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Flavangenol attenuates stress responses in mice exposed to unpredictable chronic mild stress

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ABSTRACT

Flavangenol, a pine bark extract rich in polyphenols, has been shown to exhibit antioxidative stress properties. Moreover, it is well known that increased oxidative stress is linked with mental stress. Therefore, we hypothesized that flavangenol may offer some beneficial effects to mice under stressful conditions. A high level of flavangenol (600 mg/ 10 ml/kg body weight) was administered daily to mice by gavage with or without unpredictable chronic mild stress (through daily exposure to different stressors) for 35 days. It was found that the stressors applied in this experiment greatly increased the amount of stress experienced by the mice, as evidenced by the reduction in body weight, in the distance traveled in the inner area in the open field test, in the concentration of brain-derived neurotrophic factor in the hippocampus, and by the increase in the concentration of norepinephrine (NE), epinephrine and dopamine (DA) in the plasma. However, the mice seemed not to be in a depressive state, since the result of the sucrose preference test was not significantly different from the result for the control. On the other hand, the administration of a chronically high level of flavangenol was needed to attenuate the stress, as shown by plasma corticosterone concentrations and the decrease in the plasma concentrations of DA and NE. In the non-stressed mice, an unpredicted effect of flavangenol was observed, whereby the distance traveled in the inner area in the open field test decreased. In conclusion, chronic effects resulting from a high level of flavangenol may depend on the particular state of stress, and taking flavangenol may be merited for animals suffering as a result of a stressor.

KEYWORDS: flavangenol, unpredictable chronic mild stress, mice, monoamine, anxiety, open field

INTRODUCTION

In modern society, there are several stressors that have detrimental effects on human life, and many people feel the effects of these stressors. For instance, Japanese workers spend long time on the work and feel stressed. This proportion gradually increased from 51% in 1982 to 63% in 1997 [1]. Under long-term stressful conditions, some people eventually become depressed.

Animal models of depression can be constructed by administering rodents with unpredictable chronic mild stressors (UCMS), and this method has been mostly used for assessing the pathophysiology of depression [2-4]. A recent study identified that, in mice, exposure to the UCMS not only resulted in elevated lipid peroxidation, but also led to the decreased activity of antioxidant enzymes [5].

Flavangenol is a pine bark extract which is obtained from the French maritime pine (*Pinus pinaster*) grown on the coast in the southwestern France;

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it contains oligomelic proanthocyanidin, which is a type of polyphenol, as the major component. Flavangenol exhibits antiphotoaging and anticarcinogenetic properties [6] and exerts an inhibitory effect on the accumulation of visceral fat [7]. Furthermore, flavangenol also exhibits antioxidant properties by scavenging reactive oxygen species such as $HO^{\bullet}$, $O_2^{\bullet -}$ [8], and administration of flavangenol significantly reduced the increase of serum lipid peroxide levels in B6.KOR-*Apoe*^{shl} mice, suggesting that it reduces oxidative stress [9].

Therefore, we hypothesized that flavangenol, through its antioxidative effects, may offer some benefits to mice under stressful conditions such as exposure to UCMS, as mental stress and oxidative stress strongly influence each other [10, 11]. Although the effects of flavangenol on oxidative stress have been reported, its effect on mental stress has not been investigated. The purpose of the present study was to reveal what kinds of effects resulted from the oral administration of flavangenol in mice treated with UCMS.

MATERIALS AND METHODS

Animals

Male ICR mice (3 weeks old) were purchased from Japan SLC (Hamamatsu, Japan) and maintained on a 12-h light/dark cycle (lights on at 8:00 a.m., lights off at 8:00 p.m.) at a room temperature of 22 ± 1 °C and humidity of 60%. Mice were kept in a group with free access to food and drinking water. This study was performed according to the guidelines for animal experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and Law No. 105 and Notification No. 6 of the government.

