Improvement of spontaneous alternation behavior deficit by activation of α4β2 nicotinic acetylcholine receptor signaling in the ganglioside GM3-deficient mice

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ABSTRACT

We have reported that in ganglioside GM3-deficient (GM3−/−) mice, spontaneous alternation behavior assessed by a Y-maze task was significantly lower, and total arm entries were significantly higher than in wild-type mice. The objective of the present study was to examine the role of nicotinic acetylcholine receptor (nAChR) signaling in impairment of spontaneous alternation behavior of GM3−/− mice. Nicotine treatment (0.3, 1.0 mg/kg, s.c.) dose dependently improved the spontaneous alternation deficit without affecting total arm entries in GM3−/− mice. The nicotine-induced (1.0 mg/kg, s.c.) improvement was significantly abolished by the nAChR antagonist mecamylamine (1.0 mg/kg, i.p.). The α4β2 nAChR antagonist dihydro-β-erythroidine (2.5, 10.0 mg/kg, i.p.) dose dependently counteracted the nicotine-induced improvement of spontaneous alternation in GM3−/− mice, whereas the α7 nAChR antagonist methyllycaconitine (2.5, 10.0 mg/kg, i.p.) did not. In addition, the α4β2 nAChR agonist RJR-2403 (5.0, 10.0 mg/kg, s.c.) dose dependently and significantly improved the spontaneous alternation deficit, whereas the α7 nAChR agonist PNU120596 (0.3, 1.0, 3.0 mg/kg, i.p.) did not. These findings revealed that nicotine improved spontaneous alternation behavior of GM3−/− mice via the activation of α4β2, but not α7, nAChR. Thus, the ganglioside GM3 might be responsible for α4β2 nAChR signaling in the spontaneous alternation behavior.

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 tissues and considered to have important roles in early development, cell differentiation and proliferation, and the stability of neuronal cells (8). The ganglioside GM3 synthase, encoded by a single-copy gene, is a primary glycosyltransferase for the synthesis of complex gangliosides. In previous studies (18, 24), mice with gene disruption of GM3 synthase (GM3-KO) are viable without major abnormalities. Both males and females were fertile. The major brain gangliosides present in GM3+/+ mice (GM1a, GD1a, GD1b, and GT1b) were absent in the GM3−/− mice (24). GM3−/− mice show significantly lower spontaneous alternation in the Y maze test (18). Animal studies have shown that nicotinic acetylcholine receptor (nAChR) mechanisms are involved in attentional function (3, 7). Because GM3 is concentrated in plasma membrane lipid domains that is specialized for cell signaling, there would be a possibility that spontaneous
alteration in GM3-KO mice would be derived from nAChR signaling dysfunction.

Neuronal nAChRs are composed of five subunits according to the combination of α (α2-α9) and β (β2-β4) subunits. Neuronal subunits have been categorized into two major subtypes of nAChR pentamers in the brain on the basis of their high affinity for either nicotine or α-bungarotoxin. Of the two, the former are considered to form α4β2 nAChR and the latter α7 nAChR, respectively (4, 15). The α4β2 and α7 nAChR mechanisms are critical for nicotinic involvement in cognitive functions such as attention (2, 3, 6, 7, 13).

The present study aimed to elucidate the effects of nAChR signaling on spontaneous alternation behavior in GM3−/− mice. Particular attention has focused on the characterization of nAChR subtypes involved in the effects of nicotine on spontaneous alternation behavior in GM3−/− mice, using α4β2 and α7 nAChR antagonists.

MATERIALS AND METHODS

Animals. All procedures involving animals were approved by the Animal Experiments Committee of RIKEN and Hokkaido University. All animals were cared for humanely in accordance with institutional guidelines for animal experimentation. The wild-type GM3+/+ and GM3−/− mice with C57BL/6N CrlSlc (Japan SLC, Inc., Shizuoka, Japan) genetic background (backcross generations; n = 16) were derived from a cross between +/− mice and genotyped by PCR using tail DNA (24). The mice were weaned at postnatal weeks 3 to 4 and were given free access to water and food pellets (CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan). 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 to 08:00) at 23 ± 1°C and 55 ± 5% humidity. All behavioral analyses were conducted by a well-trained experimenter who was blinded to the genotypes. Separate groups of juvenile (aged 6 weeks) male mice were used for the behavior tests.

