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Improvement of Memory by Dieckol and Phlorofucofuroeckol in Ethanol-Treated Mice: Possible Involvement of the Inhibition of Acetylcholinesterase

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Phlorotannins, the polyphonic compounds found in brown *Eisenia* and *Ecklonia* algae, have several pharmacologically beneficial effects such as anti-inflammation. In addition, our recent data show that these compounds may improve the cognitive functions of aged humans suggesting the potential ability to enhance memory in several neurodegenerative disorders. To examine the experimental hypothesis that two effective components of *Ecklonia cava*, dieckol and phlorofucofuroeckol (PFF), have memory-enhancing abilities, both were administered orally to mice before a passive avoidance test. The repeated administration of either dieckol or PFF dose-dependently reduced the inhibition of latency by the administration of ethanol. To investigate the mode of memory-enhancing actions, the levels of major central neurotransmitters in three different regions (striatum, hippocampus, and frontal cortex) of the mouse brain were measured. The levels of some of the neurotransmitters were significantly changed by ethanol. Both dieckol and PFF altered the levels of some neurotransmitters modified by the ethanol treatment. It is noteworthy that both dieckol and PFF increased the level of acetylcholine, and they exerted anticholinesterase activities. Overall, the memory-enhancing abilities of dieckol and PFF may result from, at least in part, the increment of the brain level of acetylcholine by inhibiting acetylcholinesterase.

Key words: Learning and memory, Dieckol, Phlorofucofuroeckol, Passive avoidance test, Central neurotransmitters, Acetylcholinesterase

INTRODUCTION

Learning is the acquisition of new information or knowledge and memory is the retention of learned information. The regions of the brain implicated in memory include the temporal lobes for declarative memory such as facts and events, the striatum for procedural memory such as skills, habits, and behaviors, and the neocortex for working memory. However, no single brain structure or cellular mechanism accounts for all learning and memory.

Fear-motivated avoidance tests are usually based on electric currents as the source of punishment (Myhrer, 2003). Avoidance tests are classified as passive avoidance and active avoidance. In passive avoidance, the animal has to refrain from executing a previously executed response, and the latency to refrain from performing the punished act expresses the ability to avoid (Myhrer, 2003). The effects of neurotransmitters on passive avoidance behavior following neurochemical changes have been studied extensively using agents that affect the glutamatergic and cholinergic systems. In the case of acetylcholine, muscarinic receptor antagonists result in the impairment of behavioral performance (Piercey *et al.*, 1987; Hiramatsu *et al.*, 1998), suggesting that acetylcholine is one of the major neurotransmitters playing a key role in regulating learning and memory in passive avoidance tests.

Brown algae, mostly used as sources for alginic acid in industry, are also widely consumed as food in many Asian and several European countries. Brown algae have two major chemical groups: polysaccharide and polyphenols. Polysaccharide components include alginates, laminarin

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and fucoidans, and their beneficial effects on health have been reported (Sakai and Kato, 1998, 2001). Among several categories of algal polyphenols, phlorotannin is pharmacologically prominent. The polyphenolic compounds are characterized by dibenzo-1,4-dioxin unit in the molecular skeleton (Glombitza and Gerstberger, 1985), which are found only in some specific algae such as *Eisenia* and *Ecklonia* species. Several studies using phlorotannins have been performed to examine their pharmacological actions including: the α_2-antiplasmin activity (Fukuyama *et al.*, 1989, 1990), antioxidant activity (Nakamura *et al.*, 1996; Kang *et al.*, 2003), anti-inflammatory activity by inhibiting hyaluronidase (Shibata *et al.*, 2002), or secretory phospholipase A_2, cyclooxygenases, and lipoxygenases (Shibata *et al.*, 2003). The observations from these studies support the potential use of phlortannins in the treatment of related diseases such as inflammation. However, the medicinal applications of polyphenolic compounds have not been carefully examined.

