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Research Article

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Humoral Recognition-Behavioral Stress-Coping Glycolipids Produced By Mice Given Repeated Electroconvulsive Treatment

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Abstract

Background: *Stress-coping is a core event of mammalians. Depression symptoms are induced by the stress-coping failures. Repeated electroconvulsive treatment gives a strong stress to mammalians, however, the treatment has been used to improve depression symptoms. Mammalians have recognition-behavioral stress-coping neuronal module-system followed by some humoral glycolipids. A sulfated Galbeta1-4GlcNAc-lipid promotes the serotonergic module. GalNAcalpha1-3GalNAc-lipid promotes the adrenergic module. A sulfated Fucalpha1-2Gal-lipid protects the cholinergic module keeping the stress-coping memories from the ischemia-stress. I hypothesized mammalians given repeated electroconvulsive treatment would produce these glycolipids, and would increase the stress-coping ability.*

Materials and Methods: *I examined the glycolipid productions of mice given repeated electroconvulsive treatment under general-anesthesia.*

Results: *I found mice only given the general-anesthesia produced sulfated Galbeta1-4GlcNAc-lipid and GalNAcalpha1-3GalNAc-lipid, and mice given the repeated electroconvulsive treatment under general-anesthesia further produced sulfated Galbeta1-4GlcNAc-lipid and GalNAcalpha1-3GalNAc-lipid, and increased sulfated Fucalpha1-2Gal-lipid production.*

Conclusion: *Depression symptoms are closely related to serotonergic and adrenergic module activities. I understood repeated electroconvulsive treatment would improve depression symptoms via the sulfated Galbeta1-4GlcNAc-lipid and GalNAcalpha1-3GalNAc-lipid productions.*

Keywords: Depression Symptoms, Humoral Glycolipids, Mammalian Stress-Coping System, Neuronal Module Function, Repeated Electroconvulsive Treatment

Introduction

Depression symptoms are induced by coping a serious stress. Depression patients are very tired, and complain decrease of the physical strength and loss of the eagerness. Repeated electroconvulsive treatment (r-ECT) has successfully decreased the depression symptoms, however, the treatment gives a strong stress for mammalians to show seizures. Many researchers investigated effects of r-ECT on the brain, however, the therapeutic mechanism was not yet clarified. Now, mammalians have recognition-behavioral stress-coping neuronal module-system followed by some humoral glycolipids. I previously reported the stress-coping glycolipids are produced corresponding to quality and quantity of the stressors. A sulfated Galbeta1-4GlcNAc-lipid (sG1-4GN) promoting the serotonergic module is produced to regulate the emotional behaviors for not-wasting the physical strength, GalNAcalpha1-3GalNAc-lipid (GN1-3GN) promoting the adrenergic module is produced to induce the stress-coping behaviors, and sulfated Fucalpha1-2Gal-lipid (sF1-2G) is produced to protect the cholinergic module keeping the stress-coping memories from the ischemia-stress [1]. I reported the recognition-behavioral stress-coping glycolipid-production system works in human [2]. So, I hypothesized r-ECT would produce the stress-coping glycolipids via mammalian stress-coping system, and the glycolipids would induce the anti-depression effects. Recently, r-ECT under general-anesthesia has been performed as a therapeutic method for depression patients, so, I investigate the humoral stress-coping glycolipid productions of mice given r-ECT under general-anesthesia in the present study.

Materials and Methods

Animal

Female 9 weeks-old DDY mice were purchased from Japan SLC Co. (Hamamatsu, Japan) for using in the present study. All of the experiments were conditioned in accordance with Animal Research Regulations of Akita University School of Medicine (the approval number: a-1-3019).

Treatmented Mice

Isoflurane General-Anesthesia and Electroconvulsive Treatment
For general-anesthetizing, mice were individually placed in a glass container holding 500ml filled with vapor of isoflurane (Fuji-Film and Wako-Junyaku Co. Osaka, Japan) for 10s. Sine-wave electric current (100V, 50cycles per min) was given to head of the anesthetized mice via electroconvulsive therapy apparatus (Sakai Co., Tokyo, Japan) for 3s. Validity of the electroconvulsive treatment was assessed with mice tonic convulsion.

Control Mice

A 6 mice were individually placed in the container without isoflurane vapor for 10s 6times per 2 days. They were considered as **Control mice**. They were sacrificed by the neck-dislocation, and their blood was collected 24h after the final treatment. The serum was pooled and restored at 4 °C.

Mice Given General-Anesthesia

A 6 mice were individually given the general-anesthesia 6times per 2 days. They were considered as **ANZ mice**. They were sacrificed by the neck-dislocation, and their blood was collected 24h after the final treatment. The serum was pooled and restored at 4 °C.

