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Research report

Dietary L-tyrosine alleviates the behavioral alterations induced by social isolation stress in mice

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ABSTRACT

Chronic stress induces abnormal mental state and behavior, and can be a risk factor for mental disorders. Although it is reported that L-tyrosine, an amino acid that is a precursor of catecholamine synthesis, alleviated the change of cognition and behavior induced by acute stress, knowledge about its effects on chronic stress is limited. In the present study, the effects of dietary L-tyrosine on behavioral alteration induced by chronic stress were investigated by employing a social isolation stress model in mice. Social isolation stress increased locomotor activity in both the home cage and open field. These increases of locomotor activity were suppressed by dietary L-tyrosine. Moreover, L-tyrosine increased both the concentration and turnover rate of norepinephrine metabolites. These findings partly suggest the availability of dietary L-tyrosine for psychic dysfunctions induced by chronic stress.

1. Introduction

Stress is an unavoidable part of human existence. Extreme forms of stress and chronic stress can cause an abnormal mental state and behavior, and are risk factors of some psychiatric disorders including depression and schizophrenia [9,20]. It has been studied using experimental animals such as rats or mice employing various stress models.

Social isolation rearing stress, caused by abolished social interaction among animals, including individual housing, has been reported to induce pathophysiological changes in rats and mice. For example, social isolation stress increased basal locomotor activity [32], induced hyperactivity in a novel environment [12,17,35], increased anxiety in an elevated plus-maze [23], enhanced fear response [27], increased depressive-like behavior [4], and impaired learning and memory function [5]. Social isolation stress also alters the reactivity to neurological drugs and stimulants [26,27]. Thus, social isolation stress is considered a valuable stress paradigm for investigating the effects of chronic psychosocial stress on various pathophysiological alterations in animals [5,26].

Typically, most of the disorders caused by chronic stress are treated with therapeutic drugs, but many of these drugs induce adverse reactions. Thus, many patients become noncompliant, and the situation worsens. Followed by noncompliance, a nutritional approach is expected, because food and/or dietary supplements are closer to life and can be treated without fear [16].

L-Tyrosine, a catecholamine precursor, elevates catecholamine synthesis and neuronal release [1,8]. L-Tyrosine treatment increases catecholamine synthesis especially when catecholamine neuroactivity is activated by stimulant drugs or stress. Moreover, it was reported that dietary L-tyrosine alleviated the affects of some forms of stress. In fact, some reports indicated that dietary L-tyrosine ameliorates the change of cognition and behavior caused by acute stress with preventing a reduction of norepinephrine (NE) in response to stress [2,19,29]. Although, it is known that chronic stress induces an abnormal mental state and behavior and can be a risk factor of mental disorders, the efficacy of L-tyrosine on behavioral changes caused by chronic stress is unknown. Thus, in the present study, the effects of dietary L-tyrosine on behavioral changes induced by chronic stress were investigated by employing the social isolation stress paradigm.

2. Materials and methods

2.1. Animals

Male ICR strain mice at postnatal day (PND) 21 were purchased from SLC Japan (Hamamatsu). After a minimum acclimation period of 3 days, mice were divided into two rearing groups: a social rearing group housed three per cage (22 cm × 15 cm × 12 cm), and an isolated rearing group housed individually. Isolation reared mice had visual, auditory and olfactory contact with other isolation reared and group housed mice. However, they were unable to physically contact or have social interaction with one another. Each rearing group was separated into

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two feeding groups: a control group receiving a standard powder diet (MF; Oriental Yeast, Tokyo, Japan), and a group receiving a high L-tyrosine diet comprised of standard powder diet supplemented with 4% L-tyrosine (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Chemical composition (%) of the standard diet was moisture 8.1, crude protein 23.3, crude fat 4.9, crude ash 5.6, crude fiber 2.7 and NFE 55.4. L-Tyrosine (40 g) was externally added to the standard diet (1 kg). Thus, animals were conclusively assigned to four groups ($n = 9$ or 10 each). Animals were reared in each condition from PND 24 to 57. Food and water were continuously available. Body weight of mice and food intake/mouse were measured every three days. Food intake was determined by measuring remaining food. A 12-h light/dark cycle was maintained throughout the experiment, with lights on at 0800 and off at 2000. Room temperature was maintained at 24 ± 1 °C. Behavioral tests were carried out in the following order: the home cage test at PNDs 24–40 (every three days), the open field test at PNDs 45–46, the plus-maze test at PND 49, and the forced swimming test at PND 52. At PND 57, animals were euthanized by cervical dislocation and decapitated to collect blood and brain samples. The experimental procedures followed the Guidelines for Animal Experiments of the Faculty of Agriculture and the Graduate School of Kyushu University, as well as Japanese Law (No. 105) and a Notification (No. 6) by the Japanese Government.

