid, is a precursor for the synthesis of catecholamine neurotransmitters[7]. In the central nervous system, catecholamine neurotransmitters participate in regulating activities such as awakening, attention and mood[8]. Therefore, when catecholamine insufficiency arises in the brain due to chronic stress, supplementation with tyrosine can reduce physical decline, loss of interest and slow

movement caused by chronic stress[9-10]. Previous studies indicate that tyrosine plays an active part in regulating the behavior and mood of people in such extreme environments as high altitude, oxygen deficiency and extreme cold[11-13]. However, little is known regarding the effects of tyrosine on disorders resulting from daily stresses (*e.g*., the quickened pace of life, fiercer social competition and increasing pressure at work, the disparity between efforts and gains, occupational instability and interpersonal tension). In the present study, we established a mouse model of chronic stress to investigate the effects of tyrosine intervention on disruption of the neuroendocrine network caused by chronic stress, and to clarify the underlying mechanism.

RESULTS

Quantitative analysis of experimental animals

A total of 63 healthy BALB/c mice were randomly divided into three groups with 21 mice in each group: normal control (control), chronic and unpredictable stress (CUS) and CUS plus L-tyrosine interference group (CUS-L). Experiments lasted for 4 weeks. All mice were included in the final analysis with no cases of death or infection.

Stress increased mouse body weight

After 4 weeks of chronic and unpredictable stress, the body weight of mice increased significantly compared with that 4 weeks earlier ($P < 0.05$; Table 1).

Table 1 Effect of chronic stress on body weight (g) of mice

Group	Before stress induction	Time after stress induction (week)			
		1	2	3	4
Control	23.9±1.2	24.1±1.4	24.7±1.7	25.3±1.1	26.1±1.2
CUS	22.7±1.4	23.9±1.2	21.9±1.5	25.6±1.1	26.2±1.4[a]
CUS-L	23.9±1.5	24.9±1.9	25.5±1.2	25.8±1.2	26.3±1.2

Data are presented as mean ± SD. Differences between the control group and the other two groups were tested using analysis of variance and paired sample *t*-test. [a]$P < 0.05$, *vs*. before stress induction. CUS: Chronic and unpredictable stress; CUS-L: CUS plus L-tyrosine interference.

L-tyrosine alleviated behavioral deficits in stressed mice

Spontaneous locomotor activity

Compared with normal control mice, stressed mice displayed a significant reduction in the total horizontal distance traveled in a novel environment ($P < 0.01$). L-tyrosine supplementation significantly increased the total horizontal distance traveled by mice subjected to stress ($P < 0.01$; Table 2).

Table 2 Locomotor activity (m) of mice in each group after exposure to chronic stress

Group	Before stress induction	Time after stress induction (week)			
		1	2	3	4
Control	1.31±0.21	1.39±0.29	1.38±0.17	1.52±0.21	2.01±0.31
CUS	1.30±0.48	1.73±0.20[a]	1.86±0.36[a]	1.51±0.56	1.36±0.43[a]
CUS-L	1.46±0.32	1.49±0.44[b]	2.71±0.44[ab]	1.65±0.42	1.81±0.39[b]

Data are presented as mean ± SD. Differences between the control group and the other two groups were tested using analysis of variance and paired sample *t*-test. [a]$P < 0.01$, *vs*. control group; [b]$P < 0.01$, *vs*. CUS group. CUS: Chronic and unpredictable stress; CUS-L: CUS plus L-tyrosine interference.

The Morris water maze test

After five successive tests in 4 weeks, the time taken by the control mice to find the platform gradually decreased, while mice submitted to stress showed the opposite trend, and there was a significant difference between these two groups ($P < 0.05$). Stressed mice given L-tyrosine supplementation showed greater improvement in performance in maze tests ($P < 0.05$; Figure 1).