Drug and treatments

Flavangenol® (Toyo Shinyaku Co. Ltd., Saga, Japan) was dissolved in distilled water. Mice were allowed to acclimatize themselves to the housing conditions for one week before the beginning of the experiment. They were randomized into four groups (n =10 each): group 1 was free of any stressful conditions and administered with distilled water (10 ml/kg body weight); group 2 was subjected to UCMS for five consecutive weeks and administered with distilled water (10 ml/kg body weight); group 3 was free of any stressful conditions and administered with flavangenol (600 mg/10 ml/kg body weight); and group 4 was subjected to UCMS for five consecutive weeks and administered with flavangenol (600 mg/ 10 ml/kg body weight). The level of flavangenol was determined to be high with reference to previous studies. Kimura *et al.* [6] administered flavangenol orally at 60, 200 or 600 mg/kg body weight and Yoshida *et al.* [8] administered it at 200 or 600 mg/kg body weight. Distilled water and flavangenol were administered orally once daily (13:00) using a gastric tube, but they were not administered when the open field test was performed. As a result of accidental loss, the final numbers of animals were reduced to 9 in group 1 (one died during oral administration on day 25), 7 in group 2 (three died during restraint stress on day 16), 9 in group 3 (one died during oral administration on day 12), and 9 in group 4 (one died during restraint stress on day 16).

UCMS procedures

Stressors were administered once a day for 35 consecutive days - i.e. light/dark succession every 2-h, overnight illumination, 24-h 30° cage tilting, soiled cage (100 ml water in 100 g sawdust bedding), 1-h exposure to an empty cage, 2-h physical restraint (in a tube), forced swimming for 6 min, and 24-h social isolation. The schedule for this UCMS procedure is shown in table 1.

Experimental procedures

The sucrose preference test and the open field test were performed on the 22nd and 26th day, respectively. On the 36th day, all mice were decapitated under anesthesia with isoflurane (Escain®, Mylan, Osaka, Japan), and trunk blood was collected into tubes containing heparine. The brains were quickly removed and the hypothalamus and hippocampus dissected. The samples were frozen in liquid nitrogen, and stored at -80 °C until analysis took place.

Sucrose preference test

The sucrose preference test was performed to evaluate depression-like behavior. A decrease in sucrose consumption indicates anhedonia, a main feature in depression. Forty-eight hours before the test, mice were trained to adapt to drinking a 2% sucrose solution (w/v). Mice were housed in individual cages and one bottle of 2% sucrose solution was placed in each cage. Twenty four hours before the

Table 1. Schedule for unpredictable chronic mild stress (UCMS) procedure.

	Type of stress		Type of stress		Type of stress
Day 1	Light/Dark succession every 2-h	Day 13	Soiled cage	Day 25	Soiled cage
Day 2	1-h exposure to an empty cage	Day 14	24-h 30° cage tilting	Day 26	Overnight illumination
Day 3	24-h 30° cage tilting	Day 15	24-h social isolation	Day 27	24-h 30° cage tilting
Day 4	2-h Physically restraint	Day 16	2-h Physically restraint	Day 28	Forced swimming for 6 min
Day 5	Soiled cage	Day 17	24-h 30° cage tilting	Day 29	24-h social isolation
Day 6	Overnight illumination	Day 18	1-h exposure to an empty cage	Day 30	2-h Physically restraint
Day 7	24-h social isolation	Day 19	Soiled cage	Day 31	1-h exposure to an empty cage
Day 8	1-h exposure to an empty cage	Day 20	Overnight illumination	Day 32	Light/Dark succession every 2-h
Day 9	24-h social isolation	Day 21	Light/Dark succession every 2-h	Day 33	Overnight illumination
Day 10	Light/Dark succession every 2-h	Day 22	24-h social isolation	Day 34	24-h 30° cage tilting
Day 11	2-h Physically restraint	Day 23	24-h social isolation	Day 35	2-h Physically restraint
Day 12	Overnight illumination	Day 24	24-h social isolation		

test, the bottle was replaced with tap water. The sucrose preference test was conducted from 9:00 p.m. to 1:00 a.m. and from 2:00 a.m. to 6:00 a.m., during which periods the mice were divided into two groups randomly. Mice were permitted *ad libitum* access to sucrose solution (2%, w/v) and tap water. After the test, the consumed volumes of sucrose solution and tap water were recorded and sucrose preference was calculated by the following formula: sucrose preference (%) = sucrose consumption x 100/total liquid consumption.

Open field test

The open field test was performed to evaluate the motor activity, exploratory activity and anxiety-like behavior in a novel environment. Each mouse was transferred to the open field area from its home cage.

