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Impairment of neuropsychological behaviors in ganglioside GM3-knockout mice

Kimie Niimi [a], Chieko Nishioka [a], Tomomi Miyamoto [b], Eiki Takahashi [a], Ichiro Miyoshi [b], Chitoshi Itakura [a], Tadashi Yamashita [c,d,*]

[a] Riken Brain Science Institute, Saitama 351-0198, Japan
[b] Graduate School of Medical Sciences, Nagoya City University, Nagoya 467-8601, Japan
[c] Graduate School of Advanced Life Science, Hokkaido University, Sapporo 001-0021, Japan
[d] World Class University Program, Kyungpook National University School of Medicine, Daegu, South Korea

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ABSTRACT

The ganglioside GM3 synthase (SAT-I), encoded by a single-copy gene, is a primary glycosyltransferase for the synthesis of complex gangliosides. Although its expression is tightly controlled during early embryo development and postnatal development and maturation in the brain, the physiological role of ganglioside GM3 in the regulation of neuronal functions has not been elucidated. In the present study, we examined motor activity, cognitive and emotional behaviors, and drug administration in juvenile GM3-knockout (GM3-KO) mice. GM3-KO male and female mice showed hyperactivity in the motor activity test, Y-maze test, and elevated plus maze test. In the Y-maze test, there was significantly less spontaneous alternation behavior in GM3-KO male mice than in wild-type mice. In the elevated plus maze test, the amount of time spent on the open arms by GM3-KO male mice was significantly higher than that of sex-matched wild-type mice. In contrast, there was no significant difference between GM3-KO and wild-type female mice in these tests. Thus, juvenile GM3-KO mice show gender-specific phenotypes resembling attention-deficit hyperactivity disorder (ADHD), namely hyperactivity, reduced attention, and increased impulsive behaviors. However, administration of methylphenidate hydrochloride (MPH) did not ameliorate hyperactivity in either male or female GM3-KO mice. Although these data demonstrate the involvement of ganglioside GM3 in ADHD and the ineffectiveness of MPH, the first-choice psychostimulant for ADHD medication, our studies indicate that juvenile GM3-KO mice are a useful tool for neuropsychological studies.

1. Introduction

Gangliosides (i.e., glycosphingolipids [GSLs]) containing one and more sialic acid residues are present in all mammalian cell plasma membranes and intracellular membrane structures. They are especially abundant in central nervous system tissues and are considered to have important roles in the early development, cell differentiation and proliferation, and stability of neuronal cells [1]. They are especially concentrated in plasma membrane lipid domains that are specialized for cell signaling. Plasma membranes have typical structures called rafts and caveola domain structures, with large amounts of sphingolipids, cholesterol, and sphingomyelin in the cell membranes. GM3, as the first ganglioside of the synthetic pathway, is a common precursor of following derivatives [2]. The sialyltransferase responsible for the GM3 synthesis is GM3 synthase (EC 2.4.99.9). Knockout mice in which GM3 synthase is disrupted the have been established [2–4]. Mice with gene disruption of GM3 (GM3-KO) are viable without major abnormalities but have heightened sensitivity to insulin. The basis for the increased insulin sensitivity in mutant mice was found to be enhanced insulin receptor phosphorylation in skeletal muscle [2]. Furthermore, GM3-KO mice exhibit complete hearing loss due to selective degeneration of the organ of Corti [3].

Various neurotransmitters and hormones have been shown to correlate with hyperactivity. There are many different theories that try to explain how males and females developed these different hyperactive tendencies. Generally, to ameliorate hyperactive behavior such as attention-deficit hyperactivity disorder (ADHD), methylphenidate hydrochloride (MPH) is used in human patients. MPH quickly and effectively reduces the signs and symptoms of ADHD in children under the age of 18 [5]. On the other hand, MPH is the most commonly prescribed psychostimulant and works by increasing the activity of the central nervous system [6]. It

Abbreviations: MPH, methylphenidate hydrochloride; GSLs, glycosphingolipids; ADHD, attention-deficit hyperactivity disorder; NCAM, neural cell adhesion molecule.

* Corresponding author at: Hokkaido University, Graduate School of Advanced Life Science, Sapporo 001-0021, Japan. Fax: +81 11 706 9002.
E-mail address: ty11106@sci.hokudai.ac.jp (T. Yamashita).

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produces such effects as increasing or maintaining alertness, combating fatigue, and improving attention. As a pharmacological effect, MPH belongs to the piperidine class of compounds and increases the levels of dopamine in the brain through reuptake inhibition of the dopamine transporter (DAT).

In the present study, we examined general behaviors such as motor activity and cognitive and emotional behaviors in juvenile GM3-KO mice. GM3-KO mice showed hyperactivity. Hyperactivity of GM3-KO is more remarkable in females than in males. After MPH injection, GM3-KO mice showed a more active phenotype than control mice. This suggests that GM3-KO mice are more sensitive to MPH. GM3-KO mice showed male-specific cognitive and emotional alternations. Our results indicate that these phenotypes are influenced partly by genetic factors and gender.