Mice Given Repeated Electroconvulsive Treatment

A 6 mice were individually given the electroconvulsive treatment under the general-anesthesia 6times per 2 days. They were considered as **r-ECT mice**. They were sacrificed by the neck-dislocation, and their blood was collected 24h after the final treatment. The serum was pooled and restored at 4 °C.

Humoral Glycolipid Fractionation

Glycolipids are variously charged in water. Fractionation of humoral glycolipids was performed as previous described [1]. Briefly, 2.5ml of chloroform and 5ml of methanol were added to 2ml of the pooled serum. The solution was intensively mixed for 3min and incubated for 10min at room temperature (RT). Then, 2.5ml of chloroform was added to the solution, followed by intensive mixing for 30s. A 2ml of water was added to the solution, followed by intensive mixing for another 30s. The mixture was then centrifuged at 150×g for 10min at RT. The chloroform (lower) layer was collected, and the solvent was evaporated at RT. The extracted lipids were then suspended in 2ml of water. The solution was applied to 2ml of anion-exchanger DE-52 (Whatman Co., Maidstone, UK) column, which had been saturated with 10mMNaHCO3, pH8.3 and washed with water. The lipids were eluted with 1ml consecutive washes of 50. 100, 150, 200, 250 and 300mMNaCl. Fractions eluted with 100, 150 and 250mMNaCl were then diluted to 2ml with water as the present samples.

Sulfate-Radical Eliminating Method

Humoral stress-coping glycolipid fractionated with 100 or 250mMNaCl is sulfated. Sulfate-radical of the glycolipids disturbs the terminal sugar-chain reactivity detection. Sulfate-radical of the recognition-behavioral stress-coping glycolipids was eliminated as previously described [1]. Briefly, glycolipids were re-extracted from 800µl of the sample solutions using methanol and chloroform as described above. The extracted glycolipids were added 400µl of the reagent containing silyl-agents and dehydrated pyridine of TMS-HT kit (Tokyo Chemical Industry Co., Tokyo, Japan), and then, were incubated at 90 °C for 3h. The solutions were added 800µl water, intensively mixed for 30s and were restored in 4ºC for 24h.

Measurement of the Glycolipid Production

Glycolipids are bipolar, and they attach to plastic plate in 50% ethanol condition. A modified Enzyme-Linked ImmunoSorbent Assay (ELISA) was performed for measuring the glycolipids production as previously described [1]. Briefly, the sample solution eluted with 150mMNaCl, the sulfate-radical-eliminated sample solution fractionated with 100 or 250mMNaCl, or physiological saline (PS) as a blank control, were prepared to 50% ethanol solution. A 100µl of the solution was poured into a well of a 96-well plastic plate (Sumitomo-Bakelite Co., Tokyo, Japan). The ELISA was performed with the use of 300µl of 5% bovine serum albumin (Sigma-Aldrich Co., St. Louis, MO, USA) as a blocker, a biotinylated lectin of ricinus communis recognizing Galbeta1-4GlcNAc, that of Dolichos biflorus recognizing GalNAcalpha1-3GalNAc or that of aleuria aurantia recognizing Fucalpha1-2Gal, peroxidase-conjugated-avidin (Seikagaku Co., Tokyo, Japan), 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 different 5 plates.

Statistical Analyses

Steel-Dwass test was used for finding statistical difference. A $p<0.05$ was considered as a significant difference.

Results

A Sg1-4gn Production

A sG1-4GN is produced in fraction eluted with 100mMNaCl. The production was detected in all of the samples. The ANZ mice produced the glycolipid more than the Control mice did, and the r-ECT mice produced more than the ANZ mice did [Table 1].

Table 1: Mean±Sd of Sg1-4gn Reactivity in the Samples

Sample obtained from	Light absorbance (450/655 nm)
Control mice (not-treated)	0.119±0.005
ANZ mice (Anesthetized)	※0.179±0.012
r-ECT mice (Given repeated ECT)	#※0.211±0.011
Blank (Physiological saline)	0.054±0.008

A sG1-4GN: sulfated Galbeta1-4GlcNAc-lipid promoting the serotonergic
module.
※p<0.05 compared to Control mice (Steel-Dwass test)
#p<0.05 compared to ANZ mice (Steel-Dwass test)
ECT: electroconvulsive treatment

GN1-3GN production

GN1-3GN is produced in fraction eluted with 150mMNaCl. The production was detected in all of the samples. The ANZ mice produced the glycolipid more than the Control mice, and the r-ECT mice produced more than the ANZ mice did (Table 2).

