Single L-Tyrosine administration Alters Brain Monoamine Levels in Roborovskii Hamsters

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Abstract: The Roborovskii hamster (P. robvorovskii) has high locomotor activity (hyperactivity) and low dopamine levels in the brain compared with the congeneric Djungarian hamster (P. sungorus). In the present study, we investigated the effects of acute administration of L-tyrosine, the primary precursor of dopamine, on the locomotor activity of and brain monoamine levels in Roborovskii hamsters to clarify the efficacy of L-tyrosine in ameliorating signs of hyperactivity such as those observed in hyperactive dogs. Acute administration of L-tyrosine had no effect on locomotor activity in the open field, but increases in dopamine and norepinephrine turnover rates and decrease in serotonin turnover rate in the brain were observed. These findings suggest that acute feeding of L-tyrosine may be effective in modifying brain monoamine metabolism, but not ameliorating signs of hyperactivity in the Roborovskii hamster.


Key word: Hyperactivity, locomotor activity, L-tyrosine, monoamine, Roborovskii hamster

INTRODUCTION

Hyperactivity, which is characterized by high locomotor activity, is one of the problematic behaviors seen in hyperactive dogs[14] and children with attention-deficit/hyperactivity disorder (ADHD)[15]. ADHD is one of the most common chronic neurobehavioral diseases encountered in child development. It is characterized by hyperactivity together with lack of attention and impulsivity[4, 15], with approximately 8 to 12% of children exhibiting these symptoms worldwide[4]. Furthermore, these symptoms secondarily induce learning dysfunction[15]. Similarly, hyperactive dogs have high activity levels together with features such as lack of trainability and failure to habituate to external stimulation, and the mechanism of hyperactivity in them is thought to be similar to that of ADHD. Moreover, these changes aggravate relationships between dogs and their guardians. Hyperactivity can thus decrease the quality of life (QOL)

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of both humans and companion animals.

Although the neurobiological basis of hyperactivity remains poorly understood, it appears that the cause of hyperactivity involves not only environmental factors such as lack of discipline but also biological disorders such as dysregulation of monoamine neurotransmission[2, 4]. Dopaminergic (DA) neurotransmission, in particular, is believed to play important roles in the pathology of hyperactivity[7, 13, 18]. In fact, children with ADHD are clinically treated with DA-based psychostimulants. To determine the pathology of hyperactivity, we have examined the Roborovskii hamster (Phodopus roborovskii) as a potential animal model of hyperactivity, which exhibits markedly increased locomotor activity compared with the congenic Djungarian hamster[11]. Roborovskii hamsters also have low DA levels in the brain relative to Djungarian hamster, and it was hypothesized that such levels play a role in the pathogenesis of hyperactivity. To test this hypothesis, we administered 3,4-dihydroxyphenylalanine (LDOPA), a precursor of DA synthesis, to Roborovskii hamsters and observed dose-dependent increases in DA levels in the brain and decreases in locomotor activity[10].

L-Tyrosine, an aromatic amino acid, is also a precursor in DA synthesis. There have been inconsistent findings that L-tyrosine increases the release of DA[1, 6] or that it has no effect on DA release in normal animals [5]. Moreover, DA is known to relate to other monoamine neurotransmission systems such as norepinephrine (NE), since DA is the precursor of NE. On the other hand, DA is also associated with 5-HT metabolism. For instance, the early disruption of central dopaminergic pathways is followed by increased striatal 5-HT and elevated 5-HT was implicated in hyperactivity[3]. In our previous study[11], Djungarian hamsters showed lower activity and a higher level of 5-HT compared to Roborovskii hamsters. Furthermore, Roborovskii hamsters showed lower NE levels and higher E levels compared to Djungarian hamsters. However, few studies have examined whether L-tyrosine affects DA-related disorders associated with hyperactivity. In this study, therefore, it was examined whether single administration of L-tyrosine affects the hyperactive behavior and brain monoamine levels of Roborovskii hamsters.

**MATERIALS AND METHODS**

Male Roborovskii hamsters, 3 weeks of age and reared in a controlled environment, were purchased from a local pet shop (Nomura, Fukuoka, Japan). The hamsters were housed individually in plastic cages (22 cm×15 cm×12 cm) and allowed ad libitum access to a chow standard diet (MF; Oriental Yeast, Tokyo, Japan) and water. A 12-h light/dark cycle was maintained throughout the experiments, with lights on at 0800 and off at 2000. Room temperature was maintained at 23±1°C. After one-week acclimation, the animals were subjected to the experimental procedures described below. 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.

