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# BEHAVIORAL ADAPTOGENIC HUMORAL GLYCOLIPID IN MOUSE GIVEN VARIOUS STRESSES

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**Abstract**

Stresses spoil adaptation. Animals prepare behavioral adaptogenic substances for escaping from stressful situation. Mice given forced swimming stress prepared GalNAc$\alpha_{1-3}$GalNAc-lipid inducing hyperactivity, in the humoral lipoid fraction eluted with 150 mMNaCl. Mice given various stresses may differently prepare the behavioral adaptogenic glycolipid. Disadaptation induces depression. Depression closely relates to adrenergic and serotonergic neuronal activities. The glycolipid may be produced via these neuronal activities. Mice were twice given immunization stress, given forced swimming stress or given stress restricting the mobility on a raised platform, or not given the stresses as a control. Mice were also treated with a serotonin reuptake inhibitor clomipramine or a noradrenaline reuptake inhibitor maprotiline. Reactivity of the glycolipid was increased in mice given the forced swimming stress, and in mice given the immunization stress. The reactivity was also found in mice not given the stresses, but was decreased in mice given the mobility restriction stress. Treatment of the noradrenaline reuptake inhibitor dose-dependently increased the reactivity, however, treatment of the serotonin reuptake inhibitor did not affect the reactivity. These suggest that the behavioral adaptogenic humoral glycolipid was prepared corresponding to the mobility, via not serotonergic but adrenergic neuronal activity.

**Key words** : behavioral adaptogenic humoral glycolipid, various stresses, serotonin, noradrenaline, neuronal activity

## Introduction

Stresses are harmful to adaptation. Animals prepare behavioral adaptogenic substances for escaping from stressful situation. In fact, mice given forced swimming stress producedGalNAc$\alpha_{1-3}$GalNAc-lipid inducing the escaping behavior, in the humoral lipoid fraction eluted with 150 mMNaCl[1]. Mice given various stresses may differently prepare the adaptogenic humoral glycolipid. Disadaptation induces depression. Other humoral adaptogenic substances were prepared via adrenergic and serotonergic neuronal activities relating to depression[2]. The behavioral adaptogenic glycolipid may be also prepared under these neuronal activities. In the present study, the author examines reactivity of the glycolipid in mice given various stresses, and investigates effects of antidepressants relating to the neuronal activities on the glycolipid reactivity.

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## Methods

### 1. Animal

Male 9 weeks-old DDY mice were purchased from SLC Co. (Hamamatsu, Japan) for using in the present study. All experiments were conditioned in accordance with animal research regulations at Akita University

School of Medicine (the approval number : a-1-2824).

**2. Mice given stress**

① *Mice twice given immunization stress*

Mice twice immunized are given severe stress inducing the death[2]. Ten mice were intraperitoneally injected with 1 mg/kg of ovalbumin (OVA, Grade V, Sigma-Aldrich Co., St. Louis, MO, USA) dissolved in physiological saline (PS). Ten days after this, they were given second immunization with the same OVA concentration. Seven days after the second immunization, they were sacrificed, and the serum was collected and pooled.

② Mice given forced swimming stress

Ten mice were given forced swimming stress for 5 min in the apparatus previously described[3]. They were sacrificed 10 min after given the stress, and the serum was collected and pooled.

③ Mice placed on platform

Mice placed on a raised platform are given stress restricting the mobility[4]. Ten mice were placed a raised platform (7.5 cm diameter, 25 cm height) for 5 min. They were sacrificed 10 min after the treatment, and the serum was collected and pooled.

④ Mice not given the stresses

As a control, 10 mice not given the stresses described above were prepared. They were sacrificed, and the serum was collected and pooled.

**3. Mice treated with antidepressant**

① Mice treated with a serotonin reuptake inhibitor

Antidepressant clomipramine (Wako Pure Chemical Industries Ltd., Tokyo, Japan) is a serotonin reuptake inhibitor. Ten mice were intraperitoneally injected with clomipramine solution 1, 0.5 or 0.1 mg/kg, or 100 μl PS as a control. They were sacrificed 60 min after the treatment, and the serum was collected and pooled.