The open field was a circular arena (diameter = 60 cm and height = 35 cm), which was made of wood affixed with black paper. The arena was divided into an inner area and an outer area. Measurement began as soon as mice were placed in the center of the arena under 100 lux light. The motor activity of each mouse was observed for 5 min. The total distance traveled was the indicator of motor activity. Rearing (animal standing on its hind legs with the torso perpendicular to the floor and head pointing upwards, touching or not touching the walls of the open field with the forefeet) was the indicator of exploratory activity. The distance traveled in the inner area and the number of entries into the inner area were the indicators of anxiety-like behavior. Using these measurements, the ratio of distance traveled in the inner area (distance traveled in inner area/total distance traveled) was calculated. After each test, the open field area was cleaned with an ethanol-water solution to unify the conditions of each test. The total distance traveled, the ratio of travel distance in the inner area and the number of entries into the inner area were automatically analyzed by a computer-based video-tracking system (ANY-maze; Stoelting, Illinois, United States), and the elements of exploratory activity were scored by observation.

Biochemical analysis

Plasma corticosterone assay

Total corticosterone concentrations in the plasma were measured in duplicate using a corticosterone enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer's

protocol. Intra- and inter-assay coefficient of variations were 2.8% and 8%, respectively.

Analysis of brain-derived neurotrophic factor (BDNF)

The concentration of BDNF was analyzed by enzyme-linked immunosorbent assay (ELISA). The hippocampus was homogenized in 200 µl of lysis buffer (137 mM NaCl, 20 mM Tris, 1% nonidet P-40 (NP-40), 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/ml aprotinin, 1 µg/ml leupeptin, and 0.5 mM sodium orthovanadate) and then centrifuged at 2000 × g for 20 min at room temperature. To measure the amount of BDNF, the Promega BDNF Emax ImmunoAssay System (Promega Co., Madison, Wisconsin, USA) was employed according to the manufacturer's protocol. The concentration of BDNF was expressed as pg/mg of wet tissue.

Analysis of monoamine concentrations in the brain

Total dopamine (DA), norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxy-4-hydroxyphenyl-ethyleneglycol (MHPG), serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations were measured in the hypothalamus using high performance liquid chromatography (HPLC). Samples were homogenized, and then deproteinized in 0.2 M perchloric acid containing 120 µM disodium ethylenediaminetetraacetic acid (EDTA-2Na). Samples were protected from light. After being left for 30 min for deproteinization on ice in a dark box, samples were centrifuged at 20,000 × g for 15 min at 0 °C. After centrifugation, the supernatant was filtered by a syringe-driven filter unit (0.2 µm Millex-LG, Millipore, Massachusetts, USA) and then adjusted to approximately pH 3.0 by adding 1 M sodium acetate. Samples were protected from light during this procedure. The 30 µl of filtrate was applied to the HPLC system (Eicom, Kyoto, Japan) with a 150 × 3.0 mm octadecyl silane (ODS) column (SC-50DS, Eicom) and an electrochemical detector (ECD-300, Eicom) at an applied potential of 0.75 V versus an Ag/AgCl reference analytical electrode. Changes in electric current (nA) were recorded by a computer using an interface system (Power Chrom ver.2.3.3.j; AD Ins., Tokyo, Japan). The mobile phase consisted of 0.1 M acetate-citrate buffer, pH 3.5 (500 ml of 0.1 M sodium acetate and 450 ml of 0.1 M citrate buffer), 194.6 ml methanol, 1.95 ml sodium 1 octane sulfonate (100 mg/ml) and 1.14 ml EDTA-2Na (5 mg/ml) at a flow rate of 0.5 ml/min. The concentration of monoamines and metabolites was measured and their concentrations in the brain were calculated. A standard solution was serially diluted with 0.01 N HCl (standard concentration; 3000, 1500, 750, 375, 187.5 and 93.75 pg/30 µl). The concentration of monoamines was expressed as pg/mg wet tissue.