2.2. Home cage test

Basal locomotor activity was measured in the dark period (12 h), when mice were active in their home cages. Activity was counted with an infrared beam sensor (NS-AS01; Neuroscience Inc., Tokyo, Japan) placed about 15 cm above the center of the cage, and analyzed using DAS-008 software (Neuroscience Inc.). The percentage of change of locomotor activity compare with PDN 24 (day 0) were calculated. We test the locomotor activity of isolated mice because this monitoring system was not able to count locomotor activity of multiple animals in the same cage concurrently.

2.3. Open field test

The locomotor activity in a novel environment was recorded employing the open field test. Briefly, animals were individually transferred to an open field arena from the home cages. The arena was circular (diameter 60 cm and height 35 cm), and made of black takiflex. The test was begun by placing the animal in the center of the arena. The animal's behavior was then observed for 10 min under dim light (30 lx). After each test, the field was cleaned with an ethanol–water solution. The following behavioral categories were examined: distance of path, time the animal spent moving, speed of movement, and frequency of rearing behavior. All behaviors except rearing behavior were automatically analyzed with a computer-based video tracking system (AXIS-90, Neuroscience, Inc., Japan). Frequency of rearing behavior was manually recorded.

2.4. Elevated plus-maze test

The elevated plus-maze test served as the anxiolytic behavior model in mice. The apparatus, made from black board, consisted of two open arms (27.5 cm × 5.5 cm) and two closed arms (27.5 cm × 5.5 cm × 20 cm). A 0.3 cm high edge surrounded the open arms; closed arms were covered with a removable board. The arms extended from a central platform (5.5 cm × 5.5 cm) and the maze was elevated to a height of 60.5 cm above the floor. The procedure was identical to that described previously [12] with certain modifications. Mice were gently placed individually at the central platform facing the open arm and were monitored for 5 min under dim light (30 lx). The following parameters were recorded during the total test time: (1) the number of entries into the open arms, (2) the number of entries into the closed arms, (3) time spent in the open arms, and (4) time spent in the closed arms. From these data, the rate of number of entries and time spent in each arm were calculated. All of the behaviors were analyzed automatically with a computer-based video tracking system (AXIS-90, Neuroscience, Inc., Japan). The maze was cleaned with an ethanol–water solution after each trial.

2.5. Forced swimming test

Behavioral despair was measured by the forced swimming test. The experiment was carried out as previously described [24]. Mice were placed into a cylinder (22 cm high, 9.7 cm in diameter) containing water filled up to 16 cm and maintained at 25 ± 1 °C, for a total of 6 min. The total duration of immobility was recorded by video camera during the last 4 min after a 2 min habituation period. A mouse was judged to be immobile when it remained floating in the water, making only small movements to keep its head above water.

2.6. Plasma corticosterone analysis

After decapitation, trunk blood was collected in heparinized microtubes. Blood was centrifuged at 4 °C at 10,000 × g for 4 min, and plasma was collected and stored at −80 °C until analysis. Plasma corticosterone concentration was determined using a corticosterone enzyme immunoassay kit (Assay Designs Inc., MI, USA).

