Antidepressant-Like Effects of Apigenin and 2,4,5-Trimethoxycinnamic Acid from *Perilla frutescens* in the Forced Swimming Test

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We studied the effects of apigenin and 2,4,5-trimethoxycinnamic acid (TMCA) on the behavioral despair test (forced swimming test), and the central noradrenergic, dopaminergic and serotonergic activities in mice. Apigenin at intraperitoneal doses of 12.5 and 25 mg/kg significantly decreased the duration of immobility in the forced swimming test in mice. At 100 mg/kg, the duration of immobility was returned to the control level in the test. On the other hand, TMCA treatment (25—200 mg/kg, i.p.) failed to significantly alter the duration of immobility. Based on the behavioral data, we examined changes in the monoamine turnover in mice having been subjected to forced swimming for 40 min. The monoamine turnover was measured in seven brain regions. Forced swimming exposure induced a significant decrease in dihydroxyphenylacetic acid (DOPAC)/dopamine (DA) in the striatum and amygdala and in 5-hydroxyindoleacetic acid (5-HIAA)/5-hydroxytriptamine (5-HT) in the hypothalamus, and a significant increase in DOPAC/DA in the thalamus and hypothalamus and in 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG)/norepinephrine (NE) in the amygdala, frontal cortex, hypothalamus, and midbrain. Apigenin (25 mg/kg) treatment produced attenuation of forced swim test-induced decrease of DA turnover in the amygdala and increase of DA turnover in the hypothalamus. Furthermore, intraperitoneal administration of haloperidol (0.2 mg/kg), a dopamine D_2 antagonist, blocked the apigenin (25 mg/kg)-induced decrease in immobility in the forced swimming test. These behavioral and biochemical results indicate the antidepressant properties of apigenin, which may be mediated by the dopaminergic mechanisms in the mouse brain.

Key words apigenin; forced swimming test; monoamine turnover; mouse

The leaves of *Perilla frutescens* BRITTON var. *acuta* KUDO (Labiatae) are found in Hangekoubokuto, Saibokuto, and other traditional Chinese herbal remedies which are primarily used to treat inflammatory diseases, clinical depression, and anxiety-related disorders such as anxiety neurosis and anxiety hysteria.[1—4] One of the goals in our laboratory is to characterize the bioactive compounds of *Perilla frutescens*. Although many components such as essential oils,[5,6] flavones,[7—9] and phenylpropanoids[10,11] have been identified in *Perilla frutescens*, to our knowledge, the bioactive compounds for the traditional use of the herbal medicine are still unidentified.

In most cases, traditional medicines are prepared by extraction with hot water and are orally administered so that the components present in the aqueous extract may be metabolized by gut flora before being absorbed into the body. Accordingly, to evaluate the bioactive compounds in the herbal medicines, investigation of the compounds actually absorbed into the body is necessary. In a preliminary work, we reported that apigenin and 2,4,5-trimethoxycinnamic acid (TMCA) (Fig. 1) were detected in the plasma and urine when an aqueous extract of *P. frutescens* was administered to humans.[12] Moreover, it has been reported that apigenin possesses a variety of pharmacological actions on the central nervous system, such as anxiolytic and sedative properties,[13—15] while to our knowledge, the effect of TMCA is not well characterized.

The purpose of the present study was to examine the effect of treatment with apigenin and TMCA on immobility in the forced swimming test, which has been a useful experimental method for screening antidepressant activity, as a wide range of antidepressants, including tricyclics, monoamine oxidase inhibitors, atypicals, by reducing the duration of immobility.[16,17] Moreover, in addition to the behavioral change in the forced swimming test, the levels of norepinephrine (NE), dopamine (DA), 5-hydroxytriptamine (5-HT), and their metabolites in the striatum, hippocampus, amygdala, frontal cortex, thalamus, hypothalamus, and midbrain were used as parameters for evaluating antidepressant activity of apigenin and TMCA, and the effect of dopaminergic blockade on the anti-immobility action of apigenin in the forced swimming test was also investigated.

MATERIALS AND METHODS

Animals Male ddY mice (Japan SLC, Inc.) weighting 26—30 g were used for all the experimental. Animals were housed 5 per cage in a temperature (22±2 °C), humidity (55±10%), and light (8:00—20:00) controlled room with free access to distilled water and commercial rodent chow (CE-2, Clea Japan Inc., Tokyo). All Experiments were carried out between 14:00 and 17:00 h and performed according to the Guide for Care and Use of Laboratory Animals at Tohoku Pharmaceutical University.