② Mice treated with a noradrenaline reuptake inhibitor

Antidepressant maprotiline (Wako Pure Chemical Industries) is a noradrenaline reuptake inhibitor. Ten mice were intraperitoneally injected with maprotiline solution 1, 0.5 or 0.1 mg/kg, or 100 μl PS as a control. They were sacrificed 60 min after the treatment, and the serum was collected and pooled.

**4. Humoral lipoid fractionation**

5 ml chloroform and 10 ml methanol were added to each 4 ml pooled serum. The solution was intensively mixed for 3 min and incubated for 10 min at room temperature (RT). Then, 5 ml chloroform was added to the solution, followed by intensive mixing for 30 s. 4 ml water was added to the solution, followed by intensive mixing for another 30 s. The mixture was then centrifuged (150×g) for 10 min at RT. The chloroform (lower) layer was collected, and the solvent was evaporated at RT. The extracted lipoids were then suspended in 4 ml water. The solution was applied to a 2 ml ion exchanger DE-52 (Whatman Co., Maidstone, UK) column, which had been saturated with 10 mM $NaHCO_3$, pH 8.3, and washed with water. Samples were eluted with 2 ml consecutive washes of 50. 100, 150, 200, 250 and 300 mMNaCl. Fractions collected were then diluted to 4 ml with water.

**5. Measurement of GalNAcα$_{1-3}$GalNAc-lipid reactivity**

① Detection method

Sample humoral lipoid fraction eluted with 150 mMNaCl, or 1mg/ml of globopentaosylceramide, GalNAcα$_{1-3}$GalNAc-ceramide (Sigma-Aldrich Co.) or PS as a control was prepared to 50% ethanol solution. The solution 100 μl was poured into a well of a 96-well plastic plate (Sumitomo Bakelite Co., Tokyo, Japan). Enzyme-linked immunosorbent assay (ELISA) was performed with the use of 300 μl of 5% bovine serum albumin (Sigma-Aldrich Co.) as a blocker, a biotinized lectin of Dolichos biflorus recognizing GalNAcα$_{1-3}$GalNAc in terminal of sugar chain structure (Seikagaku Co., Tokyo, Japan), peroxidase-conjugated avidin (Seikagaku Co.) and the coloring kit (Sumitomo Bakelike Co.) Then, the light absorbance was measured at the dual wavelength of 450 and 655 nm. The ELISA procedure was performed on five different plates.

② Statistical analysis

Mann-Whitney U test was used for the statistical analysis. A $p<0.05$ was considered a significant difference.

## Results

### 1. GalNAcα$_{1-3}$GalNAc-lipid reactivity in mice given various stresses

GalNAcα$_{1-3}$GalNAc-lipid reactivity was found in fraction eluted with 150 mMNaCl of mice not given the stresses. The reactivity was increased in the fraction of mice given the immunization stress, and in the fraction of the forced swimming stress. On the contrary, the reactivity of mice given the mobility restriction stress was decreased (Table 1).

### 2. The reactivity in mice treated with antidepressant

Treatment of a serotonin reuptake inhibitor clomipramine did not affect reactivity of the glycolipid. On the other hand, treatment of a noradrenaline reuptake inhibitor maprotiline increased the reactivity with a dose-dependent manner (Table 2).

## Discussion

Reactivity of the humoral glycolipid was increased not only in mice given the forced swimming stress, but also in mice treated with the noradrenaline reuptake inhibitor. Escaping behavior in mice given forced swimming stress is induced by dopaminergic neuronal activity[5], and is enhanced with adrenergic neuronal activity[6]. These suggest that the humoral glycolipid would be prepared for enhancing adrenergic neuronal activity in the behavioral

Table 1. GalNAcα$_{1-3}$GalNAc-lipid reactivity in humoral lipid fraction eluted with 150 mMNaCl of mice given various stresses

| Reactivity in the fraction | |
|---|---|
| Given stress | Light absorbance |
| Immunization twice | 0.346 ± 0.022* |
| Forced swimming 5 minutes | 0.322 ± 0.015* |
| Placed at platform 5 minutes | 0.111 ± 0.031* |
| Not given the stresses (The control) | 0.248 ± 0.056 |
| ELISA control | |
| Globopentaosylceramide (1 mg/ml) | 0.239 ± 0.019 |
| Physiological saline | 0.048 ± 0.011 |

