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Repeated Electroconvulsive Shock Treatment Induces Two Humoral Anti-Depressive Factors in Mouse

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MASADA, Y. and SUZUKI M. *Repeated Electroconvulsive Shock Treatment Induces Two Humoral Anti-Depressive Factors in Mouse.* Tohoku J. Exp. Med., 2000, **192** (4), 283-289 —— Forced swimming is considered a depression model. Repeated electroconvulsive shock treatment shows an anti-depressive effect in mice forced swimming. In serum of the mice treated with repeated electroconvulsive shock, the humoral anti-depressive factors were detected. The factors were the glycolipid having GalNAcα1-3GalNAc and mouse fibrinopeptide A having amino acid sequence TDTEKDGEFLSGGGV. The behavioral anti-depressive activity of the glycolipid was decreased by the low doses (100$\sim$10 μg/kg) of dopamine 2 receptor antagonist sulpiride. Behavioral activity of the peptide was also decreased by the low doses (100$\sim$1 μg/kg) of dopamine 1 receptor antagonist SCH-23390. The present findings clearly indicate that repeated electroconvulsive shock treatment induces the humoral anti-depressive factors affecting the dopaminergic neuronal system in mice. —— repeated electroconvulsive shock; dopaminergic neuronal activities; glycolipid having GalNAca1-3GalNAc; mouse fibrinopeptide A; mouse forced swimming

Forced swimming is a well-known depression model. Previously, we have indicated that repeated electroconvulsive shock (ECS) treatment shows a behavioral anti-depressive effect in mice which were forced swimming. It has been suggested that the effect is induced by a humoral factor produced by the treatment (Suzuki and Masuda 1999). It has already been reported that corticotropin releasing factor and thyrotropin releasing hormone relate to human depression (Contarino et al. 1999; Sattin et al. 1999). However, in this paper, we tried to detect different humoral anti-depressive factors in mice treated with repeated ECS and to speculate on the neurological mechanisms of the effects of the factors.

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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 21–25°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 anti-depressive effect

The apparatus and the procedure to measure the behavioral anti-depressive effect were previously described (Suzuki and Masuda 1999). Briefly, mice were placed into cylinders (diameter 10 cm, height 20 cm) containing 25°C water up to 10 cm height and left there for 3 minutes. The duration of climbing behavior in the next 3 minutes was measured as an index of the anti-depressive effect. The evaluation of climbing behavior was performed between 15 : 00 and 17 : 00.

The effective fractions separated from ECSed-mice serum

Mice were given 5 times per-day ECS under ether anesthetization with the use of an ECS therapy apparatus (Sakai Co., Tokyo) as previously described (Suzuki and Masuda 1999). Total 10 ml of sera was collected from these 30 mice 6 hours after the 5th treatment.

The serum was separated to the protein and the glycolipid fractions as previously described (Masuda and Sugiyama 2000). 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 the lower chloroform layer containing glycolipids. The upper layer was freeze-dried and re-solved in 2 ml of water. Chloroform of the lower layer was evaporated, and the remaining glycolipids also were re-solved in 2 ml of water.

The two solutions were fractionated as previously described (Masuda et al. 2000). Briefly, each of these solutions was applied on an ion-exchanger DE-52 (Whatman Co., Maidstone, UK) previously saturated with 10 mM $NaHCO_3$, 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 2 ml with physiological saline

(PS), and then, 200 μl of the prepared fraction was intraperioneally injected to a group of 5 mice. The duration climbing behavior of the treated mice was measured 15 minutes after the injection.

The detections of the anti-depressive factors

The protein fraction eluted with 150~200 mM NaCl and separated to 10-3 kDa was analyzed with the use of a protein sequencer (Perkin-Elmer Co., Tokyo). The glycolipid fraction eluted with 150-200 mM NaCl and separated to 50-30 kDa was analyzed with the use of 50% ethanol-lectin-ELISA method with various biotinized lectins (Masuda and Sugiyama 2000).

Reactivities of the two factors in the mice treated with ECS

Another 5 mice were given 5 times per-day ECS under ether anesthetization and the other 5 mice were given only 5 times per-day ether anesthetization as described above. Serum was collected separately from these mice 6 hours after the 5th treatment. Each one hundred μl of the serum was separated to the upper layer and the lower layer as described above, and, after freeze-dried and evaporated, the remaining proteins and glycolipids were re-solved in 100 μl of water.