Table 2: Mean±SD of GN1-3GN reactivity in the samples

Sample obtained from	Light absorbance (450/655 nm)
Control mice (not-treated)	0.066±0.005
ANZ mice (Anesthetized)	※0.113±0.021
r-ECT mice (Given repeated ECT)	#※0.189±0.036
Blank (Physiological saline)	0.049±0.004

GN1-3GN: GalNAcalpha1-3GalNAc-lipid promoting the adrenergic module.
※$p<0.05$ compared to Control mice (Steel-Dwass test)
#$p<0.05$ compared to ANZ mice (Steel-Dwass test)
ECT: electroconvulsive treatment

A sf1-2g Production

A sF1-2G is produced in fraction eluted with 250mMNaCl. The production was detected in all of the samples. The r-ECT mice produced the glycolipid more than the ANZ mice and the Control mice did [Table 3].

Table 3: Mean±Sd of Sf1-2g Reactivity in the Samples

Sample obtained from	Light absorbance (450/655 nm)
Control mice (not-treated)	0.115±0.014
ANZ mice (Anesthetized)	※0.136±0.010
r-ECT mice (Given repeated ECT)	#※0.137±0.006
Blank (Physiological saline)	0.045±0.006

A sF1-2G: sulfated Fucalpha1-2Gal-lipid protecting the cholinergic module.
※$p<0.05$ compared to Control mice (Steel-Dwass test)
ECT: electroconvulsive treatment

Supplementary Data

Raw data of light absorbance indicating the glycolipid production

Light absorbance (450/655nm)

Plate	1	2	3	4	5
sG1-4GN					
Control	0.111	0.119	0.126	0.123	0.120
ANZ	0.162	0.171	0.179	0.189	0.197
r-ECT	0.220	0.214	0.207	0.208	0.209
Blank (PS)	0.057	0.061	0.065	0.054	0.043
		GN1-3GN			
Control	0.055	0.061	0.077	0.072	0.067
ANZ	0.149	0.119	0.089	0.100	0.111
r-ECT	0.165	0.172	0.178	0.203	0.229
Blank (PS)	0.052	0.048	0.043	0.049	0.054
Control	0.205	0.199	0.193	0.194	0.205
ANZ	0.196	0.203	0.210	0.221	0.231
r-ECT	0.235	0.244	0.252	0.252	0.253
Blank (PS)	0.057	0.099	0.040	0.050	0.061

Discussion

Since it was recognized human seizures have a therapeutic potential to the psychotic symptoms, r-ECT was considered as a therapeutic method to treat the psychotic symptoms, and has been established as a method decreasing the severe depression symptoms [3]. Anti-depressive effects of r-ECT has been variously investigated [4-6], however, any investigations did not clarified the anti-depression mechanism.

Depression patients generally complain decrease of the physical strength and loss of the eagerness. This indicates depression patients have the serotonergic module dysfunction and the adrenergic module dysfunction in their stress-coping system. In fact, antidepressants improve depression symptoms by stimulating the serotonergic neuronal activity and the adrenergic neuronal activity. Now, sG1-4GN is produced to promote the serotonergic module, GN1-3GN is produced to promote the adrenergic module, and a sF1-2G is produced to protect the cholinergic module from the ischemia-stress [1]. In the present study, the Control mice produced sG1-4GN, GN1-3GN and sF1-2G. This suggests mice always prepares these glycolipids for the stress-coping. The ANZ mice produced sG1-4GN and GN1-3GN more than the Control mice did. This suggests the mice recognized the general-anesthesia as a stress to be coped with the serotonergic module activity and the adrenergic module activity. In fact, depression patients often complain insomnia, and improvement of the sleep-disturbance deceases the depression symptoms. The r-ECT mice produced sG1-2GN and GN1-3GN more than the ANZ mice did, and increased sF1-2G production. These suggest the mice recognized r-ECT not only as a stronger stress to be coped with the serotonergic module activity and the adrenergic module activity but also as an ischemia-stress decreasing the cholinergic module activity. Amnesia observed after r-ECT might be induced via ischemia-stress of the cholinergic module.

A strong stress induces secretion of gene-expressing hormones via Hypothalamus-Pituitary Axis. T3 and TSH levels of major

depression patients are lower than those of healthy controls [7], and r-ECT increases a gene-transcription [8]. Mice and depression patients given r-ECT might increase the stress-coping glycolipid-gene transcriptions via the hormones secretion. Nevertheless, mechanism of the glycolipids produced in peripheral blood is not yet clear in the present study.

Conclusion

Depression symptoms are closely related to serotonergic and adrenergic module activities. I understood repeated electroconvulsive treatment would improve depression symptoms via the sulfated Galbeta1-4GlcNAc-lipid and GalNAcalpha1-3GalNAc-lipid productions.

Ethical Approval

All of the presented experiments were conditioned in accordance with Animal Research Regulation of Akita University School of Medicine (the approval number: a-1-3019).

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