There are several episodic findings on improvements in cognitive functions of aged people by chronic ingestion of an extract containing phlorotannin. These observations provide a compelling reason to examine the potential use of these compounds for the treatment of neurodegenerative disorders that are characterized by an impairment of memory and learning functions due to the destruction of neuronal cells. Thus, the present study was designed to test whether two effective components of *Ecklonia cava* have the ability to improve learning and memory in ethanol-treated mice using a passive avoidance test, and to examine the possible mechanism of these beneficial actions by measuring the levels of major central neurotransmitters in three different parts of the brain: the striatum, hippocampus and cortex.

MATERIALS AND METHODS

Reagents and instruments

Thin-layer chromatography was performed on Merck silica gel GF-254 and an RP-18F-254 precoated plate. Identification was done with UV light and typical TLC indicating solution (*p*-anisaldehyde/sulfuric acid/acetic acid mixture). Flash column chromatography was performed with Merck silica gel (230-400 mesh). UV spectra (in ethanol) were recorded on an HP 8453 UV spectrophotometer (Hewlett Packard, U.S.A.) and IR spectra were measured on a Bomen MB-100 FT-IR spectrometer (Bomen Inc., Canada). The NMR spectra were recorded on a JEOL ECP-500 FT NMR (Jeol, Japan) at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using TMS as an internal standard. FABMS was obtained with a VG Autospec Ultma mass spectrometer (Micromass Co., England). The analysis of neurotransmitters from brain tissues was performed on an HPLC system consisted of an SCL-10A system controller, an LC-10AD pump, a CTO-10AS column temperature controller (Shimadzu, Japan), and a 1049A Programmable Electrochemical Detector (Hewlett Packard, U.S.A.). The Passive/Active avoidance system, PACS-30 (Columbus Co., OH, U.S.A.), was used for the passive avoidance test. The chemicals γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT) creatinine sulfate, 3,4-dihydroxyphenyl acetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (5-HIAA), choline, and acetylcholine chloride were purchased from Sigma Chemical Co. (MI, U.S.A.). Dopamine was from TCI Co. (Japan). Homovanillic acid (HVA) and norepinephrine hydrochloride were from Aldrich Co. (MI, U.S.A.). L-Glutamic acid was obtained from Junsei Co. (Japan). All solvents used in this work were of HPLC grade or of analytical grade. All reagents used in this study were used without further purification.

Pretreatment of algae material

Ecklonia cava was freshly collected off the coast of Jeju Island, Korea. A voucher specimen (SS-172) was deposited in the Laboratory of Aging and Degenerative Diseases, Hanbat National University, Daejeon, Korea. The whole seaweed freshly collected was washed with tap water immediately after collection, air-dried at room temperature in a darkened room until water content of 15-25% was reached. The dried algae were cut into small pieces and kept at -40°C until use.

Extraction and isolation

The seaweed (1 kg) was extracted with 95% ethanol (10 L) for 2 h in a water bath at 50°C. The solvent was evaporated *in vaccuo* to give a gummy extract, which was partitioned between ethyl acetate and water. The ethyl acetate soluble portion was subjected to reversed phase (C_{18}) column chromatography followed by gel filtration on Sephadex LH-20 with methanol. The final purification of individual compounds was accomplished by HPLC on a Spherisorb S10 ODS2 column (20×250 mm, Waters, U.S.A.) with 30% methanol at the flow rate of 3.5 mL/min to give eight compounds. By means of ^{1}H-NMR spectra, both dieckol and phlorofucofuroeckol (PFF) were identified as follows:

Dieckol

A white solid; UV(ethanol) λ_{max} 225 nm; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ5.71 (d, J=2.40, 2H), 5.79 (t, J=1.8, 1H), 5.81 (d, J=1.8, 1H), 5.93 (s, 1H), 5.97 (d, J=3.0, 1H), 6.01 (d, J=3.0, 1H), 6.13 (s, 1H), 6.15 (s, 1H), 9.17 (s, OH), 9.24s, OH), 9.25s, OH), 9.29(s, OH), 9.37s, OH), 9.45s, OH), 9.5s, OH), 9.62s, OH),, 9.70s, OH); ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 93.6, 93.7, 94.1, 94.5, 96.2, 98.1, 98.2, 98.4, 98.5, 146.0, 146.1, 122.2, 122.3, 122.6, 123.2,

123.3,124.0, 124.2, 137.1, 137.2, 141.9, 142.0, 142.6, 142.9 145.9, 146.1, 151.2, 153.1, 154.2, 155.9, 158.8, 160.3; FABMS m/z 743.1 $(M+H)^+$.