Drugs Apigenin (Funakoshi Co., Ltd., Tokyo, Japan), TMCA (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), and haloperidol (Wako Pure Chemical Industries, Ltd., Tokyo, Japan) were uniformly dispersed in 1% Tween 80 and then dissolved in distilled water. All drugs were administered in an injection volume of 4 ml/kg (i.p.); 1% Tween 80 alone

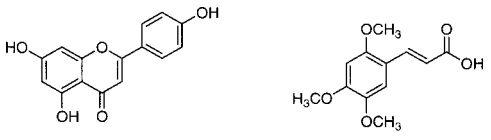

Fig. 1. Chemical Structures of Apigenin and TMCA (2,4,5-Trimethoxycinnamic Acid)

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was administered as a vehicle. The treatment schedule was chosen based on standard procedures described in the literature model.[18,19] The dose of apigenin was chosen without affecting the general motor activity that would allow us to evaluate whether the drug treatment increased or decreased the behavior.[13—15]

Forced Swimming Test The procedure was a modification of the method described by Porsolt *et al.*[16,17] Mice were placed in a glass aquarium (15 cm×12 cm diameter) containing 9 cm deep cold water (23±1 °C) for 15 min followed by a 5-min retest (test session) 24 h later. Immobility time was recorded during the test session: the mouse was considered immobile whenever it stopped swimming and remained floating in the water, with its head just above water level. Following the test the animals were dried and returned to their home cage. The vehicle or test drugs were administered 1h before a test session. Haloperidol (0.2 mg/kg, i.p.) was administered 10 min before apigenin (or vehicle) injection.

Determination of Neurotransmitter Concentrations One hour after the i.p. injection of the test drugs or vehicle, the mice were exposed to the swim stress procedure, and 40 min later they were sacrificed by decapitation. The control group (non-stress group) did not receive swimming exposure. After being sacrificed, the brain was rapidly removed and hippocampus, striatum, amygdala, frontal cortex, thalamus, hypothalamus, and midbrain were dissected on an ice-cold plate. All brain regions were homogenized by sonication in ice cold 0.1 M perchloric acid with an internal standard (Isoproterenol) at a concentration of 100 ng/ml. The homogenates were centrifuged at 10000×g, the supernatant was separated and filtered through a 0.45 μm pore size membrane filter. The filtrate was used for quantification of NE, 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG), DA, dihydroxyphenylacetic acid (DOPAC), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA) (Sigma, St Louis, MO, U.S.A.) by HPLC coupled with electrochemical detection. The HPLC system was comprised of a CCPM pump, an autosampler equipped with a cooling plate maintained at 4 °C, and CO-8010 column oven (Tosoh, Tokyo). Separation was achieved on a TSK gel ODS-100s (Tosoh, Tokyo, 250×4.6 mm i.d.). The mobile phase was 95% 50 mM sodium acetate, 10 mM citric acid, 0.15 mM EDTA, 0.45 mM SOS and 5% of acetonitrile adjusted to pH 3.6 with glacial acetic acid and filtered through 0.45 μm. The flow rate was 0.8 ml/min. Electrochemical detection was accomplished using an electrochemical detector (model EC-8020, Tosoh, Tokyo) with a glassy working electrode at a potential of +700 mV. The ratio of MHPG/NE, DOPAC/DA and HVA/DA, and 5-HIAA/5-HT were used as an index of the norepinephrine, dopamine, and serotonin turnover, respectively.

Statistics The data were analyzed using a one-way analysis of variance. If any statistically significant change was found, post-hoc comparisons were performed using Fisher's PLSD or Tukey–Kramer test. Data was deemed significant when $p<0.05$. Results are expressed as mean±S.E.M..

RESULTS

Forced Swimming Test Figure 2 shows the effect of apigenin alone on the duration of immobility after i.p. administration in the forced swimming test [$F(5, 84)=7.69$, $p<0.001$]. Post-hoc analysis revealed that apigenin produced a significant reduction in the immobility time at doses of 12.5 and 25 mg/kg in the forced swimming test. As the dose of apigenin increased from 50 to 100 mg/kg, the duration of immobility was returned to the control values. The effect of TMCA treatment at doses from 25 to 200 mg/kg did not reach statistical significance [$F(4, 72)=3.04$, $p>0.05$] under this condition (Fig. 3).

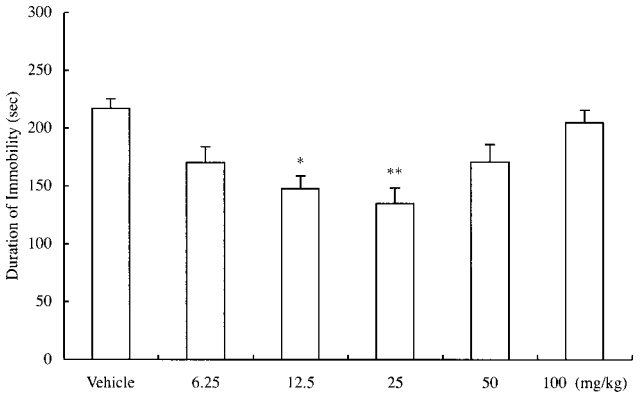

Fig. 2. Effect of Acute Apigenin Treatment on Immobility Time in the Mouse Forced Swimming Test

Data expressed as means with standard errors ($n=15$). *$p<0.05$, **$p<0.01$ *vs.* vehicle (Fisher's PLSD test).