Measurement of spontaneous alternation behavior in the Y maze test. Spontaneous alternation behavior as index of attention was evaluated using the Y maze test (19). The Y-maze test was performed using the reported procedure (17) and was conducted between 09:00 and 16:00. The mice were moved into the behavioral testing room at least 1 h before testing. The experiments were performed at 35 lux. Before behavioral testing, the mice were placed in one of the compartments and allowed to move freely on the one of the arms for 10 min. Each mouse performed one trial. An arm entry was defined as three legs entering one of the arms, and the sequence of entries was recorded manually using videotapes. An alteration was defined as entry into all three arms with consecutive choices. The percentage of spontaneous alteration was calculated as (actual alteration/maximum alteration) × 100.

Drugs. (−)-Nicotine hydrogen tartrate (nicotine) and mecamylamine hydrochloride (MEC) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Dihydro-β-erythroidine hydrobromide (DHβE), methyllycaconitine citrate (MLA), PNU120596, and RJR-2403 fumarate (RJR-2403) were purchased from Tocris Bioscience (Bristol, United Kingdom). Nicotine, MEC, DHβE, MLA or RJR-2403 were dissolved in saline solution. PNU120596 was dissolved in 100 mM in DMSO. Control mice were not treated with nAChR related drugs, and received an equivalent volume of vehicle. The doses used in this study were within the range reported to produce spontaneous alternations in various rodent models (9, 21). Nicotine, RJR-2403 or PNU120596 was administered subcutaneously (s.c.) 20 min prior to the spontaneous alternation performance. MEC, DHβE or MLA was injected intraperitoneally (i.p.) 10 min before nicotine administration.

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). The student’s t-test analyzed differences between two groups. When more than two groups were compared, the data were analyzed using analysis of variance (ANOVA), and further statistical post hoc Tukey’s test between groups was performed. The differences between groups were considered significant at P < 0.05.

RESULTS

Effects of nicotine on spontaneous alternation behavior in GM3−/− mice

The acute effects of nicotine on spontaneous alternation behavior in GM3−/− mice are shown in Fig. 1. GM3−/− mice showed a significant decrease in alternation behavior (t(18) = 3.62, P < 0.01) and a significant increase in total arm entries (t(18) = 3.34,
Acetylcholine receptor and GM3

Fig. 1 Effects of nicotine on spontaneous alternation behavior (A) and total arm entries (B) in GM3−/− mice. Nicotine (vehicle, 0.1, 0.3, 1.0 mg/kg) was administered 20 min before the test. Data are obtained from male mice (n = 10; each group) and expressed as the means ± SEM. **, P < 0.01 versus vehicle-treated GM3+/+ mice (t-test); #, P < 0.05, ##, P < 0.01 versus vehicle-treated GM3−/− mice (Tukey’s test).

Fig. 2 Effects of MEC on nicotine-induced improvement of spontaneous alternation (A) and total arm entries (B) in GM3−/− mice. MEC (vehicle, 0.2, 1.0 mg/kg) and nicotine (1.0 mg/kg) were administered 30 and 20 min before the test, respectively. Data are obtained from male mice (n = 10; each group) and expressed as the means ± SEM. **, P < 0.01 versus vehicle-treated GM3+/+ mice (t-test); $$, P < 0.01 versus vehicle-treated GM3−/− mice (t-test); ##, P < 0.01 versus vehicle- and nicotine-treated GM3−/− mice (Tukey’s test).

Effects of non-selective nAChR antagonist on nicotine-induced improvement of spontaneous alternation in GM3−/− mice

As shown in Fig. 2, the non-selective nAChR antagonist MEC significantly abolished the nicotine-induced (1.0 mg/kg) improvement of spontaneous alternation in GM3−/− mice [F(2,27) = 18.95, P < 0.01; 1.0 mg/kg, P < 0.01] (Fig. 2A). However, MEC did not affect total arm entries of GM3−/− mice (Fig. 1B).