Analysis of monoamine concentrations in the plasma

NE, epinephrine (E) and DA concentrations were measured in plasma using HPLC. Plasma was diluted with Tris buffer (pH 8.6) and100 µl of EDTA-2Na and 5 mg of alumina were added to absorb NE, E and DA from the plasma. After removal of the liquid, the alumina was rinsed with ultrapure water and transferred to centrifuge-filtration units (0.22 µm Ultra Free-MC, Millipore, Massachusetts, USA). Alumina-absorbed NE, E and DA were separated by adding 2% acetic acid solution containing 100 µm EDTA-2Na, and then the solution was filtrated at 2,000 × g for 5 min at 4 °C. The 30 µl of filtrate was injected into an HPLC system (Eicom, Kyoto, Japan) with a 150 × 3.0 mm octadecyl silane (ODS) column (SC-50DS, Eicom) and an electrochemical detector (ECD-300, Eicom) at an applied potential of 0.45 V versus an Ag/AgCl reference analytical electrode. The mobile phase consisted of 0.1 M phosphoric buffer, pH 5.7 (2 l of 0.1 M sodium dihydrogen-phosphate and 170 ml of 0.1 M disodium hydrogen-phosphate), 296 ml methanol, 1.48 g sodium 1 octane sulfonate (600 mg/l), and 0.12 g EDTA-2Na (50 mg/l) at a flow rate of 0.5 ml/min. A standard solution was serially diluted with 0.01 N HCl (standard concentration; 3200, 1600, 800, 400, 200 and 50 pg/30 µl). The concentration of monoamines was expressed as pg/µl.

Statistical analysis

Body weight was analyzed by three-way repeated analysis of variance. The sucrose preference test, open field test, plasma corticosterone assay and analysis of BDNF and monoamine concentrations were analyzed by two-way ANOVA. When a significant interaction was detected, a *t*-test was applied in the

same treatment groups. Significance was set at $P < 0.05$. All analysis was performed with StatView (version 5, SAS Institute Cary, United States, SAS 1998). Outlying data were eliminated by Thompson's test criterion for outlying observations ($P < 0.05$).

RESULTS

Changes in body weight are shown in fig. 1. Body weight in the stressed group was significantly lower than that in the non-stressed group ($F (1, 30) = 5.174$, $P < 0.05$). The main effect of flavangenol on body weight was not significant. From day 0 to day 36, body weight significantly increased in all groups ($F (35, 1050) = 117.608$, $P < 0.0001$). Significant interactions between day and stress treatment ($F (35, 1050) = 1.609$, $P < 0.05$), and among day, stress treatment and flavangenol treatment ($F (35, 1050) = 1.569$, $P < 0.05$) were observed in relation to body weight. These results revealed that mice under UCMS showed lower body weight when they were treated with flavangenol during the course of the experiment, but this reduction in body weight ceased as the days went on.

The result of the sucrose preference test is shown in fig. 2. The decrease in sucrose consumption indicates anhedonia. However, neither stress nor flavangenol treatment altered sucrose preference. No significant interaction between stress and flavangenol treatment was observed in the sucrose preference test.

The result of the open field test is shown in fig. 3. Significant interactions between stress and flavangenol treatment were observed in relation to total distance traveled ($F (1, 27) = 7.545$, $P < 0.05$) and rearing ($F (1, 26) = 6.728$, $P < 0.05$), implying that both of these indicators increased in mice experiencing UCMS without flavangenol, compared with mice treated with neither stressful conditions nor flavangenol and mice treated with both stressful conditions and flavangenol. A significant ($F (1, 27) = 5.503$, $P < 0.05$) interaction between stress and flavangenol treatment was observed in terms of the ratio of distance traveled in the inner area, suggesting that this indicator was higher in mice experiencing neither stressful conditions nor flavangenol, compared with mice experiencing UCMS without flavangenol and mice administered with flavangenol without stressful conditions. In terms of the number of entries into the inner area, mice

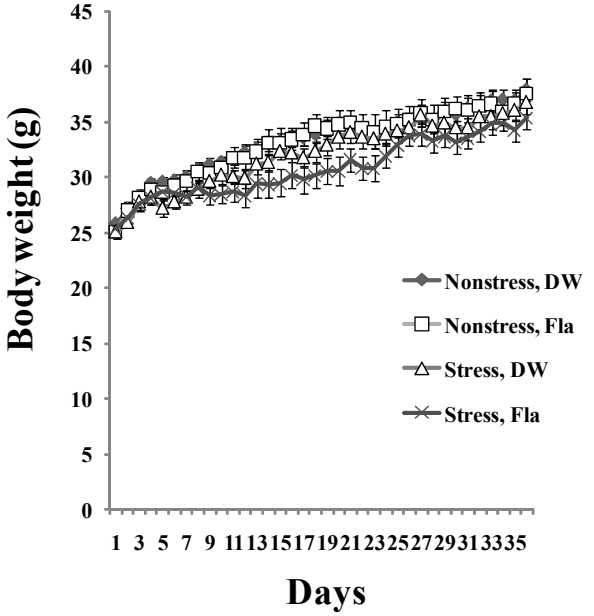

Fig. 1. Influence of flavangenol on body weight of mice experiencing unpredictable chronic mild stress. DW: Distilled water, Fla: Flavangenol. Stress: $p < 0.05$, Fla: $p > 0.05$, Stress × Fla: $p > 0.05$, Days: $p < 0.05$, Days × Stress: $p < 0.05$, Days × Fla: $p > 0.05$, Days × Stress × Fla: $p < 0.05$.

