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TWO ADAPTOGENIC HUMORAL LIPOIDS PREPARED IN MOUSE GIVEN VARIOUS STRESSES

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Abstract

Stresses induce disaptation. Disadaptation induces depression and death. Animals prepare internal stress-coping substances for keeping their adaptation. Mouse given forced swimming stress produced an adaptogenic substance, in humoral lipoid fraction eluted with 100 mM NaCl, for keeping the physical strength, and mouse given repeated immunization stress prepared another substance, in humoral lipoid fraction eluted with 250 mM NaCl, for preventing the death. Mouse given various stresses may differently prepare these adaptogenic humoral lipoids. Depression closely relates to serotonergic and adrenergic neuronal activities. Production of the adaptogenic lipoids may be affected by antidepressants. Cerebrosides are lipoids primarily contented in brain sensing stresses. Cerebrosides may have adaptgenic activity same to the humoral lipoids. Mice were twice given immunization stress, given forced swimming stress or given fear stress 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. A humoral lipoid preventing the death was produced in the fraction eluted with 100 mM NaCl of mice given the immunization stress, of mice given the forced swimming stress, or of mice treated with clomipramine or maprotiline. Another humoral lipoid preventing the death was deduced in the fraction eluted with 250 mM NaCl of mice given the immunization stress or of mice treated with maprotiline. Porcine brain cerebroside sulfates, fractionated with 100 mM NaCl and with 250 mM NaCl, dose-dependently prevented the death. These suggest that the adaptogenic humoral lipoids were cerebroside sulfates prepared via serotonergic and adrenergic neuronal activities, corresponding to quality of the stresses.

Key words : various stresses, adaptogenic humoral lipoids, serotonin, noradrenaline, neuronal activity, cerebroside sulfates

Introduction

Animals in disadaptation are forced to be dead. They prepare stress-coping substances for maintaining their adaptation. In fact, mice given forced swimming stress produced a humoral lipoid inducing motionlessness for recovering the physical strength[1]. Mice surviving after three times given immunization stress prepared a humoral lipoid preventing the death[2]. Mouse given various stresses may differently produce these adaptogenic humoral lipoids. Disadaptation also induces depression. The adaptogenic lipoids may be prepared via serotonergic and adrenergic neuronal activities relating to depression. Brain sensing stresses would produce adaptogenic substances. Cerebrosides, primarily contained in brain, may have adaptogenic activity same to the humoral lipoids. In the present study, the author sets a system assessing mouse death induced by immunization stress,

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and examines production of the adaptogenic humoral lipoids in mice given various stresses and relation of the production and antidepressant treatment in this system. The author also detects adaptogenic activity of cerebroside sulfates extracted from porcine brain in the assessment system.

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. Assessment of mouse death

① Immunization

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 injected with OVA solution 1mg/kg, and sample humoral lipoid fraction or PS as a control. Death of the mice was assessed at 1 h after the last immunization.

② Statistical analysis

Fisher's exact test was used for the statistical analysis. A $p<0.05$ was considered a significant difference.

3. Mice given stress

① Mice twice given immunization stress

Ten mice were twice immunized with the procedure described above. They were sacrificed just before the third immunization, 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[1]. They were sacrificed 10 min after given the stress, and the serum was collected and pooled.

③ Mice given fear stress on a platform

Mice placed on a platform are given fear stress[3]. 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 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 mg/kg. They were sacrificed 90 min after the treatment, and the serum was collected and pooled.

⑤ 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 mg/kg. They were sacrificed 90 min after the treatment, and the serum was collected and pooled.

⑥ 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.

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 mM NaCl. Fractions collected were then diluted to 4 ml with water.

5. Effect of fraction eluted with 100 mM NaCl or eluted with 250 mM NaCl obtained from mice given various stresses

Mice were intraperitoneally injected with 1 mg/kg of

OVA solution. Ten days after this, they were given second immunization with the same OVA concentration. Seven days after the second immunization, 10 of them were treated with OVA solution 1 mg/kg, and 200 μl of sample fraction eluted with 100 mM NaCl or eluted with 250 mM NaCl obtained from mice given the stresses described above. Death of the mice was assessed at 1 h after the last immunization.

6. Effect of the cerebroside sulfates fractions

40 mg of cerebroside sulfate isolated from porcine brain (Avanti Polar Lipids Inc., Alabaster, AL, USA) was dissolved in water and fractionated by ion exchange chromatography, eluting with 100 and 250 mM NaCl as described above. The fractions collected were diluted to 4 ml with water.

Mice were immunized twice. At 20 min before the third immunization, 10 of them were intraperitoneally injected with 200, 100 or 50 μl of these fractions or with 200 μl PS as a control. At 1 h after the third immunization, death of the mice was assessed.