Effects of subtype-selective nAChR antagonists on nicotine-induced improvement of spontaneous alternation in GM3−/− mice

The effects of subtype-selective nAChR antagonists on the nicotine-induced improvement of spontaneous alternation in GM3−/− mice are shown in Figs. 3 and 4. The selective α4β2 nAChR antagonist DHβE dose dependently counteracted the nicotine-induced improvement of spontaneous alternation in GM3−/− mice [F(2,27) = 15.45, P < 0.01]. Statistical significance was noted at a dose of 10.0 mg/kg (P < 0.01)
Effects of subtype-selective nAChR agonists on spontaneous alternation in GM3−/− mice

The effect of a selective α4β2 nAChR agonist RJR-2403 and a selective α7 nAChR agonist PNU120596 were examined. RJR-2403 dose dependently and significantly improved the lowered spontaneous alternation in GM3−/− mice [F(3,36) = 11.87, P < 0.01; 5.0 mg/kg, P < 0.05; 10.0 mg/kg, P < 0.01] (Fig. 5A). However, RJR-2403 did not affect total arm entries (Fig. 5B). On the other hand, the selective α7 nAChR agonist PNU120596 failed to improve the lowered spontaneous alternation in GM3−/− mice (Fig. 6A). Moreover, PNU120596 did not affect total arm entries (Fig. 6B).

DISCUSSION

The α4β2 and α7 nAChR mechanisms are critical for nicotinic involvement in cognitive function, such
as attention (2, 3, 6, 7, 13). Both α4β2 and α7 nAChR agonists have been shown to improve performance in cognitive tasks (14). However, it is not clear which nAChR subtypes are involved in spontaneous alternation deficit in GM3−/− mice. The present study demonstrated that acute administration of nicotine dose dependently and significantly improved the lowered spontaneous alternation performance in juvenile GM3−/− mice without affecting total arm entries. The selective α4β2 nAChR antagonist DHβE dose dependently and significantly counteracted the nicotine-induced improvement of spontaneous alternation performance in GM3−/− mice, whereas the selective α7 nAChR antagonist MLA failed to affect it. In addition, the selective α4β2 nAChR agonist RJR-2403 dose dependently and significantly improved the spontaneous alternation deficit in GM3−/− mice. However, the α7 nAChR agonist PNU120596 did not improve the spontaneous alternation deficit. These findings suggested that the nicotine-induced improvement of spontaneous alternation behavior in GM3−/− mice was mediated by the activation of α4β2, but not α7, nAChR.

It has been reported that nicotine improves attention via the activation of α4β2 nAChR in the five-choice serial reaction time task, which assesses both selective and sustained attention (3, 7). The selective α4β2 nAChR activation improved spontaneous alternation behavior in juvenile stroke-prone spontaneously hypertensive rats (SHRSP), an animal model of attention deficit hyperactivity disorder (ADHD) (21). The selective α4β2 nAChR agonist, ABT418
was shown to possess efficacy in adults with ADHD (23). These reports indicate that α4β2 nAChR is involved in attention function. On the other hand, the selective α7 nAChR activation improved performance in the 17-arm radial maze (1) and in the social recognition tests (22), and enhanced hippocampal long-term potentiation (10). In clinical trials with healthy volunteers, selective α7 nAChR agonist, GTS-21 improved episodic memory (12). Infusion of selective α7 nAChR antagonist MLA into the hippocampus impaired learning in a similar radial arm maze task (6). These reports indicate a role for α7 nAChRs in learning and memory, rather than in attention processes. In this study, the selective α7 nAChR agonist PNU120596 did not affect spontaneous alternation performance in both GM3+/− and GM3−/− mice. The present results imply that juvenile GM3−/− mice may exhibit impairment of spontaneous alternation behavior based on an attention deficit rather than learning and memory dysfunctions.

As a different viewpoint, interesting evidence about gangliosides and receptor phosphorylation was reported. GD1a, one of the ganglioside species, enhanced the phosphorylation of epidermal growth factor receptor (EGFR), whereas treatment with GD3 enhanced the phosphorylation of epidermal growth factor receptor (EGFR), whereas treatment with GM3 reduced the EGFR phosphorylation (10). As for the in vivo phosphorylation of the EGFR in skin, GD3−/− mice show an enhancement of EGFR phosphorylation as compared with wild-type and GM3−/− mice (5). From these results, the status of phosphorylation of the receptors may be under the selectivity caused by ganglioside species. As a same manner, it is not an extreme speculation that ganglioside may have a subtype-selective effect to the nicotine receptors. As conclusion, further studies using subtype-selective receptor modulators promise to provide further new findings between ganglioside GM3 and the α4β2 and α7 nAChR mechanisms, and a better understanding of the mechanism of cognitive functions of GM3−/− mice.

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