**Effects of acute L-tyrosine administration**

L-Tyrosine was purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. On the day of experiment, the animals were measured for body weight and then randomly divided into three groups (n=6), which received intraperitoneal administration of L-tyrosine at 0, 100, or 200 mg/kg body weight dissolved in 0.9% saline according to the previous reports[1, 10]. At 50 min after L-tyrosine administration, animals were subjected to the open field test (0900-1300).

**Open field test**

Locomotor activity in a novel environment was determined using 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 at the center of the arena. The behavior of animals was then observed for 5 min under dim light (100 lux). 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 defecation. All behaviors except defecation
were automatically analyzed with a computer-based video tracking system (AXIS-90, Neuroscience, Inc., Japan). Frequency of defecation was manually recorded. All animals were killed by cervical dislocation and decapitated immediately following completion of the open field test. Whole brains were immediately removed, weighed, and kept at −80°C until analyzed.

Analysis of monoamines in the brain

Levels of monoamines and their metabolites (contents/g wet tissue) were analyzed using a previously described method[17] 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 10,000× 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 150×2.1 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 2.3.2j; 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 DA, NE, 5-HT, the DA metabolite 3,4-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 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.

Analysis of L-tyrosine in the brain

Levels (contents/g wet tissue) of L-tyrosine were determined using a previously described method[9] 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 (μl) were applied to a Waters HPLC system (Pico-Tag free amino acid analysis column [3.9×300 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 [97:25]) 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, and concentration of L-tyrosine was determined and its level in the brain calculated.

Statistical Analysis

The statistical significance of differences in locomotor activity and levels of monoamines and L-tyrosine in the brain among groups were examined by one-way ANOVA followed by Fisher’s PLSD test.
Table 1   Effects of acute administration of L-tyrosine on levels of monoamines and their metabolites in whole brain of Roborovskii hamsters

<table>
<thead>
<tr>
<th></th>
<th>DA</th>
<th>DOPAC</th>
<th>NE</th>
<th>MHPG</th>
<th>5-HT</th>
<th>5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2558±53</td>
<td>177±5</td>
<td>440±23</td>
<td>37±2</td>
<td>464±13</td>
<td>262±10</td>
</tr>
<tr>
<td>L-Tyrosine 100mg/kg</td>
<td>2562±148</td>
<td>212±13</td>
<td>453±17</td>
<td>46±3</td>
<td>476±30</td>
<td>253±6</td>
</tr>
<tr>
<td>L-Tyrosine 200mg/kg</td>
<td>2447±65</td>
<td>204±7</td>
<td>443±21</td>
<td>58±4</td>
<td>463±21</td>
<td>273±7</td>
</tr>
</tbody>
</table>

DA: dopamine, DOPAC: 3,4-dihydroxyphenylacetic acid, NE: norepinephrine, MHPG: 3-methoxy-4-hydroxyphenylglycol, 5-HT: serotonin, 5-HIAA: 5-hydroxyindoleacetic acid.
Values are means pmol/g wet tissue±S.E.M.
Groups with different letters are significantly different (P<0.05)

Table 2   Effects of acute administration of L-tyrosine on monoamine turnover rates in whole brain of Roborovskii hamsters

<table>
<thead>
<tr>
<th></th>
<th>DOPAC/DA</th>
<th>MHPG/NE</th>
<th>5-HIAA/5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.072±0.003a</td>
<td>0.086±0.008a</td>
<td>0.564±0.024</td>
</tr>
<tr>
<td>L-Tyrosine 100mg/kg</td>
<td>0.083±0.005b</td>
<td>0.100±0.009b</td>
<td>0.540±0.038</td>
</tr>
<tr>
<td>L-Tyrosine 200mg/kg</td>
<td>0.083±0.002a</td>
<td>0.128±0.007a</td>
<td>0.595±0.019</td>
</tr>
</tbody>
</table>

DA: dopamine, DOPAC: 3,4-dihydroxyphenylacetic acid, NE: norepinephrine, MHPG: 3-methoxy-4-hydroxyphenylglycol, 5-HT: serotonin, 5-HIAA: 5-hydroxyindoleacetic acid.
Values are means±S.E.M.
Groups with different letters are significantly different (P<0.05)

RESULTS

Open field test

There were no significant differences in locomotor activity (distance of path in cm) in the open field among administrations of various doses of L-tyrosine (Control, 4110±274; L-tyrosine 100 mg, 4243±533; L-tyrosine 200 mg, 4159±168). Similarly, no significant differences in any other items of measurement (time spent moving, speed of movement of animals, and frequency of defecation) were observed among groups (data not shown).