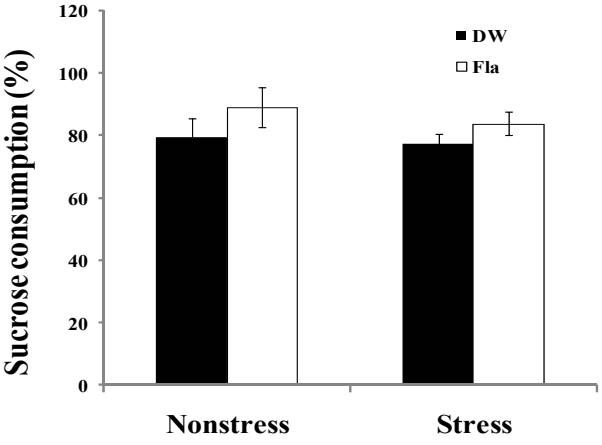

Fig. 2. Influence of flavangenol on sucrose consumption of mice experiencing unpredictable chronic mild stress. DW: Distilled water, Fla: Flavangenol. Stress: $p > 0.05$, Fla: $p > 0.05$, Stress × Fla: $p > 0.05$.

administered with flavangenol showed a significant ($F (1, 26) = 8.632$, $P < 0.01$) decrease compared with mice administered with distilled water. However, stress treatment did not significantly alter the number

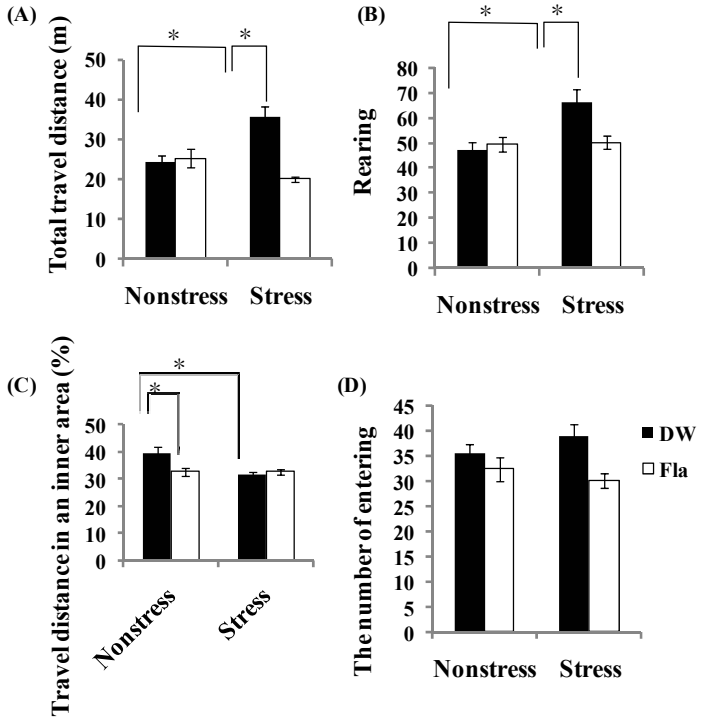

Fig. 3. Influence of flavangenol in the open field test of mice experiencing unpredictable chronic mild stress. In the open field test, we measured total distance traveled, rearing, the ratio of distance traveled in the inner area and the number of entries into the inner area. DW: Distilled water, Fla: Flavangenol. *$p < 0.05$. (A) Stress: $p < 0.05$, Fla: $p < 0.05$, Stress × Fla: $p < 0.05$. (B) Stress: $p < 0.05$, Fla: $p < 0.10$, Stress × Fla: $p < 0.05$. (C) Stress: $p < 0.05$, Fla: $p > 0.05$, Stress × Fla: $p < 0.05$. (D) Stress: $p > 0.05$, Fla: $p < 0.05$, Stress × Fla: $p > 0.05$.

of entries into the inner area, and no significant interaction between stress and flavangenol treatment was observed.