PFF

A white solid; UV(ethanol) λ_{max} 227 nm; ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 5.73 (d. J=1.85, 1H), 5.76 (d, J=1.85, 1H), 5.83 (t, J=1.85, 1H), 5.84 (t, J=1.85, 1H), 6.29 (s, 1H), 6.43 (s, 1H), 6.72 (s, 1H), 8.22 (s,OH), 9.19 (s, OH), 9.22 (s, OH), 9.45 (s, OH), 9.86 (s,OH), 9.88 (s, OH), 10.15 (s, OH) ; ^{13}C-NMR (DMSO-d_6, 125 MHz) δ 94.3, 94.0, 95.3, 96.9, 97.0, 98.8, 99.6, 103.8, 103.9, 120.6,123.0, 123.1, 126.9, 134.5, 137.4, 142.5, 145.3, 147.0, 147.5, 150.0, 150.9, 151.4, 159.4, 159.5, 160.5, 160.8; FABMS m/z 603.1 $(M+H)^+$.

These components of *Ecklonia cava* were identical to those described previously (Fukuyama *et al.*, 1989, 1990). Fig. 1 shows their chemical structures.

Animals and dosing regimens

Male ICR mice (weighing 18-20 g each) were purchased from Daehan Biolink Co. (Eumsung, Korea). They were housed in a ventilated animal room at 24±1°C with 55±5% relative humidity and controlled lighting (from 6:00 to 18:00 daily). The animals had free access to standard food and water. The compounds were suspended in 5% Tween-80. For the memory tests, PFF (0.2 and 2 mg/kg) and dieckol (1 and 10 mg/kg) were administered orally once a day for 7 days at a volume of 1 mL/100 g of body weight. Ethanol was given to the mice in a single oral administration at a dose of 3 g/kg body weight as a 38 v/v% solution in water. For the analysis of neurotransmitters, higher doses of PFF (2 mg/kg) and dieckol (10 mg/kg) were administered. All procedures were conducted in accordance with the Guide for National Institutes of Health, the Guide for the Care and Use of Laboratory Animals as approved by the Chungnam National University Animal Care and Use Committee.

Passive avoidance test

Memory tests were carried out by the method previously published (Lee *et al.*, 1999). The apparatus consisted of two equal compartments (15 × 15 × 22 cm) separated by a wall with a guillotine door (4 × 3.5 cm) in the lower middle part. One of the two compartments was illuminated and the other was dark. The animals were placed in a dark room 1 h before trials began. The tests were conducted for two consecutive days at the same time of the day. On the first day, each mouse was placed in the illuminated compartment. After 30 s, the guillotine door was raised, allowing access to the dark compartment. Once the mouse entered the dark compartment, the guillotine door was lowered and the mouse received an electric shock (0.6 mA for 5 s). On the second day, each testing trial was carried out with the same test procedure of the learning trial. Each mouse was again placed in the illuminated compartment, the guillotine door was opened and the step through latency was measured. An upper cut off time was set, allowing mice to be exposed in the light compartment for 300 s. The latency and the number of mice that did not enter the dark compartment were recorded in the testing trials.