The reactivity of the peptide with TDTEKDGEFLSEGGGV in the protein solution was measured with the ELISA method. Briefly, 100 μl of each of the protein solutions or 100 μl of synthesized peptide TDTEKDGEFLSEGGGV prepared to 1 mg/ml with PS as a control was poured into a well of a 96 holes plastic plate (Sumitomo Bakelite Co., Tokyo). After 2 hour incubation at RT, the ELISA was performed with the use of 5% bovine serum albumin (Sigma Co., St. Louis, MO, USA) as a blocking reagent, 0.1 mg/ml of the anti-TDTEKDGEFLSEGGGV rabbit IgG which had been previously made and purified, 1 μg/ml of peroxidase-conjugated anti-rabbit IgG sheep IgG (BioRad Co., Richmond, CA, USA) and washing solution which was PS containing tris-HCl pH 7.4 and 0.005% tween 20 (Wako Co., Osaka). The well-coloring was performed using a coloring kit (Sumilon; Sumitomo Bakelite Co., Tokyo) and the light absorbance was measured at the wave length 450 and 655 nm.

The reactivity of GalNAcα1-3GalNAc-lipid in the glycolipid solution was measured with the use of 50% ethanol-lectin-ELISA method with biotinized lectin of *Dolichos biflorus*, using 100 μl of globopentaosylceramide having GalNAcα1-3GalNAc (Sigma) prepared to 2.5 mg/ml with PS as a control, as previously described (Masuda and Sugiyama 2000).

The neurological mechanisms of the anti-depressive effects induced by the factors

The synthesized peptide TDTEKDGEFLSEGGGV and the previously purified globopentaosylceramide were prepared with PS. The peptide was intraperitoneally injected to 13 groups of 5 mice at the dose of 1 mg/kg and the

glycolipid was injected to other 13 groups of 5 mice at the dose of 2.5 mg/kg. Fifteen minutes after the treatment, the group of mice was injected with an antagonist, 100 μg/kg of prasozin (α1), yohimbine (α2), alprenolol (β), NAN-190 (serotonin 1A), ketanserin (serotonin 2) or LY-278 • 584 (serotonin 3), 100, 10 or 1 μg/kg of SCH-23390 (dopamine 1) or sulpiride (dopamine 2), or 100 μl of 0.5% carboxymethylcellulose (CMC) sodium (Wako) as a control. Fifteen minutes after the second injection, the duration of climbing was measured.

Statistical analysis

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

Results

The glycolipid fraction eluted with 150-200 mM NaCl and separated to 50 ~30 kDa (the fraction A) and the protein fraction eluted with 150-200 mM NaCl and separated to 10~3 kDa (the fraction B) showed the anti-depressive activity (the fraction A; 69.2±1.2, the fraction B; 98.8±2.6, the other fractions; 0, the mean±S.E. of the climbing duration in seconds).

The glycolipid in the fraction A was only recognized by the lectin of *Dolichos biflorus* and the peptide in the fraction B had the amino acid sequence TDTEKD-GEFLSEGGGV. These indicate that the glycolipid is GalNAcα1-3GalNAc-lipid (Backer et al. 1983) and the peptide is mouse fibrinopeptide A (the DBGET integrated database retrieval system, Genome Net).

The reactivities of the glycolipid and the peptide in the ECSed mice were higher than those in the mice only given ether anesthetization. In the ECSed mice, the reactivity of the peptide was higher than that of the glycolipid (Table 1).

The anti-depressive effect of the synthesized peptide was decreased by specific dopamine 1 antagonist SCH-23390 (Pazo et al. 1993) dose-dependently, and that of globopentaosylceramide was decreased by specific dopamine 2 antagonist sulpiride (Pazo et al. 1993) with a dose-dependent manner. The other antagonists did not affect the anti-depressive activities of the synthesized peptide and globopentaosylceramide (Table 2).