The result concerning total corticosterone concentrations in plasma is shown in fig. 4. In mice administered with flavangenol, corticosterone concentrations significantly decreased ($F (1, 27) = 8.383$, $P < 0.01$) compared with mice administered with distilled water. However, stress treatment did not significantly influence plasma corticosterone concentrations, and no significant interaction between stress and flavangenol treatment was observed.

The result concerning BDNF concentration is shown in fig. 5. No main effect of stressful conditions and flavangenol was detected. No significant interaction was observed, but it was nearly significant ($F (1, 29) = 3.274$, $P = 0.081$). In brief, in the distilled-water group, the BDNF concentration in the stressed group was lower than that in the non-stressed group, but the reverse tendency was observed in the flavangenol group.

The result concerning monoamine concentration in the brain is shown in fig. 6 and table 2. Stress and flavangenol treatment did not alter total NE, DOPAC, HVA and 5-HIAA concentrations. No significant interactions between stress and flavangenol treatments were observed in terms of total NE, DOPAC, HVA and 5-HIAA concentrations. Mice administered with flavangenol showed a significant decrease compared with mice administered with distilled water in terms of DA ($F (1, 25) = 7.345$, $P < 0.05$) and MHPG ($F (1, 25) = 5.269$, $P < 0.05$) concentrations. In terms of DA concentration, the non-stressed group showed a tendency for it to decline ($F (1, 25) = 3.584$, $P = 0.07$) compared with the stress group. However, stress treatment did not significantly alter the concentration, and no significant interactions between stress and flavangenol treatment were observed in DA and MHPG

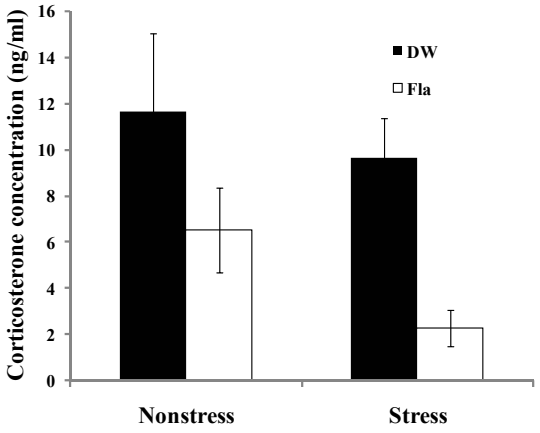

Fig. 4. Influence of flavangenol on corticosterone concentration of mice experiencing unpredictable chronic mild stress. DW: Distilled water, Fla: Flavangenol. Stress: $p > 0.05$, Fla: $p < 0.01$, Stress × Fla: $p > 0.05$.

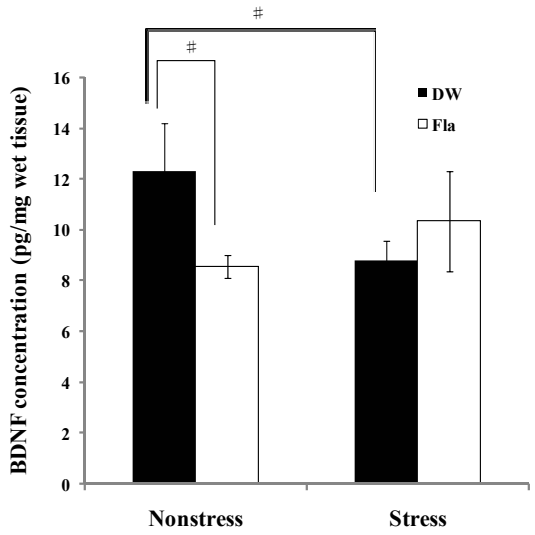

Fig. 5. Influence of flavangenol on BDNF concentration of mice experiencing unpredictable chronic mild stress. DW: Distilled water, Fla: Flavangenol. $^{\#}p < 0.10$. Stress: $p > 0.05$, Fla: $p > 0.05$, Stress × Fla: $p < 0.10$.

concentrations. In terms of 5-HT concentration, mice administered with flavangenol did not show a significant effect, but they showed a tendency for the concentration to decline ($F(1, 26) = 4.189$, $P = 0.0509$) compared with mice administered with distilled water. However, stress treatment did not significantly alter the concentration, and no significant interaction between stress and flavangenol treatment was observed in 5-HT concentration.