Result

1. Effect of the fraction eluted with 100 mM NaCl or with 250 mM NaCl

All mice treated with lipoid fraction eluted with 100 mM NaCl, of mice given the immunization stress or of mice given the forced swimming stress, escaped from the death. But 5 mice treated with the fraction of mice given the fear stress, and 6 mice treated with the fraction of mice not given the stresses did not. All mice treated with the fraction of mice pretreated with clomipramine or maprotiline escaped from the death (Table 1). On the other hand, all mice treated with lipoid fraction eluted with 250 mM NaCl, of mice given the forced swimming stress or the fear stress, escaped from the death. All mice treated with the fraction of mice not given the stresses also escaped from the death, but 6 mice treated with the fraction of mice given the immunization stress did not. All mice treated with the fraction of mice pretreated with clomipramine escaped from the death, but 4 mice treated with the fraction of mice pretreated with maprotiline did not (Table 2).

Table 1. The effect of 200 μl (eq.serum) humoral lipid fraction eluted with 100 mM NaCl obtained from mice given various stresses

	Dead (/total 10)
Given stress	Fraction eluted with 100 mM NaCl
Not given the stresses (the control)	6
Immunization twice	0*
Forced swimming 5 minutes	0*
Placed at platform 5 minutes	5
Clomipramine 1 mg/kg	0*
Maprotiline 1 mg/kg	0*

*$p<0.05$ compared to the control (Fisher's exact test)

Table 2. The effect of 200 μl (eq.serum) humoral lipid fraction eluted with 250 mM NaCl obtained from mice given various stresses

	Dead (/total 10)
Given stress	Fraction eluted with 250 mM NaCl
Not given the stresses (the control)	0
Immunization twice	6*
Forced swimming 5 minutes	0
Placed at platform 5 minutes	0
Clomipramine 1 mg/kg	0
Maprotiline 1 mg/kg	4*

*$p<0.05$ compared to the control (Fisher's exact test)

2. Effect of cerebroside sulfate fractions

The cerebroside sulfates fractioned from porcine brain suppressed mouse death in a dose-dependent manner. In mice treated with the 100 mM NaCl fraction, all treated with the 200 μl, 7 treated with the 100 μl and 6 treated with the 50 μl escaped from the death. In mice treated with the 250 mM NaCl fraction, all treated with the 200 μl, 8 treated with the 100 μl and 7 treated with the 50 μl escaped from the death. In mice treated with

Table 3. Effect of the cerebroside sulfate fraction on anaphylactic death

Dead (/total 10)				
Fractioned with NaCl (mM)	The cerebroside sulfate fraction (μl) 200	100	50	Control 200 μl of PS
100	0*	3	4	5
250	0*	2	3	

40 mg of porcine brain cerebroside sulfates was fractioned with 100 mMNaCl and 250 mMNaCl, and they were individually prepared to 4 ml with water.
*$p<0.05$ compared to the control (Fisher's exact test)
PS : physiological saline

200 μl of PS as a control, 5 did not (Table 3).

Discussion

A lipoid in the fraction eluted with 100 mM NaCl, of mice given the immunization stress or of mice given the forced swimming stress, prevented mouse death. As these stresses are considered to induce physical load to the mice, the adaptogenic lipoid is suggested to be produced for keeping the physical strength. Mice treated with a serotonin reuptake inhibitor clomipramine or treated with a noradrenaline reuptake inhibitor maprotiline also produced the lipoid. As physical strength is maintained by activities of alpha$_1$-adrenoceptor and 5-HT1 receptor[4,5], the adaptogenic lipoid would be produced via the serotonergic and adrenergic neuronal activities. On the other hand, a lipoid in the fraction eluted with 250 mM NaCl of mice not given the stresses prevented mouse death. Mouse would originally maintain production of the adaptogenic lipoid. Mice given the immunization stress or mice treated with maprotiline, however, deduced the lipoid. Noradenaline is increased in mice not enabling to escape from the death induced by immunization stress[6]. The neurotransmitter mediates emotional memory forming via the beta$_2$-adrenoceptor activity[7]. Hippocampus superintends memory. GinsenosideRb1 protects the hippocampal neurons from the ischemic damage[8]. It was reported that this adaptogen also prevented mouse death induced by immunization stress[9].The adaptogenic humoral lipoid in 250 mM NaCl would be prepared for protecting the hippocampus from the excessive adrenergic activity.

In the present study, mouse is suggested to prepare the adaptogenic lipoids, corresponding to the stress quality, via serotonergic and adrenergic neuronal activities in the brain sensing stresses. As porcine brain cerebroside sulfates prevented mouse death, the adaptogenic humoral lipoids are suggested to be cerebroside sulfates produced by the brain. The producing mechanism has not been well understood, however, the present findings will help to increase understanding of stress coping system.

Competing Interests

The author declares that he has no competing interests.

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