ELISA : enzyme-linked immunosorbent assay
Globopentaosylceramide : GalNAcα$_{1-3}$GalNAc-ceramide
*p<0.05 compared to the control (Mann-Whitney U test)

Table 2. Effect of antidepressant on GalNAcα$_{1-3}$GalNAc-lipid reactivity

| Antidepressant | | |
|---|---|---|
| | Light absorbance | |
| Dosage (mg/kg) | Clomipramine | Maprotiline |
| 1 | 0.277 ± 0.060 | 0.406 ± 0.026* |
| 0.5 | 0.281 ± 0.061 | 0.326 ± 0.038* |
| 0.1 | 0.271 ± 0.058 | 0.298 ± 0.049 |
| Physiological saline (The control) | 0.253 ± 0.063 | 0.266 ± 0.039 |
| ELISA control | | |
| Globopentaosylceramide (1mg/ml) | 0.245 ± 0.027 | |
| Physiological saline | 0.043 ± 0.022 | |

ELISA : enzyme-linked immunosorbent assay
Globopentaosylceramide : GalNAcα$_{1-3}$GalNAc-ceramide
*p<0.05 compared to the control (Mann-Whitney U test)

adaptation system activated by dopaminergic neuronal activity. Reactivity of the humoral glycolipid was also found in mice given the immunization stress, and in mice not given the stresses, but was decreased in mice given mobility restriction stress on a raised platform. This suggests that the glycolipid would be prepared for maintaining mobility of mouse. Serotonergic neuronal activity induces fear emotion decreasing mobility in rats[7], however, reactivity of the glycolipid was not affected in mice treated with the serotonin reuptake inhibitor. These suggest that the glycolipid would function not in the emotional behavior system.

In the present study, mouse is suggested to prepare the behavioral adaptogenic humoral glycolipid corresponding to the mobility, via not serotonergic but adrenergic neuronal activity. The brain senses stresses. Globopentaosylceramide, primarily contented in the brain, induced the escaping behavior as well as the behavioral adaptogenic glycolipid did[8]. The glycolipid might be also produced in the brain. The production mechanism has not been clear, however, the present findings will contribute to understand behavioral stress coping system.

## Competing Interests

The author declares that he has no competing interests.

## References

1) Masuda, Y., Ohnuma, S. and Sugiyama, T. (2000) Relationship between antidepressants and glycolipids in the forced swimming test in mice. *Methods Find. Exp. Clin. Pharmacol.,* **22**, 667–670.

2) Masuda, Y. (2017) Twoadaptogenic humoral lipids prepared in mouse given various stresses. *Akita J. Med.*, **44**, (in print)

3) Masuda, Y., Ishigooka, S. and Matsuda, Y. (2001) Behaviors of mice given forced swimming. *Exp. Anim.*, **50**, 331–335.

4) Masuda, Y. and Matsuda, Y. (2004) Slip-down from a raised platform : Another behavior of Slc : ddY mice treated with methamphetamine. *Exp. Anim.,* **53**, 61–62.

5) Perona, M.T., Waters, S., Hall, F.S., Sora, I., Lesch, K.P., Murphy, D.L., Caron, M. and Uhl, G.R. (2008) Animal models of depression in dopamine, serotonin, and norepinephrine transporter knocked mice : prominent effects of dopamine transporter deletions. *Behav. Pharmacol.,* **19**, 566–574.

6) Villegier, A.S., Drouin, C., Bizot, J.C., Marien, M., Glowinski, J., Colpuert, F. and Tassin, J.P. (2003) Stimulation of postsynaptic alpha1b-and alpha2-adrenergic receptors amplifies dopamine-mediated locomotor activity in both rats and mice. *Synapse*, **15**, 277–284.

7) Zangrossi, HJr, Viana, M.B., Zanoveli, J., Bueno, C., Nogueria, R.L. and Graeff, F.G. (2001) Serotonergic regulation of inhibitory avoidance and one-way escape in the rat elevated T-maze. *Neurosi. Biobehav. Rev.*, **25**, 637–645.

8) Masuda, Y., Sugiyama, T. (2000) The effect of glybopentaosylceramide on a depression model, mouse forced swimming. *Tohoku. J. Exp. Med.,* **191**, 47–54.