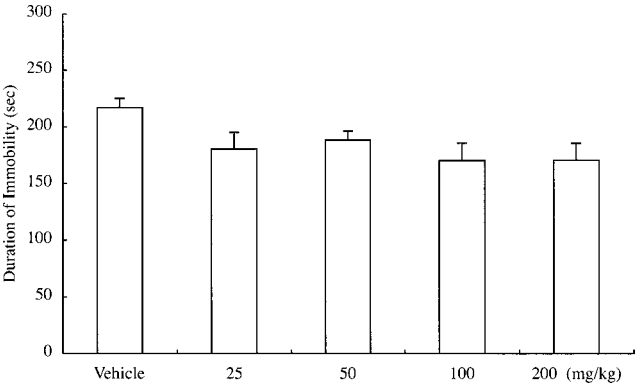

Fig. 3. Effect of Acute TMCA Treatment on Immobility Time in the Mouse Forced Swimming Test

Data expressed as means with standard errors ($n=15$).

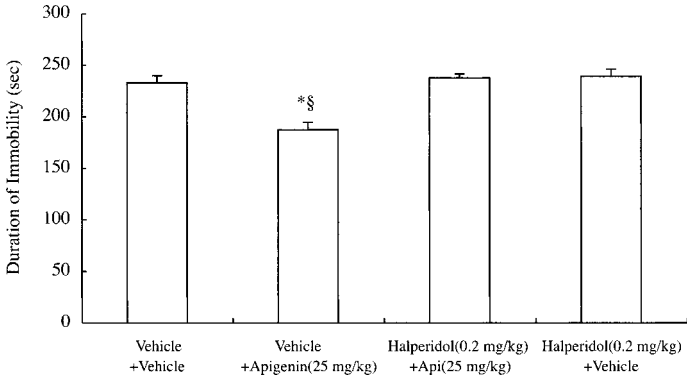

Fig. 4. Effects of Pretreatment of Haloperidol on the Apigenin-Induced Decrease in the Duration of Immobility in the Mouse Forced Swim Test

Data expressed as means with standard errors ($n=16$). *$p<0.01$ *vs.* vehicle+vehicle, §$p<0.01$ *vs.* haloperidol+vehicle (Tukey–Kramer test).

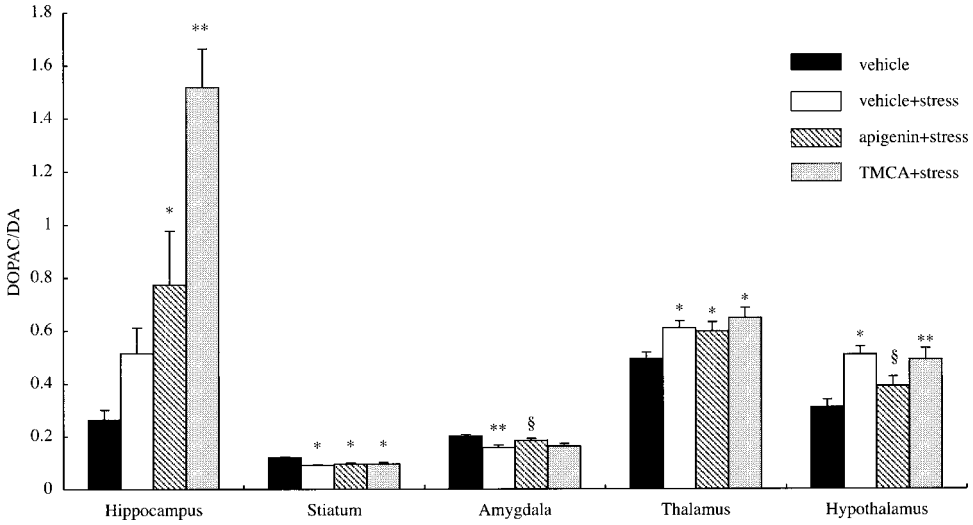

Fig. 5. Effects of Acute Apigenin and TMCA Treatment on Forced Swim Test-Induced Changes in the DOPAC/DA in Mouse Brain

Data expressed as means with standard errors (n=9—10). $*p<0.05$, $**p<0.01$ *vs*. vehicle, $\S p<0.05$ *vs*. vehicle+stress (Fisher's PLSD test).