Analysis of monoamines in the brain

Tables 1 and 2 show the levels of monoamines, their metabolites, and their turnover rates in whole brain. Levels of DA, NE and 5-HT in whole brain were unaffected by L-tyrosine. There were significant differences in the levels of DOPAC and MHPG and the turnover rates of DA (DOPAC/DA) and NE (MHPG/NE) among groups. Both 100 and 200 mg/kg doses of L-tyrosine increased DOPAC level and DA turnover rate. Similarly, 200 mg/kg doses of L-tyrosine increased MHPG level and NE turnover rate. The levels of 5-HT and 5-HIAA and the turnover rate of 5-HT were unaffected by L-tyrosine.

Analysis of L-tyrosine in the brain

Fig. 1 shows L-tyrosine levels in the brain. L-Tyrosine
significantly and dose-dependently increased L-tyrosine level in the brain.

**DISCUSSION**

In the present study, it was examined whether L-tyrosine could alter the locomotor activity of and brain monoamine levels in Roborovskii hamsters as a potential animal model of hyperactivity.

It was found that acute administration of L-tyrosine did not affect locomotor activity in the open field, although it was reported that L-tyrosine increased the behavior in the open field of normal animals[8]. Gibson et al.[8] reported that L-tyrosine raised its own concentration, but did not alter either that of the other amino acids or of DA, NE and 5-HT. This pattern was similar to the present study. Therefore, Roborovskii hamster may have some differences in neurotransmission in the brain involved in regulation of locomotor activity. It may be dependent upon differences in the receptor levels and/or signal transduction. Further studies remain to be done in future.

The increase in turnover rate of DA by L-tyrosine suggests that L-tyrosine may have enhanced the release of DA and activity of DA neurotransmission in the brain. This conclusion is supported by some studies[1, 6]. It was observed that L-DOPA, a precursor of DA and increased DA release and neurotransmission[12], decreased the locomotor activity of Roborovskii hamster accompanied by increase DA turnover rate[10]. In this study, L-tyrosine increased DA turnover rate similar to L-DOPA, but had no effect on the locomotor activity of Roborovskii hamster. There were large differences between the previous report using L-DOPA[12] and the present study. L-DOPA even at 50 mg/kg body weight greatly enhanced DA, DOPAC, NE, MHPG in the brain, but the reverse was true for 5-HT and 5-HIAA[12]. The continuous or magnitude of increased DA release may be related to the decrease in locomotor activity through lowering 5-HT levels, since elevated 5-HT concentration was suggested to cause hyperactivity. On the other hand, L-tyrosine at both 100 and 200 mg/kg body weight weakly increased DOPAC and MHPG, but no significant differences were detected in others. Particularly, 5-HT was not changed in both concentration and turnover rate. These facts may be the major reason for the weak effect of L-tyrosine compared with L-DOPA.

In conclusion, acute supplementation of L-tyrosine did not ameliorate the locomotor activity of Roborovskii hamsters, but L-tyrosine altered levels of monoamine metabolites and turnover rates in the brain.

**ACKNOWLEDGMENTS**

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L-チロシンの単回投与はロボロフスキーハムスターの脳内モノアミン含量を変化させる

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要 約：ヒメキヌゲネズミ属のハムスターであるロボロフスキーハムスターは、同属のジャンガリアンハムスターに比べて多動行動を示し、脳内ドーパミン含量が低いことが分かっている。本研究ではペットの多動行動の改善を目的として、ドーパミンの前駆アミノ酸であるL-チロシンの単回投与がロボロフスキーハムスターの自発運動量および脳内モノアミン含量に及ぼす影響を調べた。オープンフィールド試験における自発運動量に変化は見られなかったが、脳内のノルエピネフリンの代謝物であるMHPG含量とドーパミンおよびノルエピネフリンの代謝回転率の亢進が認められた。以上の結果から、L-チロシンの単回投与で脳内モノアミン代謝は変化するが、多動性改善に至らないことが示唆された。

Key words：Hyperactivity, locomotor activity, L-tyrosine, monoamine, Roborovskii hamster