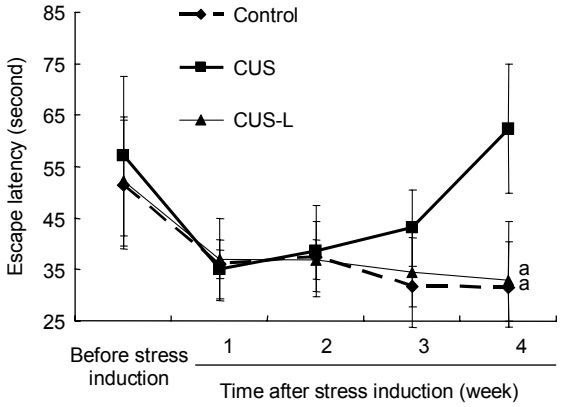

Figure 1 Escape latency trends at 1–4 weeks in the Morris water maze test.

Data are presented as mean ± SD. Differences between the control group and the other two groups were tested using analysis of variance and paired sample *t*-test. [a]$P < 0.05$, *vs*. CUS group. CUS: Chronic and unpredictable stress; CUS-L: CUS plus L-tyrosine interference.

L-tyrosine increased serum levels of total thyrotropin (TT3) and total triiodothyronine (TT4) in stressed mice

Compared with mice in the control group, serum levels of TT3 and TT4 were significantly decreased in stressed mice ($P < 0.05$). TT3 and TT4 serum levels were significantly increased by L-tyrosine supplementation ($P < 0.05$; Table 3).

Table 3 Effect of chronic stress on serum levels of TT3 and TT4 (ng/mL)

Group	TT3	TT4
Control	0.74±0.22	48.20±3.41
CUS	0.55±0.27[a]	29.91±12.8[a]
CUS-L	0.64±0.13[b]	35.62±7.1[b]

Data are presented as mean ± SD. Differences were tested using analysis of variance and paired sample *t*-test. [a]*P* < 0.05, *vs*. control group; [b]*P* < 0.05, *vs*. CUS group. TT3: Total thyrotropin; TT4: total triiodothyronine; CUS: chronic and unpredictable stress; CUS-L: CUS plus L-tyrosine interference.

L-tyrosine supplementation modulated neurochemical changes in brain tissues of stressed mice

After 4 weeks of chronic stress, dopamine and norepinephrine levels in the pallium, hippocampus and hypothalamus were significantly decreased (*P* < 0.01 or *P* < 0.05). Levels of dopamine in the pallium and hippocampus, as well as levels of norepinephrine in the pallium and hypothalamus, were restored by L-tyrosine supplementation (*P* < 0.05 or *P* < 0.01; Figure 2).

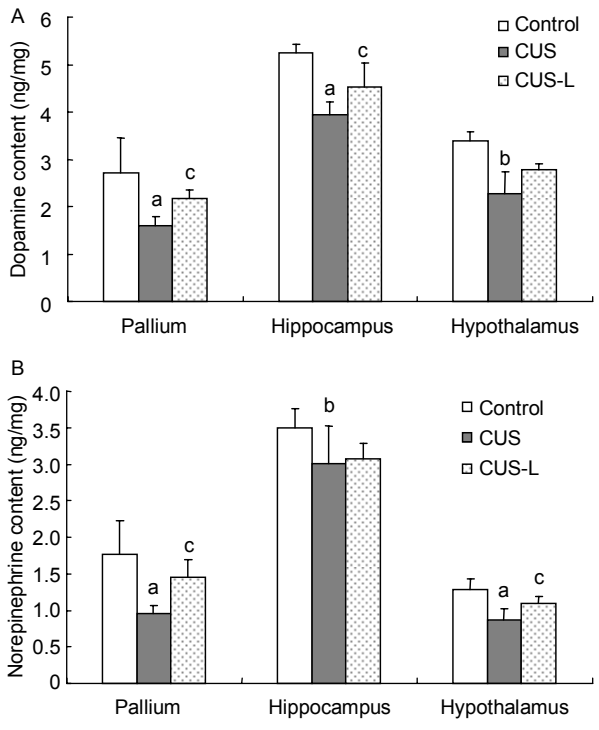

Figure 2 Dopamine (A) and norepinephrine (B) content in the pallium, hippocampus and hypothalamus (ng/mg).