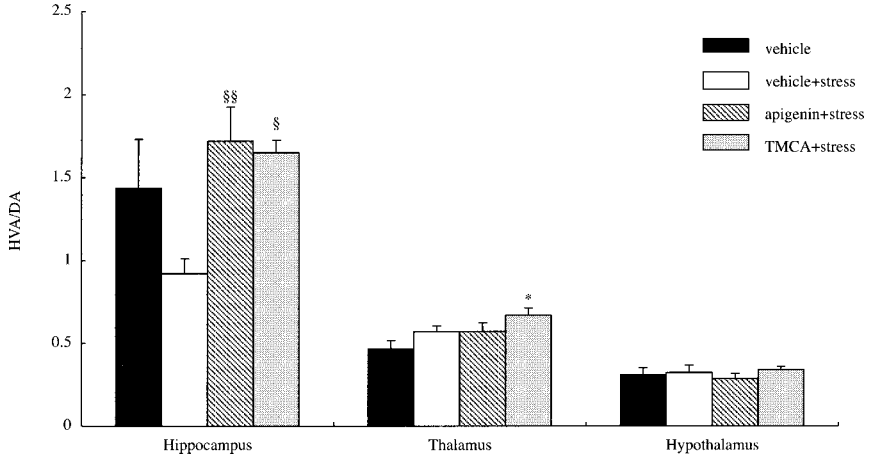

Fig. 6. Effects of Acute Apigenin and TMCA Treatment on Forced Swim Test-Induced Changes in the HVA/DA in Mouse Brain

Data expressed as means with standard errors (n=9—10). $*p<0.05$ *vs*. vehicle, $\S p<0.05$, $\S\S p<0.01$ *vs*. vehicle+stress (Fisher's PLSD test).

Effect of Haloperidol on Apigenin-Induced Decrease in Immobility Figure 4 shows the effects of apigenin alone (25 mg/kg) or combination with haloperidol (0.2 mg/kg) on immobility time in the forced swimming test. Drug treatments modified the immobility time [$F(3,60)=5.37$, $p<0.001$]. Post-hoc analysis revealed that the vehicle plus apigenin decreased the immobility time ($p<0.01$) whereas haloperidol plus the vehicle had no effect ($p>0.05$) on the behavior.

DA Turnover There was a significant difference in the DOPAC/DA ratio between 4 groups in the hippocampus [$F(3, 32)=13.80$, $p<0.0001$], striatum [$F(3, 34)=11.21$, $p<0.0001$], amygdala [$F(3, 33)=5.25$, $p<0.005$], thalamus [$F(3, 34)=4.71$, $p<0.01$], and hypothalamus [$F(3, 31)=7.54$, $p<0.001$] and in the HVA/DA ratio in the hippocampus [$F(3, 32)=3.66$, $p<0.05$], and thalamus [$F(3, 34)=3.20$, $p<0.05$] (Fig. 5, 6). Post-hoc analysis revealed that the forced swim test exposure produced a significant decrease in the DOPAC/DA ratios in the striatum ($p<0.0001$) and amygdala ($p<0.005$—0.001), and a significant increase in the DOPAC/DA ratios in the thalamus ($p<0.01$) and hypothalamus ($p<0.0005$). Apigenin treatment at the dose of 25 mg/kg, which significantly reduces immobility in the forced swimming test, increased these forced swim test-induced decreases in the amygdala DOPAC/DA ratio ($p<0.05$), and significantly attenuated the swim stress-induced increase in the hypothalamic DOPAC/DA ratio ($p<0.05$—0.01), but failed to significantly alter the swim stress-related decreases in the DOPAC/DA ratio in the striatum and increases in the thalamus. TMCA treatment at doses of 100 mg/kg did not reach statistical significance under this condition. These changes observed in the DA turnover were due to a combination of changes in DA, DOPAC and HVA (Table 1).

5-HT Turnover There was a significant difference in the 5-HIAA/5-HT ratio between 4 groups in the hypothalamus [$F(3, 31)=14.57$, $p<0.0001$]. Post-hoc analysis revealed that the forced swim test exposure produced a significant decrease in the 5-HIAA/5-HT ratio in their hypothalamus ($p<0.0001$) (Fig. 7). Apigenin and TMCA treatment failed to significantly alter the swim stress-related decreases in the

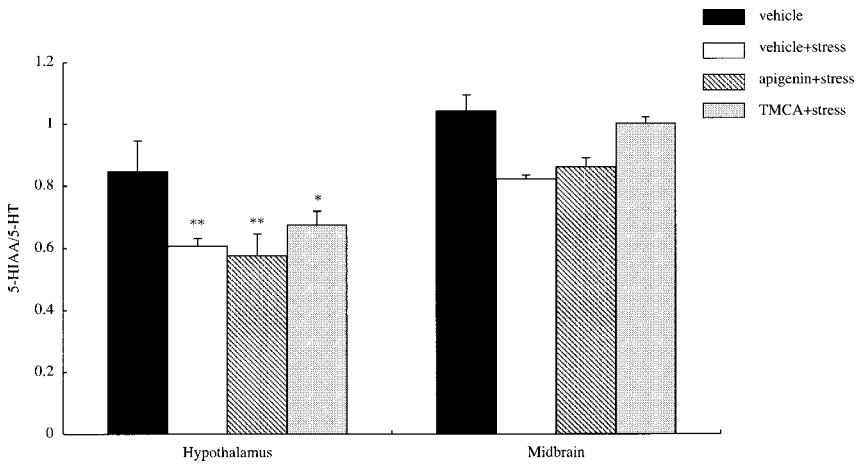

Fig. 7. Effects of Acute Apigenin and TMCA Treatment on Forced Swim Test-Induced Changes in the 5-HIAA/5-HT in Mouse Brain