2.7. Analysis of monoamines in the brain

After decapitation, brains were immediately removed, dissected to the prefrontal cortex, hypothalamus, and striatum, weighed, and kept at −80 °C until analyzed. Levels of monoamines and their metabolites (contents/g wet tissue) were analyzed using a previously described method [34] with some modifications. Briefly, the tissue was homogenized and deproteinized in 0.2 M perchloric acid containing 100 μM EDTA 2Na. The homogenate was left for 30 min for deproteinization. Then, the homogenate was centrifuged at 20,000 × g for 15 min at 0 °C. After centrifugation, the pH of supernatant was adjusted to approximately 3.0 by adding 1 M sodium acetate. The supernatant was then centrifuged with a centrifuge-filtration unit (Ultra Free C3-GV Millipore, Bedford, MA, USA) at 10,000 × g for 5 min at 0 °C. A 30 μl portion of filtrate was applied to a high performance liquid chromatography (HPLC) system (Eicom, Kyoto, Japan) with a 150 mm × 2.1 mm octadecyl silane (ODS) column (SC-5ODS, Eicom) and an electrochemical detector (ECD-300, Eicom, Kyoto, Japan) at an applied potential of +0.75 V versus Ag/AgCl reference analytical electrode. Changes in electric current (nA) were recorded in a computer using an interface system (Power Chrom ver 2.3.2.j; AD Instruments, Tokyo, Japan). The mobile phase consisted of 0.1 M aceto-citric acid buffer (pH 3.5), methanol, 0.46 M sodium 1-octane sulfonate, and 0.015 mM disodium ethylenediaminetetraacetic acid (830:170:1.9:1) at a flow rate of 0.2 ml/min. The concentrations of monoamines and metabolites including dopamine (DA), NE, serotonine (5-HT), the DA metabolite dihydroxyphenylacetic acid (DOPAC), NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG), and 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined, and their levels in the brain were calculated. Turnover rates (DOPAC/DA, MHPG/NE, and 5-HIAA/5-HT) were also calculated. The limit of detection of the system for all monoamines was 0.1 pg/sample.

2.8. Analysis of L-tyrosine in the brain

Levels (contents/mg wet tissue) of L-tyrosine in the brain were determined using a previously described method [13] with some modifications. Briefly, brains were homogenized, deproteinized, and centrifuged by the same process as for the analysis of monoamines. After centrifugation, the pH of supernatant was adjusted to approximately 7.0 by adding 1 M sodium hydroxide. The sample (20 μl) was then completely dried under reduced pressure. Dried residue was resolved with 10 μl of a 1 mol/l sodium acetate–methanol–triethylamine (2:2:1) solution. The sample was re-dried, and resolved in 20 μl of deprivatization solution (methanol–water–triethylamine–phenylisothiocyanate [7:1:1:1]). The sample was maintained at room temperature for 20 min to allow phenylisothiocyanate to react with the amino groups to produce phenylthiocarbamyl amino acid residues. The sample was dried again, and was resolved with 100 μl of Pico-Tag Diluent (Waters, Milford, USA). This diluted sample was filtered through a 0.45 μm filter (Millipore). The same method was applied to standard solutions prepared by diluting L-tyrosine with distilled water. These derivatized samples (5 μl) were applied to a Waters HPLC system (Pico-Tag free amino acid analysis column [3.9 mm × 300 mm], Alliance 2690 separation module, 2487 dual-wavelength UV detector, and Millennium 32 chromatography manager; Waters). Samples were equilibrated with buffer A (70 mmol/l sodium acetate [pH 6.45 with 10% acetic acid]–acetonitrile [975:25]) and eluted with a linear gradient of buffer B (water–acetonitrile–methanol [40:45:15]) (0, 3, 6, 9, 40, and 100%) at a flow rate of 1 ml/min at 46 °C. The absorbance at 254 nm was measured, the concentration of L-tyrosine was determined, and its level in the brain was calculated.

2.9. Statistical analysis

Data were analyzed using a factorial two-way ANOVA with respect to rearing and feeding conditions, except for home cage activity. Fishers' PLSD was performed as a means separation test when a significant interaction was detected. Home cage activity was examined with a repeated measure ANOVA followed by Student's t-test.

3. Results

Dietary and rearing conditions did not affect the body weight or food intake of mice (Figs. 1 and 2). Likewise, no significant differences were observed in plasma corticosterone concentrations (Fig. 3).

3.1. Home cage test

As shown in Fig. 4, no significant ($F(1,13) = 3.93$, n.s.) differences in locomotor activity were observed between dietary groups in isolated mice. The activity was significantly ($F(5,65) = 20.00$, $P < 0.0001$) increased as time went on. A significant ($F(5,65) = 4.61$, $P < 0.005$) interaction between diet and time was detected, imply-