Data are presented as mean ± SD. Differences between the control group and the other two groups were tested using analysis of variance and paired sample *t*-test. [a]*P* < 0.01 and [b]*P* < 0.05, *vs*. control group; [c]*P* < 0.05, *vs*. CUS group.

CUS: Chronic and unpredictable stress; CUS-L: CUS plus L-tyrosine interference.

DISCUSSION

In daily life, chronic stress-related hazards are generally caused by various complex, low-intensity and long-term life events[14]. Single stress factors (*e.g*., amputation of the tail, high-frequency-voltage convulsion and long-term restraint) were, in the past, frequently used to reproduce stress in animal models in a relatively short period of time[15]. However, single stress factors do not fully reproduce chronic stress because they are too intense or animals develop resistance easily[16]. While multiple chronic and unpredictable factors are better able to reproduce chronic stress in animal models, this approach has its own shortcomings, including a lengthy model production period, high demands on laboratory resources and the requirement to frequently change stimulants, which results in a heavy workload for the researcher[17-18].

After 4 weeks of chronic stress, the body weight of mice in the CUS group showed a significant increase compared with that before the experiment. In previous studies, some mice suffered a loss of weight, while some gained weight. Thus, our result is not contradictory to those previously reported by various groups[15, 19-20]. The distance covered per unit time by CUS mice in the first and second weeks of testing increased gradually, but their performance declined in the spontaneous movement experiment from the third week. This indicates that damage caused by chronic stress to physical and psychological health begins to manifest from the third week, and that chronic stress can weaken the animal physically, leading to increased fatigability, which hinders their ability to explore the surrounding environment in a limited time. The increased escape latency of the stressed mice suggests that the animals' learning and memory abilities decline with increasing duration of stress, and that chronic stress may damage their learning and memory functions.

Rodents' memory ability is related to prefrontal cortex dopamine content, and proper functioning of the dopaminergic system is critical to good working memory[21-24]. Studies show that the locus coeruleus releases substantial amounts of norepinephrine during awakening, decreases release significantly in the period of slow-wave sleep, and completely halts the release of the neurotransmitter in the period of paradoxical sleep, which causes people not to remember their dreams. This indicates that norepinephrine regulates the plasticity of nerve cells and promotes memory[25-28]. In this experiment, supplementation with tyrosine increased levels of catecholamine neurotransmitters in the brain, thereby improving the learning and memory abilities of the mice.

The lowered serum TT_3 and TT_4 levels in the stressed mice clearly show that the hypothalamus-hypophysis-thyroid axis plays an important role in the stress response. Under stress, reduced activities of thyroid peroxidase and oxidase, which are necessary for TT_3 synthesis, leads to decreased serum levels of TT_3 in the peripheral blood and quicker transformation of TT_4 into the non-bioactive TT_3. Central nervous system norepinephrine functions to inhibit the hypophysis-adrenal cortex axis and activate the hypophysis-thyroid axis, both of which affect thyroid function, which depends on the regulation of the hypothalamus-hypophysis-thyroid axis. Other central nervous system regions act on the arcuate nucleus in the center of the hypothalamus, causing it to produce and release thyrotropin-releasing hormone, which induces anterior pituitary cells to secrete thyroid-stimulating hormone, which regulates thyroid hormone levels[29-32]. Normal levels of thyroid hormone help regulate energy metabolism in the body, quicken the repair and the renewal of damaged cells, and strengthen resistance to chronic stress. Clinically, many patients with depression caused by social stress present with weakened immunity and easy fatigability due to decreased serum levels of TT_3 and TT_4. Supplementation with tyrosine can increase norepinephrine in the brain and induce thyrotropin-releasing hormone neurons to release more thyrotropin-releasing hormone, which acts on the hypophysis to release more thyroid-stimulating hormone. As a result, the synthesis and release of thyroid hormones increase[33-35].