Data expressed as means with standard errors (n=9—10). * $p<0.05$, ** $p<0.01$ *vs*. vehicle (Fisher's PLSD test).

Table 1. Effects of Forced Swim Test Exposure and Apigenin and TMCA Treatment on the DA, DOPAC, and HVA Concentrations in the Mouse Brain

Region	Group	DA	DOPAC	HVA	DA+DOPAC+HVA
Hippocampus	Vehicle	49.89±8.96	10.69±0.61	49.86±7.94	110.44±17.51
	Vehicle+stress	60.89±10.24	28.45±6.78	55.52±5.66	144.86±22.68
	Apigenin (25)	39.14±4.4	31.12±9.49[a)]	65.31±9.24	135.56±23.12
	TMCA (100)	50.29±5.02	72.41±6.53[b,d)]	82.45±8.92[b,c)]	205.15±20.47[b,d)]
Striatum	Vehicle	23257.25±1135.57	2783.36±169.3	1458.46±114.42	27499.07±1419.29
	Vehicle+stress	24742.5±705.7	2227.32±92.13[b)]	1430.42±73.44	28400.24±871.27
	Apigenin (25)	25510.33±1041.08	2431.36±139.02	1467.8±104.67	29409.49±1284.77
	TMCA (100)	26617.92±603.87	2571.17±143.31	1802.52±60.48[a,d)]	30991.61±807.67
Amygdala	Vehicle	1211.62±95.67	240.1±17.22	197.19±16.04	1648.9±128.93
	Vehicle+stress	2294.24±362.53[b)]	339.18±37.33[a)]	293.86±34.97[b)]	2927.27±434.84[b)]
	Apigenin (25)	1691.65±165.03	304.57±24.69	245.24±17.54	2241.46±207.26
	TMCA (100)	2242.99±247.13[b)]	344.86±22.60[b)]	312.6±12.53[b)]	2900.46±282.25[b)]
Thalamus	Vehicle	668.03±76.4	317.91±20.7	296.37±27.35	1282.31±124.44
	Vehicle+stress	876.9±125.27[b)]	532.52±77.41[b)]	472.62±46.59[b)]	1882.04±249.27
	Apigenin (25)	794.71±89.81[a)]	451.65±25.28[a)]	426.6±34.20[b)]	1672.96±149.29
	TMCA (100)	742.18±35.07[a)]	471.6±12.45[a)]	482.44±10.69[b)]	1696.23±58.21[b,d)]
Hypothalamus	Vehicle	999.88±77.42	302.94±30.11	279.6±36.02	1582.42±143.55
	Vehicle+stress	1215.89±93.56	602.24±31.81[b)]	385.64±51.85	2203.76±177.22[b)]
	Apigenin (25)	1265.92±69.83[a)]	518.9±39.45[b)]	357.72±39.21	2142.55±148.49[b)]
	TMCA (100)	1339.56±81.29[b)]	639.82±32.57[b)]	447.13±25.56[b)]	2426.52±139.42[b)]

Mice were sacrificed 40 min following forced swim test exposure. Concentrations are expressed as ng per g fresh weight of brain tissue. Data expressed as mean±S.E.M. (n=9—10). [a)] $p<0.05$; [b)] $p<0.01$ *vs*. vehicle. [c)] $p<0.05$; [d)] $p<0.01$ *vs*. vehicle+stress (Fisher's PLSD test).

Table 2. Effects of Forced Swim Test Exposure and Apigenin and TMCA Treatment on the 5-HT and 5-HIAA Concentrations in the Mouse Brain

Region	Group	5-HT	5-HIAA	5-HT+5-HIAA
Hippocampus	Vehicle	618.67±77.04	688.84±55.53	1307.51±132.56
	Vehicle+stress	379.19±57.47[a)]	448.21±65.73[b)]	827.4±123.19
	Apigenin (25)	404.84±63.68[a)]	456.44±58.33[a)]	861.28±122.01
	TMCA (100)	264.9±59.96[b)]	332.61±70.30[b)]	597.51±130.26
Hypothalamus	Vehicle	2213.72±288.95	1759.25±208.08	3972.97±497.03
	Vehicle+stress	2707.32±256.45	1625.86±137.17	4333.18±393.62
	Apigenin (25)	2264.06±69.44	1293.77±53.52	3557.83±122.96
	TMCA (100)	2100.11±105.6	1422.4±89.17	3522.51±194.77
Midbrain	Vehicle	1367.06±156.11	1416.65±181.48	2783.71±337.59
	Vehicle+stress	1648.15±169.31	1322.33±107.9	2970.47±277.21
	Apigenin (25)	1532.31±110.09	1300.4±128.19	2832.71±238.28
	TMCA (100)	722.39±76.96[b,d)]	711.62±68.88[b,d)]	1434.01±145.84[b,d)]

