Glycoside in Schizophrenic Patients Sera Affects Behavior of Mouse Forced Swimming

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MASUDA, Y., OHNUMA, S., SUGAWARA, J. and SUGIYAMA, T. *Glycoside in Schizophrenic Patients Sera Affects Behavior of Mouse Forced Swimming.* Tohoku J. Exp. Med., 2001, **194** (4), 223–228 — We found a glycoside in sera of schizophrenic patients. This glycoside increased climbing of mouse forced swimming and the climbing was decreased by dopamine D1 receptor antagonist SCH-23390 with a dose dependent manner. This glycoside had much reactivity of GalNAc α 1-3GalNAc, but not that of polymannoses nor that of Gal β 1-3GlcNAc α 1-serine or threonine. This strongly suggests that the glycoside is an isolated *O*-glycoside, but not *N*-glycoside nor *O*-glycoside having core 1 or 2. The present findings suggest that schizophrenic patients have the special glycoside affecting the D1 receptor activity in their serum. ——— Schizophrenic patients; glycoside; dopaminergic neuronal activities; mouse forced swimming

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We have found that a behavior climbing of mouse forced swimming is mediated by dopaminergic neuronal activities but not by the other neuronal activities (Masuda and Suzuki 2000). There are other tests detecting behavioral effects of compounds with the use of mice, but, even in open-field test that is primarily used for the detections, the stereotyped behaviors are affected by benzodiazepines. These ambiguous neurological features in other behavioral tests support that the climbing of mouse forced swimming can be utilized as a special behavioral index of the dopaminergic neuronal activities. It is said that symptoms of schizophrenic patients are closely related

with the dopaminergic neuronal activities. Considering that mice have humoral substances affecting the dopaminergic neuronal activities (Masuda and Suzuki 2000), in this paper, we detect a substance affecting the behavior of mouse forced swimming in sera of schizophrenic patients.

MATERIALS AND METHODS

Animals

Male ddY mice (SLC Co., Tokyo), 8 weeks of age and weighing 20–25 g were used. They were housed in groups of five per plastic cage (338×140×225 mm) with free access to food and water. The animal room was kept at 21–

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25°C with 50–60% humidity and lighted on from 7:00 to 19:00. The mice were forced to swim for 6 minutes 24 hours before the following experiment (Porsolt et al. 1977). All of the studies were approved by the Ethics Committee for Animal Experiments of Akita University School of Medicine.

Measurement of behavioral effect

The apparatus and the procedure to measure the behavioral affect were previously described (Masuda and Suzuki 2000). Briefly, mice were placed into cylinders (diameter 10 cm, height 20 cm) containing 10 cm of 25°C water and left there for 3 minutes. The duration of climbing behavior in the next 3 minutes was measured as an index of the behavioral effect. The evaluation of climbing behavior was performed between 15:00 and 17:00.

Subjects

Three of the authors are psychiatrists. Each of the psychiatrists evaluated psychotic and physical states of subjective humans with the ICD-10 diagnostic guidelines, WHO. With common consent, the psychiatrists identified 7 healthy volunteers (4 females and 3 males, age 24–43) and 7 schizophrenic patients without physical illness (3 females and 4 males, age 23–40). The patients were suffered from schizophrenia for 3 years or more and were previously treated, but did not take the medicines a month or more before the late consultation.

The effective fraction separated from the sera

A total 10 ml of sera was collected from the healthy volunteers and another total 10 ml of sera was collected from the schizophrenic patients one week after the medical treatment of antipsychotics, antiparkinsonisms and benzodiazepines, under the informed consent. These sera were deprived of lipids (Masuda and Suzuki 2000) and separated 28 fractions (Masuda et al. 2000) as previously described. Briefly, the 10 ml of sera was added with 25 ml

of methanol and 12.5 ml of chloroform. The solution was agitated for 2 minutes and left for 10 minutes at room temperature (RT). After added with 12.5 ml of chloroform, the solution was agitated for 30 seconds. Following addition of 12.5 ml of water, the solution was also agitated for 30 seconds. The solution was centrifuged in 150 g for 5 minutes at RT, and was separated to the upper methanol-water layer containing proteins and glycosides and the lower chloroform layer containing lipids and glycolipids. The upper layer was freeze-dried and re-solved in water. The solution was applied on an ion-exchanger DE-52 (Whatman Co., Maidstone, UK) previously saturated with 10 mM NaHCO₃, pH 8.3, and was eluted with the buffer, 50, 100, 150, 200, 250 and 300 mM NaCl. These were separated furthermore to above 30, 30–10, 10–3 and below 3 kDa with the use of an ultra-filtration membrane (Centricon, Amicon Co., Tokyo).

Each of these 28 fractions was prepared to 10 ml with physiological saline (PS), and then, 200 μ l of the prepared fraction was intraperitoneally injected to a group of 5 mice. The climbing duration of the treated mice was measured 40 minutes after the injection.