Brain sample preparation for neurotransmitter analysis

The animals were sacrificed 1 h after the ethanol treat-

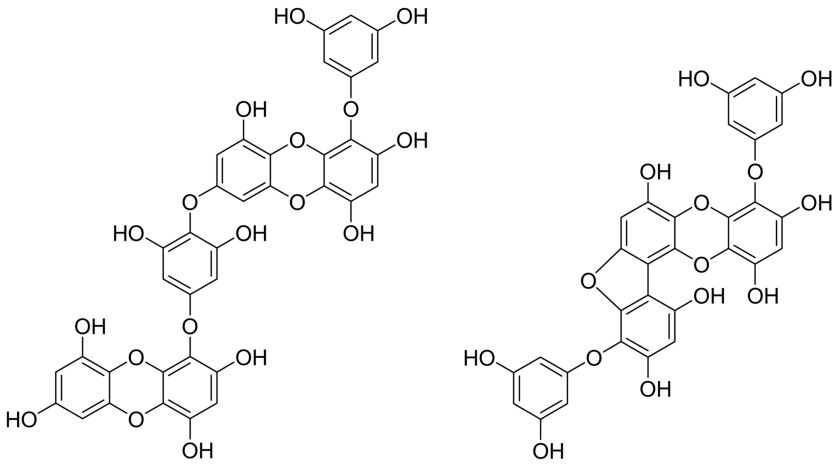

Fig. 1. Chemical structures of dieckol and phlorofucofuroeckol (PFF). By means of ^{1}H-NMR spectra and comparisons with published data, both dieckol and PFF were identified as shown in "Materials and Methods".

ment by exposure to microwave irradiation for 1.4 s at the power of 4.5 kW of 2450 MHz on a Microwave irradiator (TMW-6402C, Muromachi Co., Japan), which provided rapid inactivation of the enzymes associated with neurotransmitter metabolism, particularly, choline and acetylcholine. After irradiation of the animals, the brains were quickly removed from the skulls and dissected into three brain regions, the frontal cortex, striatum, and hippocampus, according to the method of Glowinsky and Iversen (Glowinski and Iversen, 1966; Palkovits and Brawnstein, 1988). Each region was stored at -70°C until used.

Analysis of neurotransmitters

The neurotransmitters were analyzed by the published method (Linh *et al.*, 2000). Choline and acetylcholine were analyzed on a column of ACH-3 (3.0 × 150 mm, ESA Inc., MA, U.S.A.) connected with an ACH-SPR solid phase reactor (ESA Inc., MA, U.S.A.) using the mobile phase of a phosphate buffer (100 mM, pH 8) containing 0.5 mM of tetramethylammonium chloride and 2 mM octanesulfonic acid sodium salt. The column was maintained at 35°C during HPLC analysis and the flow rate of the eluent was 0.35 mL/min. Ethyhomocholine was used as the internal standard. Detection was carried out on an electrochemical detector using a platinum electrode, and the working electrode was maintained at 0.3 V vs. Ag/AgCl.

Measurement of the activity of acetylcholinesterase

Inhibition of the activity of acetylcholinesterase (AChE) was measured using Ellman's coupled enzyme assay (Ellman *et al.*, 1961). The principle of this particular enzyme reaction is that AChE hydrolyzes acetylthiocholine (ATCh) to yield thiocholine and this product then reacts with DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) to form a dithio compound and 5-thio-2-nitrobenzoate. By measuring the rate of thiobenzoate formation, the enzyme activity was determined. Briefly, 5 mM ATCh (20 μL, Sigma Co.), 5 mM DTNB (40 μL, Sigma Co.) and a test sample (50 μL of test compounds in physiological saline with 5% Tween-80) were added to make the final volume of 100 μL in a 1 mL cuvette. The AChE solution (10 μL, 10 units, Sigma Co.) was then added and the absorbance was measured at 412 nm for 60 seconds. All enzymes, ATCh and DTNB were dissolved in phosphate buffer (pH = 7.3). The same amount (50 μL) of phosphate buffer as used for the test sample was used as the control. There was no effect of vehicle (5% Tween-80 in physiological saline) on the activity of acetylcholinesterase.