Data expressed as mean±S.E.M. (n=9—10). [a)] $p<0.05$; [b)] $p<0.01$ *vs*. vehicle. [c)] $p<0.05$; [d)] $p<0.01$ *vs*. vehicle+stress (Fisher's PLSD test). Mice were sacrificed 40 min following forced swim test exposure. Concentrations are expressed as ng per g fresh weight of brain tissue.

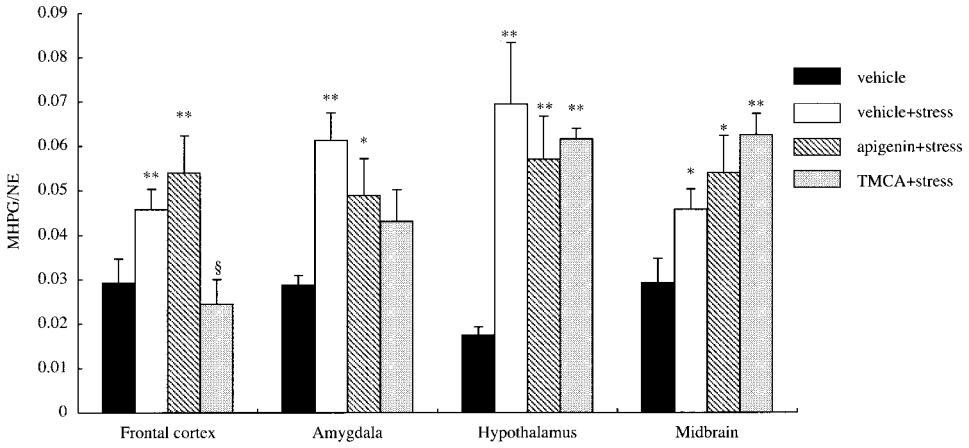

Fig. 8. Effects of Acute Apigenin and TMCA Treatment on Forced Swim Test-Induced Changes in the MHPG/NE in Mouse Brain

Data expressed as means with standard errors (n=9—10). $*p<0.05$, $**p<0.01$ *vs.* vehicle, $\S p<0.05$ *vs.* vehicle+stress (Fisher's PLSD test).

Table 3. Effects of Forced Swim Test Exposure and Apigenin and TMCA Treatment on the NE and MHPG Concentrations in the Mouse Brain

Region	Group	NE	MHPG	NE+MHPG
Hippocampus	Vehicle	705.65±27.56	10.8±0.54	716.44±28.1
	Vehicle+stress	619.44±70.23	21.41±2.00[a)]	640.85±72.23[b)]
	Apigenin (25)	672.9±40.51	21.16±3.52[a)]	694.06±44.02[a)]
	TMCA (100)	653.48±24.31	24.66±4.97[b)]	678.14±29.28[b)]
Frontal cortex	Vehicle	596.53±28.59	17.58±3.04	614.11±31.64
	Vehicle+stress	601.7±28.94	26.86±2.34[a)]	628.55±31.28
	Apigenin (25)	584.28±34.93	29.94±3.24[b)]	614.22±38.17
	TMCA (100)	559.22±23.95	13.7±3.05[d)]	572.92±27
Amygdala	Vehicle	595.42±25.95	16.95±1.27	612.37±27.22
	Vehicle+stress	647.72±63.41	40.04±5.72[b)]	687.76±69.13
	Apigenin (25)	527.56±33.25	27.66±4.67	555.22±37.92
	TMCA (100)	519.75±15.79	22.97±4.33[d)]	542.72±20.12
Hypothalamus	Vehicle	2094.76±94.22	37.09±3.31	2131.85±97.53
	Vehicle+stress	2190.42±70.7	160.82±36.43[d)]	2351.23±107.13
	Apigenin (25)	1860±84.61[a,d)]	102.73±15.27[a)]	1962.73±99.88[d)]
	TMCA (100)	1762.18±59.00[b,d)]	108.99±5.27[a)]	1871.17±65.26[a,d)]
Midbrain	Vehicle	771.88±34.67	24.05±2.88	771.9±37.55
	Vehicle+stress	766.75±22.67	40.79±6.24[a)]	807.54±28.91[a,c)]
	Apigenin (25)	633.91±42.73[b,c)]	35.39±5.51	669.3±48.25
	TMCA (100)	725.36±41.84	44.24±2.16[b)]	769.59±44

Mice were sacrificed 40 min following forced swim test exposure. Concentrations are expressed as ng per g fresh weight of brain tissue. Data expressed as mean±S.E.M. (n=9—10). [a)] $p<0.05$; [b)] $p<0.01$ *vs.* vehicle. [c)] $p<0.05$; [d)] $p<0.01$ *vs.* vehicle+stress (Fisher's PLSD test).