Discussion

The humoral anti-depressive factors detected in the present studies were the glycolipid and the peptide previously reported (Masuda et al. 2000; Masuda and Sugiyama 2000). The peptide, mouse fibrinopeptide A, is induced by peripheral dopaminergic neuronal activity (Masuda et al. 2000) and the glycolipid having GalNAcα1-3GalNAc in the terminal is induced by peripheral serotonergic neuronal activities (Masuda and Sugiyama 2000). As ECS induces peripheral

TABLE 1. *Reactivities of the glycolipid and the peptide in mouse serum fractions*

A) The GalNAcα1-3Gal-lipid reactivity in the glycolipid fraction

	Light absorbance at 450 and 655 nm
Negative control	
5% BSA	0.007
Mouse only given anesthetization	0.071±0.004
Mouse given repeated ECS	0.153±0.012*
Positive control	
2.5 mg/ml of Globopentaosylceramide	0.602

B)The peptide reactivity in the protein fraction

	Light absorbance at 450 and 655 nm
Negative control	
5% BSA	0.004
Mouse only given anesthetization	0.041±0.004
Mouse given repeated ECS	0.282±0.009*
Positive control	
1 mg/ml of the synthesized peptide	0.463

Value indicates the mean±S.E. of 5 mice. *$p<0.01$ (Mann-Whitney U-test).
BSA, bovine serum albumin. The synthesized peptide, TDTEKDGEFLSEGGGV.

cholinergic neuronal hyperaction due to convulsion, repeated ECS may also stimulate peripheral dopaminergic and serotonergic neuronal activities, although the mechanisms have not been clear. A glycolipid, ceramide trihexoside goes into brain through the blood-brain barrier (Kaye et al. 1988). The humoral glycolipid detected in the present studies would reach the brain in the same fashion. As mouse fibrinopeptide A is a small molecule, it would reach the hypothalamus having a high permeability in the blood-brain barrier.

Mouse forced swimming is a well-known depression model, and has been used for screening test of anti-depressive properties of drugs (Porsolt et al. 1977). Many pharmacological studies have confirmed that the anti-depressive effect on the model is induced by the adrenergic and serotonergic neuronal activities of drugs. However, as indicated in Table 2, dopamine antagonists mediated the anti-depressive effects of the factors on the model. The present findings strongly suggest that the dopaminergic activities are also important in mediating the neuronal mechanism of the anti-depressive behavior in the model. The anti-depressive effects of dopaminergic drugs have not been clear (Nikulina et al. 1991), but the present findings suggest that the humoral anti-depressive factors have an essential role in anti-depressive behaviors much more than the pharmacological activities of the dopaminergic drugs. Furthermore, as shown in Table 1, repeated

TABLE 2. *Effects of receptor antagonists on climbing durations of mice treated with the synthesized peptide or globopentaosylceramide*

Antagonist	Dose (μg/kg)	The duration of climbing (second)	
		1 mg/kg of the peptide	2.5 mg/kg of Globopentaosylceramide
Prazosin	100	97.6±3.8	99.0±4.2
Yohimbine	100	95.8±4.2	96.8±3.8
Alprenolol	100	100.2±4.6	98.4±4.6
NAN-190	100	96.4±3.3	102.0±5.0
Ketanserin	100	92.2±2.9	101.2±5.2
LY-278,584	100	98.2±3.6	100.8±4.9
SCH-23390	100	0*	93.6±4.1
	10	18.0±1.2*	94.4±4.8
	1	64.4±2.9*	92.0±5.1
Sulpride	100	88.6±3.1	10.0±1.3*
	10	94.4±4.4	52.4±3.2*
	1	93.2±3.6	88.8±4.4
(Control) 0.5% CMC	—	102.2±3.9	94.0±4.0

Value in this table indicates the mean±S.E. of climbing durations in 5 mice. The peptide-SCH-23390 and globopentaosylceramide-sulpiride ($k=3$, $n1=n2=n3=5$, $n=15$, $H=12.5$, $p<0.01$: Kruskal-Wallis rank test) $^*p<0.01$ compared to the control (Mann-Whitney U-test). The peptide, TDTEKDGEFLSEGGGV. CMC, carboxymethylcellulose sodium.

ECS increased the reactivity of the peptide more than that of the glycolipid. This suggests that the anti-depressive effect of repeated ECS on mouse forced swimming is mainly the function of the peptide. We have found that the anti-depressive effect of repeated ECS on the depression model is mediated by dopamine 1 neuronal activity (Suzuki and Masuda 1999). This finding would be supported by the present result.

ECS has been often used for human depression. The findings in the present studies strongly suggest that ECS may also induce a humoral anti-depressive factor in the patients. Further investigations will clarify the humoral mechanisms of the ECS therapy.

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