Statistical analysis

The data were processed by analyses of variance followed by individual comparisons using the Student's *t*-test, and were expressed as the mean ± S.E.M. The inhibitory activity of acetylcholinesterase was calculated as follows:

$$\text{Inhibition \%} = 100 - S/E \times 100$$

where S is the initial rate of reaction with the test sample and E is the initial rate of the control reaction.

RESULTS AND DISCUSSION

Effect of dieckol and PFF on memory

To examine the effects of dieckol and PFF on the improvement of learning and memory, pure dieckol and PFF were administered in ethanol-pretreated mice and a passive avoidance test was performed. Fig. 2 shows that the ethanol treatment (3 g/kg) significantly caused a retardation of learned information. Since alcoholic dementia is known to be very similar to Alzheimer's disease with regard to chemical mediators and brain regions involved (Arendt *et al.*, 1988, 1995; Renis *et al.*, 1996; Guerri and Renau-Piqueras, 1997), an ethanol-treated mice model has been used as an animal model for studying learning and memory related to Alzheimer's disease-associated dementia (Arendt *et al.*, 1995; Raghavendra and Kulkarni 2001). Thus, in the current study, an ethanol-treated mouse model was used as a memory-impaired model. Although an ethanol treatment influences spontaneous locomotor activity in a dosage-dependent manner (Ryback 1973;

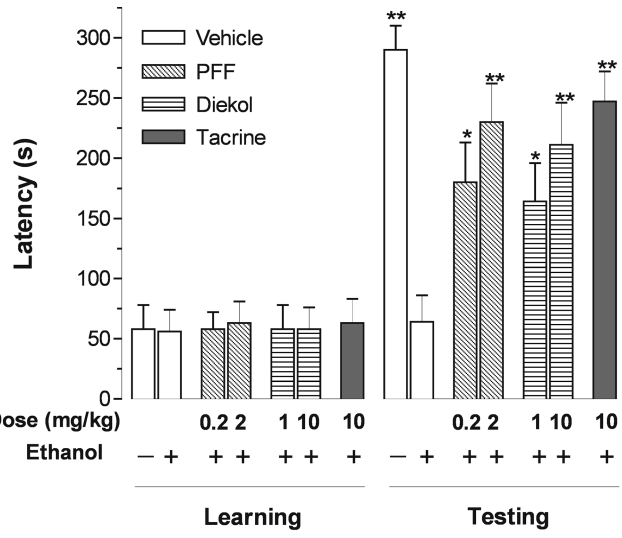

Fig. 2. Latency for the acquisition (learning) and retention (test) phases of the passive avoidance test by various concentrations of dieckol and phlorofucofuroeckol (PFF). The values are expressed as mean ± S.E.M. of at least ten mice. The difference between the effect of a treatment of saline and that of dieckol on the retention of ethanol-treated mice was statistically significant (*$p < 0.05$ for 1 mg/kg, **$p < 0.01$ for 10 mg/kg). Similarly, the difference between the effect of saline and that of PFF was statistically significant (*$p < 0.05$ for 0.2 mg/kg, **$p < 0.01$ for 2 mg/kg).

MacInnes and Uphouse 1973; Matchett and Erickson 1977), the present study used 3.0 g/kg of ethanol and this was not the highest dose (more than 4.0 g/kg) that depresses spontaneous locomotor activity. Throughout our experiments, the ethanol treatment did not cause any depressed, inactive and sluggish behavior, affecting the results of the passive-avoidance test. Nonetheless, we could not exclude the possibility of an involvement of ethanol on mice movement in the memory-impaired model used in this study.

The repeated administration of either dieckol or PFF significantly reduced the inhibition of latency by the administration of ethanol. This occurred in a dose-dependent manner. A small amount of either dieckol (1.0 mg/kg) or PFF (0.2 mg/kg) significantly improved the retardation of learned information caused by ethanol ($p<0.05$). The effect of a 10-fold large amount of either dieckol or PFF on the reduction of ethanol-induced latency inhibition was similar to that of tacrine (10 mg/kg), a cholinesterase inhibitor. These results suggest that both dieckol and PFF have a memory-enhancing ability and this mode of action may be due to the inhibition of cholinesterase. Thus, we hypothesized that the memory-enhancing abilities of dieckol and PFF are caused by an alteration of central chemical transmission, especially the level of acetylcholine.

