Research Article

Stress-Coping Humoral Glycolipids Produced by Mice Given Controlled Bathing Treatments

Yutaka Masuda,1 Hiroto Narita,2 and Hiroaki Hasegawa2

1Psychosomatic Division, Graduate School of Medicine, Akita University, Hondo 1-1-1, Akita 010-8543, Japan
2Department Mechanical and Intelligent Engineering, School of Engineering, Utsunomiya University, Youtou 7-1-2, Utsunomiya 321-8585, Japan

Correspondence should be addressed to Yutaka Masuda; y-masuda@hos.akita-u.ac.jp

Received 10 June 2019; Revised 1 August 2019; Accepted 17 September 2019; Published 3 November 2019

Academic Editor: Michael Ryan Hunsaker

Copyright © 2019 Yutaka Masuda et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Mammalians have recognition-behavioral stress-coping neuronal module system followed by some humoral glycolipids. A sulfated Galbeta1-4GlcNAc-lipid promotes the serotonergic module regulating the emotional behaviors for not-wasting the physical strength; GalNAcalpha1-3GalNAc-lipid promotes the adrenergic module inducing the behaviors escaping from the uneasy situation, and sulfated Fucalpha1-2Gal-lipid protects the cholinergic module keeping the stressor-memory from the ischemia-stress. Mouse given bathing recognizes the stressors to be coped with in the treatment. We previously observed mouse given CO2-microbubble-bathing increased the behavior escaping from the bathing situation. Mouse given CO2-microbubble-bathing would recognize the other stressors to be coped with in the treatment. We examined stress-coping glycolipids produced by mice given controlled bathing treatments, and got the following results. A sulfated Galbeta1-4GlcNAc-lipid production was increased by the acidic bathing condition and the dissolved CO2, GalNAcalpha1-3GalNAc-lipid production was increased by the dissolved CO2, and sulfated Fucalpha1-2Gal-lipid production was increased by the acidic bathing condition. We understood the mice treated with CO2-microbubble-bathing would recognize the acidic bathing condition and the dissolved CO2, but not the microbubble, as the other stressors to be coped.

1. Introduction

1.1. Background. We have investigated mammalian recognition-behavioral stress-coping system. We previously found mouse given CO2-microbubble-bathing showed the behavior escaping from the bathing situation, more than mouse given bathing did [1].

1.2. Theory. Mammalian brains work via network of the functional neuronal modules [2]. We found some humoral glycolipids following the recognition-behavioral stress-coping modules. A sulfated Galbeta1-4GlcNAc-lipid (3-O-Sulfo-Beta-D-Galactosyl-(1->4)-N-Acetyl-Beta-D-Glucosamine-lipid; sG1-4GN) promotes the serotonergic module regulating the emotional behaviors for not-wasting the physical strength [3, 4]. A GalNAcalpha1-3GalNAc-lipid (GalNAcalpha1->3GalNAc-lipid; GN1-3GN) promotes the adrenergic module inducing the behaviors escaping from the uneasy situation [5, 6]. And sulfated Fucalpha1-2Gal-lipid (Fucalpha1-2[6OSO3]Galbeta1-4Glcbeta-lipid; sF1-2G) protects the cholinergic module keeping the stressor-memory from the ischemia-stress, as an adaptogen did [4, 7, 8]. Mammalians produce the stress-coping glycolipids corresponding to the stressors.

1.3. Hypothesis. Mouse given bathing recognizes the stressors to be coped in the treatment. We hypothesized mouse given CO2-microbubble-bathing would recognize the other stressors to be coped with in the treatment. In the present study, we examine the stress-coping glycolipids produced by mice given controlled bathing treatments.

2. Materials and Methods

2.1. Animals. Female 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).
2.2. Bathing Apparatus. A water-tank (the diameter 40 cm, the depth 70 cm) was prepared at room temperature (RT). A 20°C tap-water was poured in the tank to 50 cm depth. A microbubble-generator and absorb pipe were settled at a depth of the tank, and an electric pump and a CO₂-cylinder were settled outside of the tank.

2.3. Bathing pH Condition and Microbubble-Generator. A bath salt generally marketed was prepared for keeping pH 5 bathing condition. Slit-type microbubble-generator was prepared. The generator produces microbubble by passing-through high-speed water-flow containing gas [9].