The detection of the effective substance

The fraction of the volunteers sera or the schizophrenic patients sera, which was eluted with 200–250 mM NaCl and separated to 10–30 kDa, was analyzed with the use of HPLC, and was also analyzed with lectin-ELISA method (Masuda and Hishikawa 1993). Briefly, lectin MAM, which recognizes Sial α 2-3Gal (Seikagaku Co., Tokyo), was prepared to 2 μ g/ml with PS, and 100 μ l of the MAM solution was poured into a well of a 96-holes plastic plate (Sumitomo Bakelite Co., Tokyo). Two hours after this, the well was three times washed with washing solution (WS; PS containing 0.005% tween 20; Seikagaku Co.). After 30 minutes blocking with 5% bovine serum albumin (BSA; Sigma Co., St. Louis, MO, USA), 100 μ l of the sera

fractions was poured into the well. One hour after the incubation at RT, the well was three times washed with WS. A hundred ml of three different biotinized lectins recognizing the sugar chain structure (ConA: polymannoses, ABA: Gal β 1-3GalNAc α 1-serine or threonine, DBA: GalNAc α 1-3GalNAc; Seikagaku Co.), which were prepared to 2 μ g/ml with PS, was poured into the well. One hour after incubation at RT, the well was three times washed with WS. A hundred ml of peroxidase-conjugated avidine (Seikagaku Co.) prepared to 0.1 μ g/ml with PS was poured into the well. Fifteen minutes after the incubation at RT, the well was 4 times washed with PS. Coloring of the wall was performed with the coloring kit (Sumitomo-Bakelite Co.) and the light absorbance was measured at the dual wavelength 455 and 650 nm.

Furthermore, the fractions were applied on DBA affinity column (Seikagaku Co.) and eluted with 6 M urea. The eluted solutions were desalted and freeze-dried. The obtained substances were re-solved in water to 10 μ g/ml. A hundred ml of the solutions prepared to 10 μ g/ml was poured into another 96-holes plastic plate which was previously treated with 2 μ g/ml of DBA, and following lectin ELISA was performed with five different biotinized lectins (DSA: Gal β 1-4GlcNAc, SSA: Sial α 2-6Gal, AAL: Fuc α 1-2Gal and MAM; Seikagaku Co.) prepared to 2 μ g/ml with PS, and peroxidase-conjugated avidine as described above.

The neurological mechanism of the behavioral effect induced by the glycoside

The glycosides were intraperitoneally injected to 5 mice at the dose of 5, 1 or 0.2 mg/kg. Another 5 mice were injected with 100 μ l of PS. Forty minutes after this, the climbing duration of the mice was measured.

The glycoside of the schizophrenic patients was injected to other mice at the dose of 5 mg/kg. Ten minutes after this, the 5 mice were injected with 100, 10 or 1 μ g/kg of a dopamine

D1 receptor antagonist SCH-23390 or a dopamine D2 receptor antagonist sulpiride (Sigma Co.), or 100 μ l of PS. Thirty minutes of these second injections, the climbing duration of the mice was measured.

Statistical analysis

Kruskal-Wallis rank test was used to find significant differences among the clusters. After the significant differences were found (p -values of less than 0.01), Mann-Whitney U-test was used.

RESULTS

The behavioral effect was showed in the mice treated with the fraction eluted with 200–250 mM NaCl and separated to 10–30 kDa from schizophrenic patients sera (the climbing duration; 53.0 ± 4.1 : mean $\pm$ s.e. in 5 mice). The other fractions and all of the volunteers sera fractions did not show the effect (the duration; 0).

HPLC indicated that the effective fraction and the corresponding fraction of the volunteers sera did not contain proteins, which strongly suggests that an acidic glycoside was in the fractions. On the plate coated with MAM, the glycoside of the schizophrenic patients sera was showed much more GalNAc α 1-3GalNAc reactivity than that of the volunteers sera. The reactivities of polymannoses and Gal β 1-3GalNAc α 1-serine or threonine were not found (Table 1). On the plate coated with DBA, several differences were showed between the glycoside of the schizophrenic patients and that of the volunteers (Table 2).

The glycoside of the schizophrenic patients sera increased the climbing duration with a dose dependent manner, but that of the volunteers sera did not (Table 3). The behavioral effect of the glycoside of the schizophrenic patients was suppressed by SCH-23390 dose-dependently, but not by sulpiride (Table 4).

TABLE 1. *The sugar chain reactivities of the glycosides on the plate coated with MAM*

	The glycoside of		(Negative control)
	The volunteers sera	The schizophrenic patients sera	PS
Polymannoses	0.082	0.091	0.093
Galb1-3GalNAc1-serine or threonine	0.018	0.021	0.024
GalNAc1-3GalNAc	0.105	0.290	0.060

Values in this table indicate the light absorbances at 455 and 650 nm.
MAM, lectin of *Macckia amurensis*; PS, physiological saline.