Effect of dieckol and PFF on the levels of central neurotransmitters

To investigate this hypothesis, the levels of major central neurotransmitters in different regions of the brain were measured. Fig. 3 shows that the treatment of ethanol caused alterations of major central neurotransmitters in three different regions: the striatum, hippocampus, and frontal cortex. While the level of norepinephrine in the striatum was significantly increased, this level in the hippocampus was decreased (Fig. 3A). The level of GABA in

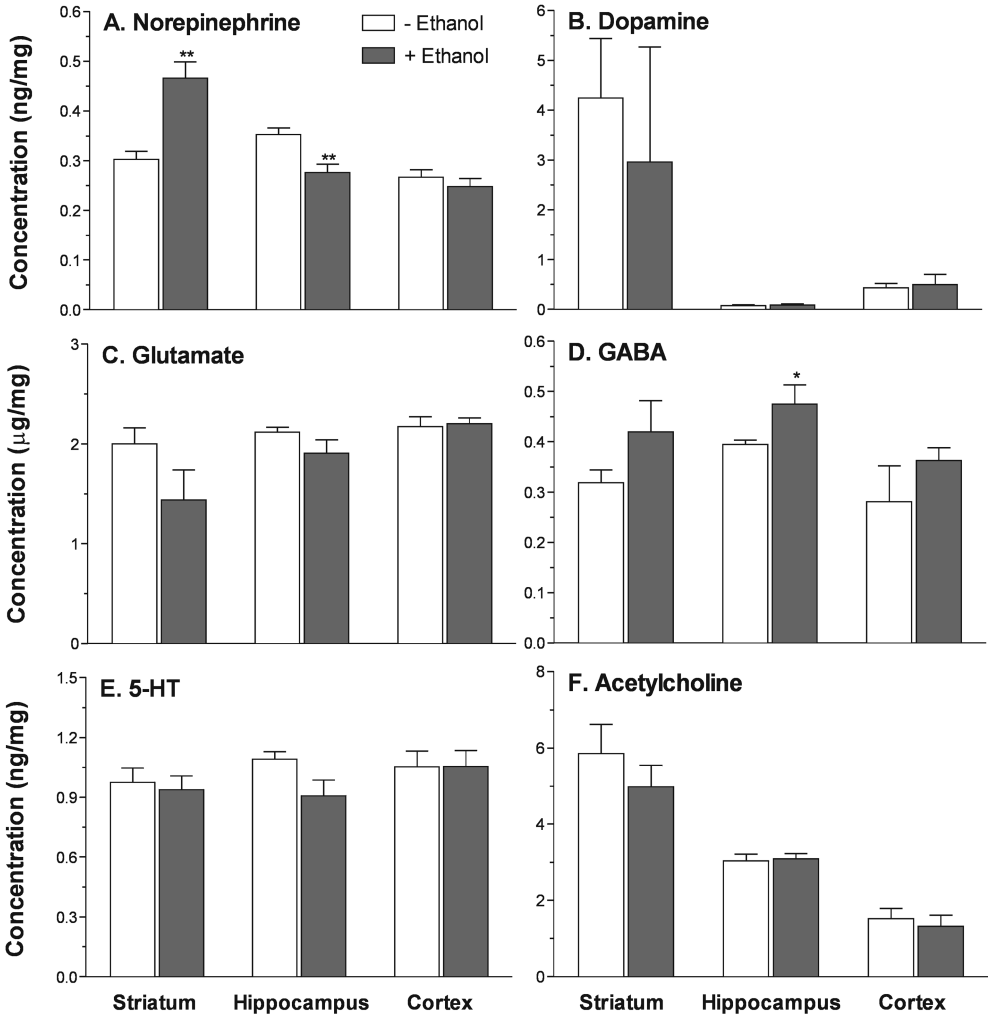

Fig. 3. Effect of an ethanol-treatment on the levels of major central neurotransmitters in the average mouse brain. The values are expressed as mean ± S.E.M. of at least ten mice. A statistical difference from the non-ethanol-treated group is illustrated by either *($p < 0.05$) or **($p < 0.01$). GABA; γ-aminobutyric acid, 5-HT; 5-hydroxytryptamine.