2.4. Bathing Procedure. Mice were treated with bathing in the water-tank filled with the tap-water for 3 min as pre-treatment. One day after this, 6 mice were treated with the tap-water bathing for 3 min (Bathing group: B group) as Positive Control, another 6 mice were treated with the tap-water bathing in pH 5 bathing condition for 3 min (pH condition group: PH group), another 6 mice were treated with the tap-water bathing generated air-microbubble for 3 min (Microbubble-bathing group: MB group), and the other 6 mice were treated with the tap-water bathing generated CO₂-microbubble for 3 min (CO₂-microbubble-bathing group: CM group). Immediately after these treatments, the mice were sacrificed by the neck-location, and blood was collected. The sera were pooled and restored at 4°C.

2.5. Humoral Lipid Fractionation. Humoral lipid fractionation was performed as previously described [4]. Briefly, 1.25 ml of chloroform and 2.5 ml of methanol were added to each 1 ml of the pooled serum. The solution was intensively mixed for 3 min and incubated for 10 min at RT. Then, 1.25 ml of chloroform was added to the solution, and followed by intensive mixing for another 30 s. A 1 ml of water was added to the solution, and followed by intensive mixing for another 30 s. The mixture was then centrifuged at 150 gravitudes for 10 min at RT. The lower chloroform layer was collected, and the solvent chloroform was evaporated at RT. The extracted lipids were then suspended in 1 ml of water. The solution was applied to 0.5 ml of an ion exchanger DE-52 (Whatman Co., Maidstone, UK) column, which had been saturated with NaHCO₃, pH 8.3, and washed with water. Samples were then diluted to 1 ml of solutions fractionated with 100, 150, 200, 250, and 300 mM NaCl. Fractions eluted with 100, 150, 200, 250, and 300 mM NaCl were then diluted to 1 ml with water as the present samples.

2.6. Sulfate-Radical Elimination. Stress-coping humoral glycolipids fractionated with 100 mM NaCl and 250 mM NaCl are sulfated. Sulfate-radical was eliminated from the glycolipids for measuring the terminal sugar-chain reactivity as previously described [4]. Briefly, lipids were extracted again from 800 µl of the sample solutions by using methanol-chloroform method as described above. To the extracted lipids were added 400 µl of the reagent containing silyl-agents of TMS-HT kit (Tokyo Chemical Industry Co. Tokyo, Japan), and then, incubated at 90°C for 3 h. To the solution was added 800 µl of water, and intensively mixed for 30 s.

2.7. Measurement of the Glycolipid Production. Bipolar glycolipids attach to a plastic plate in 50% ethanol solution. A modified Enzyme-Linked ImmunoSorbent Assay (ELISA) was performed for measuring the glycolipid production as previously described [4]. Briefly, the sample solutions eluted with 150 mM NaCl, the sulfate radical-eliminated sample solutions fractionated with 100 mM NaCl and 250 mM NaCl, or physiological saline (PS) as Negative Control, was prepared to 50% ethanol solution. A 100 µl of the solution was poured into a well of a 96-well plastic plate (Sumitomo-Bakelite Co., Tokyo, Japan). ELISA was performed with the use of 300 µl of 5% bovine serum albumin (Sigma-Aldrich Co., St. Louis, MO, USA) as a blocker, a biotinized-lectin of Recinus communis recognizing Galbeta1-4GlcNAc, that of Dolichos biflorus recognizing GalNacalpha1-3GalNAc or that of Aleuria aurentia recognizing Fucalpha1-2Glc (Seikagaku Co., Tokyo, Japan), peroxidase-conjugated-avidin (Seikagaku Co.), and the coloring kit (Sumitomo Bakelite Co.). Then, the light absorbance was measured at the dual wavelength of 450/655 nm. The ELISA procedure was individually performed on 5 different plates.

2.8. Statistical Analyses. Mann-Whitney U-test was used for finding difference from Positive Control. A p < 0.05 (n₁ = n₂ = 5) was considered as a significant difference.

3. Results

3.1. sG1-4GN Production. A sG1-4GN is produced in the fraction eluted with 100 mM NaCl. The glycolipid production was detected in all of the samples. The PH group mice increased the glycolipid production, and the CM group mice further increased the glycolipid production (Table 1).

3.2. GN1-3GN Production. A GN1-3GN is produced in the fraction eluted with 150 mM NaCl. The glycolipid production was detected in all of the samples. The CM group mice increased the glycolipid production (Table 2).

3.3. sF1-2G Production. A sF1-2G is produced in the fraction eluted with 250 mM NaCl. The glycolipid production was detected in all of the samples. The PH group mice increased the glycolipid production (Table 3).