5-HIAA/5-HT ratio in the hypothalamus. These swim stress-related changes in the 5-HT turnover were due to a combination of changes in 5-HT and 5-HIAA (Table 2).

NE Turnover There was a significant difference in the MHPG/NE ratio between 4 groups in the amygdala [$F(3, 33)=4.13$, $p<0.05$], frontal cortex [$F(3, 34)=5.10$, $p<0.01$], hypothalamus [$F(3, 31)=6.24$, $p<0.005$], and midbrain [$F(3, 34)=4.80$, $p<0.01$]. Post-hoc analysis revealed that the forced swim test exposure produced a significant increase in the MHPG/NE ratio in the amygdala ($p<0.01$), frontal cortex ($p<0.05$), hypothalamus ($p<0.01$), and midbrain ($p<0.05$) (Fig. 8). TMCA treatment protected the forced swim test-induced increase in the MHPG/NE ratio in the frontal cortex ($p<0.05$). Apigenin treatment failed to significantly alter the swim stress-related decreases in the MHPG/NE ratio. These swim stress-related changes in the NE turnover were due to alterations in the concentrations of the MHPG as opposed to no change in the concentrations of the parent amine (Table 3).

DISCUSSION

In this study, we examined the effects of apigenin and TMCA on the behavioral despair test in mice. Apigenin at intraperitoneal doses of 12.5 and 25 mg/kg significantly decreased the duration of immobility in the test in mice, whereas at higher concentrations (100 mg/kg), the duration of immobility was returned to the control level in the test. The reason for the U-shaped dose responses is obscure. These results suggest that apigenin may have an antidepressant-like activity in a certain dose range. However, it is also possible that the ability of apigenin to decrease the immobility in the forced swimming test may be simply due to the increased spontaneous motor activity such as psychostimulants, but Viola *et al*. and Zanoli *et al*. reported that at least apigenin doses up to 100 mg/kg did not increase the spontaneous motor activity.[13,15)] Thus, the apigenin induced decrease in the immobility seems not to be mediated by stimulation of the overall motor activity of the animals. On the

other hand, TMCA treatment (25—200 mg/kg, i.p.) failed to significantly alter the duration of immobility.

There are substantial reports to indicate that swim-stress for 30—40 min induced alterations in the levels of monoamines and its metabolites in different functional regions of the rat and mouse brain. Clinically used antidepressant drugs are effective in blocking the stress induced the changes.[20—26] The data presented in this study also demonstrated that forced swim stress for 40 min increased in MHPG levels in the hippocampus, frontal cortex, hypothalamus, and midbrain, indicating enhanced NE turnover. However, the sum of their amounts was not changed, except in the hippocampus, suggesting no remarkable change in the NE biosynthesis. These results support the view that under an acute uncontrollable stress, the enhanced release of NE may increase its catabolism more than its synthesis, which results in a reduction in the NE concentration.[27] The DA and HVA levels increased in the amygdala and thalamus, whereas DOPAC increased in the amygdala, thalamus, and hypothalamus, but decreased in the striatum. The DOPAC/DA ratio, reflecting the DA turnover, decreased in the striatum and amygdala and increased in the thalamus and hypothalamus, while HVA/DA did not change. The sum of their amounts, an index of the DA synthesis, was enhanced in the amygdala, thalamus and hypothalamus. These data suggest that in the DA system, forced swimming affected both the synthesis and turnover. The synthesis of DA increased by stress is considered to be due to an increase in the activity of the rate-limiting enzyme, tyrosine hydroxylase. In the mouse brain, tyrosine hydroxylase activity was reported to be enhanced *in vivo* and *in vitro* by acute immobilization stress.[28] On the other hand, the 5-HT and 5-HIAA levels and the synthesis did not change except in the hippocampus. The turnover decreased in the hippocampus.