2. Materials and methods

2.1. Animals

All procedures involving animals were approved by the Animal Experiment Committees of RIKEN, Hokkaido University, and Nagoya City University. Wild type (+/+) and homozygote (−/−) mice with the C57BL/6JJmsSlc (Japan SLC, Inc., Shizuoka, Japan) genetic background (backcross generations; $N = 15$) were derived from a cross between heterozygote (+/-) mice and genotyped by PCR using tail DNA [2]. The mice were weaned at postnatal weeks 3–4 and were given free access to water and food pellets (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan). Juvenile (aged 6 weeks) male and female mice were studied. The mice were individually housed until use in a microisolation cage (MBS7115RHMV; Allentown, NJ, USA) with bedding (TEK-FRESH; Harlan Laboratories, Madison, WI, USA), under a 12:12 h light: dark cycle (dark period, 20:00–08:00) at 23 ± 1 °C and 55 ± 5% humidity. All behavioral analyses were conducted by a well-trained researcher who was blinded to the genotypes. Separate groups of mice were used for the behavioral tests.

2.2. Motor activity test

Motor activity was monitored in every 30 min period for 72 consecutive hours, starting at 17:00 with LOCOMO system (Melquest, Toyama, Japan). In the light phase (light period, 08:00–20:00), the light intensity in the center of the home cage was 70 lux. Mice were given free access to water and food without interruption of the photo beams. Data were obtained from male (+/+, −/−; $n = 10$ each) and female (+/+, −/−; $n = 10$ each) mice.

2.3. Measurement of spontaneous alternation behavior in the Y-maze test

Working memory, as an index of cognitive functions, was evaluated using the Y-maze test [7]. The Y-maze test was performed using the reported procedure [8] and was conducted between 09:00 and 16:00. Each mouse performed one trial. The percentage of spontaneous alteration was calculated as (actual alteration/maximum alteration) × 100. Data were obtained from male mice (+/+, −/−; $n = 8$ each) and female (+/+, −/−; $n = 8$ each) mice.

2.4. Measurement of emotional-related behavior in the elevated plus maze test

Emotional-related behavior, as an index of impulsivity, was evaluated using the elevated plus maze test [9]. The elevated plus maze test was performed using the reported procedure [10] and was conducted between 09:00 and 16:00. The number of transitions between the arms, the number of entries into open arms, and the time spent in open arms were measured. Data were obtained from male mice (+/+, −/−; $n = 8$ each) and female (+/+, −/−; $n = 8$ each). The number of entries into the open arms is expressed as a percentage of the total number of arm entries. The time spent on open arms is expressed as a percentage of the total time of arm entries.

2.5. Drug

Methylphenidate hydrochloride (MPH, Sigma, Saint Louis, MO, USA) was dissolved in saline solution just prior to administration. The drug and vehicle were intraperitoneally administered. Mice were given MPH (0, 0.02, 0.2, or 2.0 mg/kg) 15 min after the motor activity test of day 3 and immediately placed in the test cages again. Motor activity was evaluated in every 5 min period with LOCOMO system. The doses (0–2.0 mg/kg) used were within the range reported to produce alterations of motor activity in rodent models [11]. The effect of the drug on motor activity was examined for two consecutive hours after injection in the light phase. Data were obtained from male (+/+: vehicle, 0.02, 0.2, 2.0 mg/kg; $n = 10$ each, −/−: vehicle, 0.02, 0.2, 2.0 mg/kg; $n = 10$ each) and female (+/+: vehicle, 0.02, 0.2, 2.0 mg/kg; $n = 10$ each, −/−: vehicle, 0.02, 0.2, 2.0 mg/kg; $n = 10$ each) mice.

2.6. Statistical analysis

The data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were conducted using Excel Statistics 2006 (SSRI, Tokyo, Japan). When more than two groups were compared, the data were analyzed using analysis of variance (ANOVA), and a further *post hoc* Tukey's test between groups was performed. The student's *t*-test was used to analyze differences between two groups. The differences between groups were considered significant at $P < 0.05$.

3. Results

3.1. Motor activity

The activities of each group were plotted for each of the three recording days in the male (Fig. 1) and female (data not shown) mice. All groups displayed increased in activity following lights off and exhibited surges of activity when the lights were turned on. In the male mice, a genotype effect ($F(1, 54) = 155.95$, $P < 0.01$), day effect ($F(2, 54) = 41.09$, $P < 0.01$), and genotype × day interaction ($F(2, 54) = 5.41$, $P < 0.01$) were seen in the 72-h activity. The total activity of male −/− mice was significantly higher than that of sex-matched +/+ mice on day 1, day 2, and day 3 (day 1; $P < 0.01$, day 2; $P < 0.01$, day 3; $P < 0.01$, Tukey's test). In the female mice, a genotype effect ($F(1, 54) = 82.79$, $P < 0.01$), day effect ($F(2, 54) = 1.00$, $P > 0.05$), and genotype × day interaction ($F(2, 54) = 1.49$, $P > 0.05$) were also seen in the 72-h activity. The total activity of female −/− mice was significantly higher than that of sex-matched +/+ mice on day 1 (day 1; +/+, −/−: 11623.8 ± 1275.61, 23298.5 ± 2384.80; $P < 0.01$, Tukey's test), day 2 (day 2; +/+, −/−: 9159.0 ± 1176.92, 22586.8 ± 2501.29; $P < 0.01$, Tukey's test), and day 3 (day 3; +/+, −/−: 8090.9 ± 1492.72, 25624.7 ± 2819.50; $P < 0.01$, Tukey's test). The activity in the mice during 12 h dark and light periods on day 1, day 2, and day 3 showed statistical difference between genotypes. In the dark phase, the activity of −/− mice was significantly higher than that of sex-matched +/+ mice on day 1, day 2, and day 3 (data not shown). In the light phase, the activity of −/− mice was also significantly higher than that of sex-matched +/+ mice on day 1, day 2,