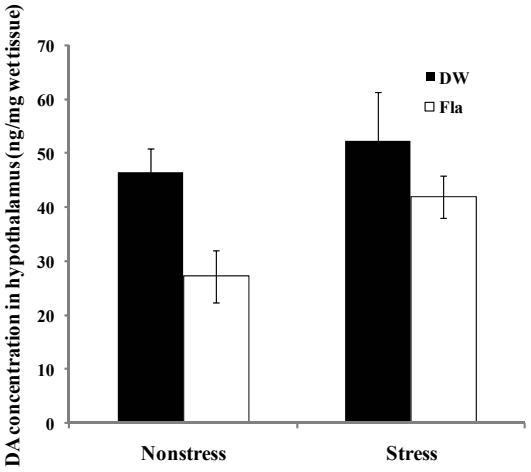

Fig. 6. Influence of flavangenol on DA concentration in hypothalamus of mice experiencing unpredictable chronic mild stress. DW: Distilled water, Fla: Flavangenol. Stress: $p < 0.10$, Fla: $p < 0.05$, Stress × Fla: $p > 0.05$.

The result concerning monoamine concentration in the plasma is shown in fig. 7. Significant interactions between stress and flavangenol treatment were observed in DA ($F(1, 25) = 6.164$, $P < 0.05$) and E ($F(1, 26) = 5.584$, $P < 0.05$) concentrations. In brief, in the distilled-water groups, the total DA and E concentrations in the stressed group were significantly higher than those in the non-stressed group. In the stressed groups, total DA concentration in the group administered with flavangenol was significantly lower than that in the group administered with distilled water. In terms of NE concentration, stress treatment induced a significant ($F(1, 29) = 8.650$, $P < 0.01$) increase. However, flavangenol treatment did not affect the concentration, and no significant interaction between stress and flavangenol treatment was observed in the total NE concentration.

DISCUSSION

The present study evaluated whether administration of a high level of flavangenol has any effects on mice treated with UCMS. It was considered that stressors administered in the present study led to stress in mice, as reductions in body weight, distance traveled in the inner area in the open field test and BDNF concentration were observed, together with increases in the concentration of NE, E and DA in plasma. In the open field test, reduction in distance traveled in the inner area represented anxiety.

Table 2. Effects of flavangenol on monoamine contents in the hypothalamus of mice under unpredictable chronic mild stress.

	Nonstress		Stress		Effects of ANOVA		
	DW	Fla	DW	Fla	Stress	Fla	Stress and Fla
5-HT	47683 ± 6134	26192 ± 5982	45908 ± 6410	38470 ± 8530	NS	$p = 0.0509$	NS
NE	153503 ± 13130	108856 ± 21801	157186 ± 28667	154653 ± 27845	NS	NS	NS
DOPAC	33727 ± 5070	28786 ± 2194	34757 ± 5056	27444 ± 3382	NS	NS	NS
5-HIAA	95758 ± 10424	85396 ± 8786	102846 ± 15795	94480 ± 12326	NS	NS	NS
HVA	19571 ± 1970	12574 ± 2216	21308 ± 3937	20321 ± 2986	NS	NS	NS
MHPG	247197 ± 25883	170324 ± 8726	246698 ± 40832	194379 ± 26234	NS	$p < 0.05$	NS

Values are means ± S.E.M in pg/mg wet tissue. NS indicates no significance.
DW: Distilled water, Fla: Flavangenol.
5-HT: serotonin, NE: norepinephrine, DOPAC: 3,4-dihydroxyphenylacetic acid, 5-HIAA: 5-hydroxyindoleacetic acid, HVA: homovanillic acid, and MHPG: 3-methoxy-4-hydroxyphenylethyleneglycol.