In summary, chronic stress causes behavioral changes and disorders in the neuroendocrine network, and L-tyrosine can relieve or inhibit these changes. However, the stress response itself is dynamic, and examining only one specific time point cannot fully clarify the complexities of this process.

MATERIALS AND METHODS

Design

A randomized, controlled, animal experiment.

Time and setting

The experiments were performed at a laboratory in the Department of Pathophysiology of Peking Union Medical College in China from March 2008 to September 2010.

Materials

Animals

Sixty-three genetically similar male BALB/c mice, weighing 23–24 g and 8–9 weeks of age, were provided by the Experimental Animal Center, Peking Union Medical College in China (certification No. SYXK (Jing) 2008-0098). The animals were housed at a controlled temperature (22 ± 2°C) and humidity (45–65%) in artificially lighted rooms with a 12-hour light/dark cycle (lights on at 7: 00 a.m.), with free access to food and water. During the first week after arrival, animals were allowed to habituate to their new environment without handling. The experiments were performed in a different room with the same temperature.

Drugs

L-tyrosine (Sigma, St. Louis, MO, USA) was dissolved in sterile 0.9% saline[36].

Methods

Stress induction

Mice in the CUS and CUS-L groups, which were housed alone, were subjected to nine types of stressors, including cage tilting (45°, 12 hours), an empty cage with water on the bottom (12 hours), inversion of the light/dark cycle (12 hours), vibration (45 minutes), immersion in water (4°C, 5 minutes), restraint (2 hours), food deprivation (12 hours), water deprivation with empty water bottles (12 hours) and unpredictable 1-second foot shocks (0.3 mA). Two of these stressors were applied daily in a random manner to ensure that mice could not predict which type of stress would be presented[37-39]. The mice in the control group were housed together (21 mice in one cage) and did not receive any stimulation. The weights of the mice were monitored before experiments every week

L-tyrosine administration

Mice from the CUS-L group (L-tyrosine suspension, 5 mL, 2.032 g/kg), control group and CUS group (distilled water, the same volume as for the CUS-L group) received intragastric administration of the appropriate solution daily before stress induction (lasting for 28 successive days)[36].

Behavioral observations

The stress procedure lasted for 4 weeks, and behavioral testing was performed at the end of every week.

Spontaneous locomotor activity

Each mouse was individually placed in an automated locomotor activity chamber (50 × 50 × 40 cm^3) equipped with horizontal and vertical infrared beams (Institute of Materia Medica, Peking Union Medical College, Beijing, China). The chambers were placed in a dimly lit room illuminated by four overhead 15 V projection lamps mounted 200 cm above the chambers. The animal was placed in the chamber and allowed to move spontaneously for 120 seconds (18:00 and 21:00). The

total horizontal activity distance (m) traveled by mice in the test chamber was recorded with a video camera placed above the chamber and analyzed with DigBehv software (Version 2.0; Shanghai Jiliang Software Technology Co., Ltd., Shanghai, China). Data were collected before the stress was applied and on the last day of every week[40-42].