Table I. Comparison of the effect of an ethanol treatment on the metabolism of central neurotransmitters in three different regions of the mouse brain

	HVA/Dopamine			DOPAC/Dopamine			Glutamate/GABA			5-HIAA/5-HT			Choline/Acetylcholine		
	Str	Hipp	Cor	Str	Hipp	Cor	Str	Hipp	Cor	Str	Hipp	Cor	Str	Hipp	Cor
– Ethanol	0.57 ± 0.35	1.05 ± 0.19	0.66 ± 0.06	0.27 ± 0.16	0.34 ± 0.15	0.25 ± 0.03	6.35 ± 0.31	5.24 ± 0.17	6.04 ± 1.20	0.67 ± 0.06	0.68 ± 0.02	0.39 ± 0.03	2.15 ± 1.07	1.70 ± 0.31	1.54 ± 0.19
+Ethanol	1.27 ± 0.55	1.23 ± 0.29	0.83 ± 0.15	0.94 ± 0.43	0.31 ± 0.07	0.51 ± 0.18	[a] 4.87 ± 0.63	[a] 4.24 ± 0.57	6.33 ± 0.54	[a]0.89 ± 0.06	0.64 ± 0.07	0.34 ± 0.04	1.69 ± 0.42	2.22 ± 0.74	1.65 ± 0.18

Significant differences in response to a saline treatment in comparison with corresponding ethanol, [a]$p<0.05$. GABA; γ-aminobutyric acid, 5-HT; 5-hydroxytryptamine, DOPAC; 3,4-dihydroxyphenyl acetic acid, 5-HIAA; 5-hydroxyindole-3-acetic acid, HVA; homovanillic acid, Str; striatum, Hipp; hippocampus, Cor; frontal cortex.