4. Discussion

A sG1-4GN is produced for maintaining the physical strength, GN1-3GN is produced for inducing the behaviors escaping from the uneasy situation, and sF1-2G is produced for keeping the stressor-memory. In the present study, the B group mice produced sG1-4GN, GN1-3GN and sF1-2G. This suggests the tap-water bathing gave a stressor depriving of the physical strength, an uneasiness to be avoided, and a stressor to be memorized, to the mice. The PH group mice and the CM group mice, but not the MB group mice, increased sG1-4GN production. Furthermore, the production of the CM group mice was larger than that of the PH group mice. These suggest the mice recognized the acidic bathing condition as another stressor.
The mice might recognize the PO2 elevation to be avoided. The PH group mice, but not the MB group mice nor the CM group mice, recognized the dissolved CO2 as an uneasy situation factor to be avoided. As the PO2 elevation frequently occurs, the dissolved CO2 might not be recognized as a stressor to be memorized.

Mouse has the recognition-behavioral stress-coping system followed by some humoral glycolipids. We investigated the stress-coping glycolipids produced by mice given controlled bathing treatments, and found the mice recognized the dissolved CO2 and the acidic bathing condition as the different stressors to be coped.
Funding

The present study was performed under financial support of Japan Society for Promotion of Science [JSPS, grant number 17K19892], which Prof. H. Hasegawa gained.

Acknowledgments

The late Dr. Toshihiro Sugiyama, Professor Emeritus of Graduate School of Medicine, Akita University, gave experimental advice to us. We profoundly thank him.

Supplementary Material

Raw data of light absorbance indicating the glycolipid production. (Supplementary Materials)

References

[1] S. Sakai, H. Hasegawa, S. Uemura, and Y. Masuda, “Stress-coping effect induced by microbubble carbonated bathing,” Akita Journal of Medicine, vol. 42, pp. 125–128, 2018.

[2] E. Bullmore and O. Sporn, “The economy of brain network organism,” Nature Reviews Neuroscience, vol. 13, pp. 336–349, 2012.

[3] Y. Masuda, “Two adaptogenic humoral lipoids prepared in mouse given various stresses,” Akita Journal of Medicine, vol. 44, pp. 63–67, 2017.

[4] Y. Masuda, “Analysis of sugar-chain in stress-coping humoral lipoids,” Akita Journal of Medicine, vol. 45, pp. 27–30, 2018.

[5] Y. Masuda, S. Sugawara, S. Ohnuma, and T. Sugiyama, “Humoral GalNAcα1-3GalNAc-lipid reactivity of humans in Hypomanic state,” The Tohoku Journal of Experimental Medicine, vol. 197, no. 2, pp. 115–118, 2002.

[6] Y. Masuda, “Behavioral adaptogenic humoral glycolipid in mouse given various stresses,” Akita Journal of Medicine, vol. 44, pp. 69–72, 2017.

[7] L. Liu, T. Hoang-Gia, H. Wu et al., “GinsenosideRb1 improves spatial learning and memory by regulation of cell genesis in hippocampal subregions of rats,” Brain Research, vol. 1382, pp. 147–154, 2011.

[8] Y. Masuda, “GinsenosideRB1 preventing anaphylactic death in mouse,” Akita Journal of Medicine, vol. 43, pp. 105–107, 2016.

[9] H. Hasegawa, Y. Nagasaka, and H. Kataoka, “Electrical potential of microbubble generated by shear flow in pipe with slits,” Fluid Dynamics Research, vol. 40, no. 7–8, pp. 554–564, 2008.

[10] Y. Komoto, T. Nakao, M. Sunakawa, and H. Yorozu, “Elevation tissue PO2 with improvement of tissue perfusion by topically applied CO2,” Advances in Experimental Medicine and Biology, vol. 222, pp. 637–645, 1988.

[11] D. Q. She, M. Wang, D. M. Zhu, L. G. Sun, and D. Y. Ruan, “Effect of ganglioside on synaptic plasticity of hippocampus in lead exposed rats in vivo,” Brain Research, vol. 1060, no. 1–2, pp. 162–169, 2005.

[12] D. T. Downing, M. E. Stewart, P. W. Wertz, S. W. Colton, W. Abraham, and J. S. Strauss, “Skin lipids: an update,” Journal of Investigative Dermatology, vol. 88, no. s3, pp. 2s–6s, 1987.