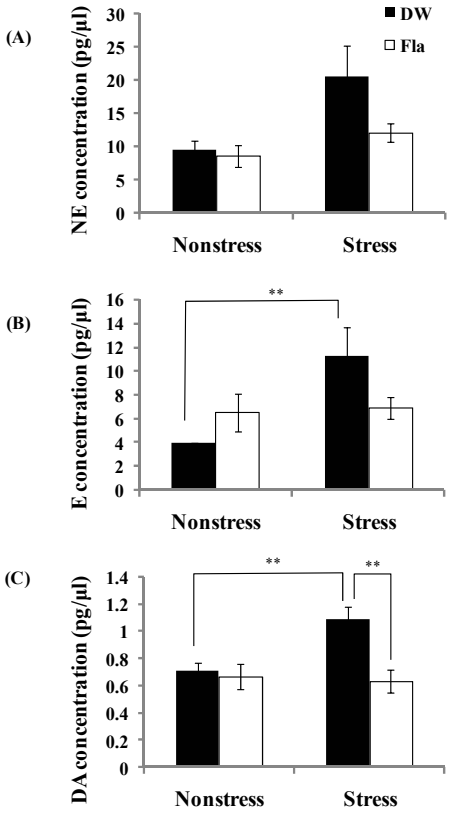

Fig. 7. Influence of flavangenol on monoamine concentrations in plasma of mice experiencing unpredictable chronic mild stress. This monoamine indicates NE, E, and DA. DW: Distilled water, Fla: Flavangenol. **$p < 0.01$.
(A) Stress: $p < 0.01$, Fla: $p < 0.10$, Stress × Fla: $p > 0.05$.
(B) Stress: $p < 0.05$, Fla: $p > 0.05$, Stress × Fla: $p < 0.05$.
(C) Stress: $p < 0.05$, Fla: $p < 0.01$, Stress × Fla: $p < 0.05$.

Moreover, the concentration of NE and E increased in rats treated with a combination of prenatal and postnatal behavioral stressors [12], and the concentration of DA in plasma increased in mice exposed to high-speed railway noise stress [13]. However, the mice were not in a depressive state according to the result of the sucrose preference test. In previous studies, UCMS induced a reduction in sucrose preference. One of the stressor procedures in that study consisted of food and water deprivation, stroboscopic illumination (150 flashes/min), white noise, light/dark succession every 2-h, overnight illumination, 45° cage tilt, soiled cage, and pair-housing [14]. In other procedure, the UCMS consisted of food and water deprivation for 24-h, 45° cage tilt for 24-h, shaker stress for 40 min, soiled cage, lights on overnight, tail pinch for 2-h, cold stress, heat stress, and intermittent white noise for 1-h [15]. Therefore, we suggest that the stressors used in the present study (Table 1) were mild compared with the previous studies, and not enough to lead to a depressive state.

In the present study, we presumed that flavangenol may have a beneficial effect on mice treated with UCMS. In terms of corticosterone concentrations, mice administered with flavangenol showed a significant decrease compared with mice administered with distilled water. Furthermore, in the stressed groups, mice administered with flavangenol showed a significant decrease in DA concentration in the plasma, and NE concentration in the plasma tended

to be lower compared with mice administered with distilled water. From these results, we suggest that flavangenol has a stress-relief effect. On the other hand, from the results of total distance traveled and rearing in the open field test, it was considered that mice were in a restless state through the application of UCMS but that their restless state was attenuated by the administration of flavangenol. In other words, from these results, we suggest that flavangenol may have a regulatory function for hyperactivity. Furthermore, according to Demet and Halaris [16], the levels of MHPG are a valid index of central NE activity and increase under stressed conditions. MHPG levels in the hypothalamus were decreased by flavangenol, which also implied that flavangenol attenuates the stress response under UCMS.

MHPG levels in the hypothalamus were decreased by flavangenol, under both UCMS and non-stressed conditions. According to this result, flavangenol attenuates NE activation and is beneficial for the subject before any stressors are received. On the other hand, flavangenol may not have beneficial effects where there are no stressful conditions. In the non-stressed groups, both the ratio of distance traveled in the inner area in the open field and the BDNF concentration in the group administered with flavangenol were significantly lower than those in the group administered with distilled water. Furthermore, it was observed that administration of flavangenol induced anxiety-like behavior in terms of number of entries into the inner area in the open field test. In previous studies, it was reported that there was a significant decrease in DA neuronal population in terms of activity in the ventral tegmental area for rats experiencing chronic mild stress [17], and that prenatal stress affected anxiety and led to the reduction of DA in the hypothalamus [18]. Therefore, we suggest that this induction of anxiety-like behavior is related to the reduction of DA concentration. Whatever the case, the flavangenol levels applied here were too high. This may be the reason for the unpredicted effects observed under non-stressed conditions.

CONCLUSION

In conclusion, administration of flavangenol has beneficial effects for stressed mice, and the effects of flavangenol may depend on the particular state of stress.

CONFLICT OF INTEREST STATEMENT

None to declare.

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