Miura *et al*. also demonstrated that swim stress increased the NE turnover without changing the NE biosynthesis in all the mouse brain regions, while it decreased the 5-HT turnover in the thalamus-hypothalamus.[22] The HVA/DA ratio did not change in the striatum, while the sum of their amounts was enhanced in thalamus-hypothalamus. This report seems consistent with our present data. However, there are discrepancies in reporting the swim stress-induced neurochemical changes in different regions of the brain. Connor *et al*. reported that DA turnover in the striatum and 5-HT turnover in the frontal cortex, amygdala and hippocampus without affecting the amines and the metabolites due to swim-stress in rats.[23] Other studies found reduced striatal DA content and significant changes in the 5-HT levels in the frontal cortex and hypothalamus in the rat brain using *in vivo* microdialysis following swim-stress for 30 min.[24,25] Thus, the variations in the stress induced neurochemical changes depend on the experimental conditions adopted by different experimenters. As Kirby *et al*. pointed out, alterations induced by stressors depend on the intensity of the stress induced and the techniques used for the assay of the neurotransmitters.[26]

The data presented in this study also demonstrated that apigenin treatment produced attenuation of the swim stress-induced decrease in the DA turnover in the amygdala and increase in the DA turnover in the hypothalamus, while the metabolic turnover of NE and 5-HT did not show any significant differences between the apigenin treated and swim-stress groups in all regions assayed. TMCA treatment protected swim-stress induced increase of NE turnover in the frontal cortex, and tended to increase the swim-stress related decreases in the 5-HT turnover in the hypothalamus and midbrain, although this effect did not reach statistical significance. Apigenin and TMCA provoked a robust "stress like" increase in the basal DA turnover in the hippocampus. This increase in the hippocampal DA turnover may account for the inability of the compound to attenuate the forced swim test-induced increase in the DA turnover in this brain region. The amygdala has been implicated as a key site of antidepressant action in the forced swim test, inasmuch as direct injection of imipramine and pargyline into particular amygdaloid nuclei, but not other brain structures, produces behavioral responses similar to i.p. injections of these drugs.[29] Connor *et al*. also reported that amygdala was particularly sensitive to swim stress exposure.[23] Our data showed that the acute apigenin treatment is effective in preventing the swim-stress induced alterations of the dopaminergic system. It also indicates that the specificity of apigenin is more pronounced towards amygdala and hypothalamus. On the other hand, TMCA is more specific towards the frontal cortex in NE system and the hypothalamus and midbrain in 5-HT system. However, it is unlikely that this effect of TMCA on these systems mediates its behavioral effects in the forced swim test.

One interesting possibility is that the effect of apigenin may be mediated by central Bz receptors. Avallone *et al*. demonstrated that apigenin was able to reduce the GABA-activated chloride currents in electrophysiological experiments.[14] The chloride channel blocker, picrotoxin, at subconvulsant doses, enhanced the anti-immobility effects in the 'forced swimming' test with rats whereas open field activity remained unaffected or was even decreased by the same treatments.[30,31] Furthermore, picrotoxin treatment potentiated the anti-immobility effects of some classical antidepressants, imipramine and desipramine.[32] Thus, it is reasonable to suggest that the decreased GABA-activated Cl^- currents, which occurs upon apigenin treatment, would result in reduced immobility in the forced swimming test. However, if apigenin decreased the GABA-activated Cl^- currents, apigenin should exhibit an anxiogenic effect, but Viola *et al*. and Salgueiro *et al*. reported that apigenin exhibits anxiolytic activity in mice without any sedative or myorelaxant effects.[13,33] Thus, there are discrepancies in reporting the ability of apigenin to GABA activity.

Another possible explanation may involve the action of apigenin in the dopaminergic systems implicated with anti-immobility behavior. Lorenzo *et al*. indicated that apigenin inhibited the activity of MAO, the major catabolic enzyme of monoamines.[34] In fact, apigenin tended to attenuate the swimming stress induced increase of the DOPAC level in the amygdala, thalamus, and hypothalamus, although these effects did not reach statistical significance. Furthermore, the treatment of the D_2 receptor antagonist, haloperidol, before the administration of apigenin, blocked the apigenin-induced decrease in immobility in the forced swimming test, while the same dose of haloperidol, *per se*, did not affect the duration of immobility. These results suggest that the effect of apigenin on behavioral changes elicited by forced swimming may be related to dopaminergic neurotransmission. Thus, the

dopamine hypothesis is simpler and more attractive, although we can not rule out the possibility that apigenin acts on the GABA neurotransmitter systems as described above and the reports that GABA could be an essential modulator of the dopamine related function.[35] Moreover, considering that the pharmacodynamic interaction between apigenin and haloperidol is based on behavioral data, additional studies are necessary to confirm it at the neurochemical level.

In summary, the results of the present study such as (1) the attenuation of the forced swimming-induced immobility, (2) protection of the stressor-induced changes in dopamine turnover by apigenin treatment, and (3) the attenuation of apigenin-induced decrease in the immobility by haloperidol treatment suggest that apigenin has an antidepressant-like effect, which may be related by the dopaminergic mechanisms. Thus, we can surmise that the antidepressant effect of *Perilla frutescens* can be ascribed to apigenin, one of the human metabolites after the administration of the extract.[12] This report could be of interest in the study of the potential therapeutic use of this plant.

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