TABLE 2. *The sugar chain reactivities of the glycosides on the plate coated with DBA*

	The glycoside of		(Negative control)
	The volunteers sera	The schizophrenic patients sera	PS
Galb1-4GlcNAc	0.063	0.040	0.034
Siala2-6Gal	0.101	0.032	0.032
Siala2-3Gal	0.172	0.103	0.056
Fucal-3Gal	0.040	0.097	0.038
Fucal-2Gal	0.039	0.028	0.028

Values in this table indicate the light absorbances at 455 and 650 nm.
DBA, lectin of *Dolichos biflorus*; PS, physiological saline.

TABLE 3. *The behavioral effects of the glycosides on mouse forced swimming*

Dose (mg/kg)	The glycoside of	
	The volunteers sera	The schizophrenic patients sera
5	0	91 ± 5.8*
1	0	25 ± 22*
0.2	0	9 ± 2.5*
(Control) PS	0	

Values in this table indicate the climbing duration (second) in the 5 mice; mean ± s.e. The glycoside of the schizophrenic patients sera has the statistical difference ($k=3$, $n_1=n_2=n_3=5$, $n=15$, $H=12.5$, $p<0.009$: Kruskal-Wallis rank test).

* $p<0.01$ compared to the control (Mann-Whitney U-test). PS, physiological saline.

TABLE 4. Effects of dopamine antagonists on the behavioral activity of the glycoside of the schizophrenic patients sera

Dose (μ g/kg)	Antagonist	
	SCH-23390 (dopamine 1)	Sulpiride (dopamine 2)
100	0*	89 ± 7.2
10	39 ± 3.8*	88 ± 6.4
1	77 ± 4.4*	89 ± 6.8
Control		
PS		92 ± 6.3

Values in this table indicate the climbing duration (second) in the 5 mice treated with the glycoside of the schizophrenic patients sera at the dose of 5 mg/kg; the mean ± s.e. SCH-23390 has the statistical difference ($k=3$, $n_1=n_2=n_3=5$, $n=15$, $H=12.5$, $p<0.009$: Kruskal-Wallis rank test). * $p<0.01$ compared to the control (Mann-Whitney U-test). PS, physiological saline.

DISCUSSION

The present findings clearly demonstrate that the schizophrenic patient's sera had the glycoside affecting the behavior of mouse forced swimming via dopamine D1 receptor activity.

In the forced swimming test, mice show the climbing as an escaping behavior (Masuda et al. 2001). At this time, the mice recognize the situation as a harmful one (Masuda 1999). This indicates that the dopamine D1 receptor activity of the glycoside has a relation with the recognition as well as the temporal behavior. In our previous experiments, we have found that treatments of antipsychotics, antiparkinsonisms and benzodiazepines do not induce the climbing and do not produce the glycoside in mice. It has been thought that recognition of schizophrenic patients is accelerated, and, in the present study, the volunteers did not have the glycoside. These may suggest that the glycoside of the patients was the primary cause of the abnormal recognition. Recently, it has been noted that the dopamine D1 receptor activity relates with the symptoms of refractory schizophrenic patients (Martin et al. 1994; Miller and Chouinard 1993). This also supports the present suggestion.

We have reported that GalNA α 1-3GalNAc-lipid mediates the behavior of mouse forced swimming via dopamine D2 receptor activity (Masuda and Sugiyama 2000). The glycolipid can go into the brain easily because of the hydrophobic character. But, as the glycoside is highly hydrophilic, the blood-brain barrier may have a specific receptor for the glycoside as previously reported (Alliegro 2000). In the present study, the glycoside affected the behavior via the dopamine D1 receptor activity. The different reactions of the brain dopamine neurons to the GalNA α 1-3GalNAc may depend on the lipid form and the glycoside form, but the mechanisms have been entirely unclear.

Structure and metabolism of the glycoside are also important points. This glycoside did

not have polymannoses reactivity nor Gal β 1-3GalNAc α 1-serine or threonine reactivity. The glycoside is not N-glycoside (asparagine-connected sugar chains with polymannoses) nor O-glycoside with core 1 or 2 (sugar chains with Gal β 1-3GalNAc α 1-serine or threonine). But, the further detection to the sugar structure depends on the future technological progress for O-glycosides (Suzuki 1998). In the present study, it is only suggested that the glycoside is an isolated O-glycoside with much GalNA α 1-3GalNAc structure. Originally, glycosides are metabolized on the binding protein or lipid. Previously, many proteins were investigated in the relation with schizophrenics. One of them was apolipoprotein E (Chen et al. 1999). This protein has many serine and threonine that can bind an O-glycoside. The protein may be related with production of the glycoside, but mechanism of the isolation has not been also clarified.

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