The Morris water maze test

The Morris water maze test was conducted in a large circular pool with a diameter of 80 cm and four side walls approximately 70 cm high, containing no internal cues, stimuli, markings or objects, but surrounded by stable, salient extra-maze cues (Institute of Materia Medica, Peking Union Medical College, China). The pool was filled with water at a temperature of 22 ± 1°C to a depth of about 35 cm. The pool was conceptually divided into four quadrants with equal areas (NE, SE, SW and NW). A circular escape platform (8 cm in diameter and 34 cm in height) was placed into the tank at a fixed position in the center of the NW quadrant, and was 20 cm away from the wall. Black nontoxic carbon ink (Chinese ink) was added to the pool to make the water opaque. The top surface of the platform was 1 cm below the water surface, with a rough surface to make it easy for the mice to climb on. The test was given before the stress had been applied and was conducted on the last day of every week between 21:00 and 23:00. During the test trial, the mouse was released into the water at one of the three different starting positions (in three different quadrants that did not contain the platform) with its head facing the wall. And then the mouse was allowed to swim for 120 seconds to search for the hidden platform. If it failed to locate the platform within 120 seconds, escape would be assisted and escape latency was recorded as 120 seconds. At the end of each trial, each mouse would stay on the platform for 3 seconds. The sequence of the starting positions remained the same for all the mice within one session, but changed each session. During each trial session, escape latency (the time taken to find the platform) was recorded by a computerized video imaging analysis system[43-47] (supplementary Figure 1 online).

Sampling

Mice were sacrificed by cervical dislocation at the end of the last behavioral tests, and serum and brain tissues (pallium, hippocampus and hypothalamus[48-49]) were immediately collected and stored at −80°C for experiments.

Determination of serum levels of thyroid hormone

Thyroid hormone levels were analyzed using radioimmunoassay kits with ^{125}I as a tracer (China Institute of Atomic Energy, Beijing, China). All assays were performed in duplicate according to the manufacturer's instructions. Analyses were conducted at room temperature on 50-μL samples for TT4 and TT3. The reaction system was composed of standard (50 μL, 0, 0.5, 1, 2, 4, 8 ng/mL), samples (50 μL) and ^{125}I tracer (200 μL), each of which was at an appropriate volume. After incubation, the reaction system was separated by adding a separation reagent (500 μL), except for the tubes set to measure total counts, and centrifuged at 4 000 r/min for 25 minutes. The supernatant was decanted, and the radioactivity of the pellet was counted with a gamma counter (GAMMA-C12, DPC, USA)[50-51].

Measurement of catecholamines by high performance liquid chromatography

Assays for norepinephrine and dopamine were performed with high performance liquid chromatography. The pallium, hippocampus and hypothalamus were sonicated in 1.5 mL 0.1 M $HClO_4$ and 40 μL 3,4-dihydroxy-benzylamine as the internal standard and centrifuged at 14 000 r/m for 15 minutes. Supernatants (20 μL) were injected onto a 4.5 mm × 250 mm, 10 μm chromatography column (Shimadzu, Japan) in a mobile phase containing KH_2PO_4 (100 mM), sodium 1-octanesulfonate (1.0 mM), ethylenediamine tetraacetic acid-Na_2 (0.5 mM), methanol (11% v/v) and pure water. Sample amounts were calculated by comparing the relative peak areas of sample peaks to internal standards. Norepinephrine and dopamine levels were measured in a single chromatogram. Concentrations were expressed as nanogram of norepinephrine or dopamine per milligram of sample tissue wet weight[52-55].

Statistical analysis

Data were analyzed using SPSS version 17.0 (SPSS, Chicago, IL, USA) and were expressed as mean ± SD. One-way analysis of variance was performed for intergroup comparisons for the same rearing condition at different time points. The paired sample *t*-test was used for intergroup comparisons for different rearing conditions. A value of $P < 0.05$ was considered statistically significant.

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Author contributions: Zhihua Wang and Jinghua Li participated in the study design and preparation of chronic stress animal models. Zhiming Wang and Lingyan Xue were in charge of behavioral tests. Yingjie Chen was responsible for testing endocrine and neurochemical changes. Yi Zhang and

Jun Su participated in data analysis and statistical analysis. Zhongming Li was responsible for study design, manuscript writing and statistical analysis.

Conflicts of interest: None declared.

Ethical approval: This study was approved by the Animal Ethics Committee of Bethune International Peace Hospital of Chinese PLA, China.

Supplementary information: Supplementary data associated with this article can be found, in the online version, by visiting www.nrronline.org.

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