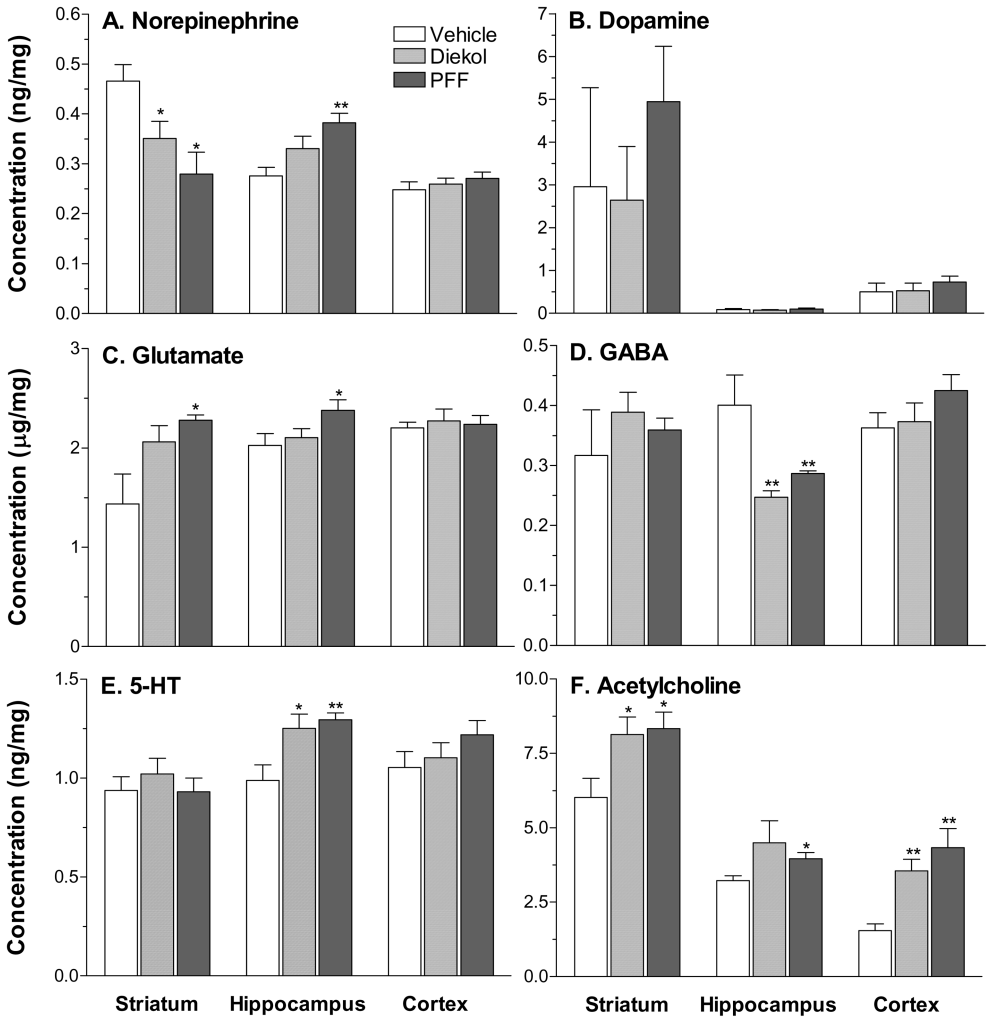

Fig. 4. Effect of either dieckol or phlorofucofuroeckol (PFF) on the levels of major central neurotransmitters in the brains of ethanol-treated mice. The values are expressed as mean ± S.E.M. of at least ten mice. A statistical difference from the vehicle (saline-treated) is illustrated by either * ($p < 0.05$) or ** ($p < 0.01$). GABA; γ-aminobutyric acid, 5-HT; 5-hydroxytryptamine.

the hippocampus was significantly increased, but not in the striatum or cortex (Fig. 3D). The levels of the major neurotransmitters, dopamine, glutamate, GABA, 5-HT, and acetylcholine, were not significantly changed in three different regions of the brain. To compare the effect of the ethanol treatment on the metabolism of these central neurotransmitters, we measured the levels of major metabolites: HVA and DOPAC for dopamine, GABA for glutamate, 5-HIAA for 5-HT, and choline for acetylcholine. Table I shows a comparison of the effects of the ethanol

treatment on the metabolism of central neurotransmitters in three different regions of the average mouse brain. The treatment of ethanol significantly caused an alteration of the ratio of glutamate/GABA in both the striatum and the hippocampus, indicating that the level of glutamate was decreased and the level of GABA was increased. The ratio of 5-HIAA/5-HT in the striatum was also significantly changed, indicating that the metabolism of 5-HT was increased (Table I). The metabolism of both dopamine and acetylcholine was not significantly changed by the treatment of ethanol.

Fig. 4 illustrates that both dieckol (10 mg/kg) and PFF (2 mg/kg) altered the levels of some of the major central neurotransmitters modified by the ethanol treatment. Both dieckol and PFF significantly reduced the level of norepinephrine in the striatum, which was increased by ethanol ($p<0.05$). In contrast, only PFF significantly increased the level of norepinephrine, which was decreased by ethanol in the hippocampus. PFF significantly increased the level of glutamate in both the striatum and the hippocampus, and both dieckol and PFF decreased the level of GABA in the hippocampus. Both dieckol and PFF increased the level of 5-HT in the hippocampus. Note that both dieckol and PFF also increased the level of acetylcholine in all three regions of the average mouse brain (Fig. 4F). There may be two possibilities to increase the level of acetylcholine: (1) by increasing the supply of choline, and (2) by inhibiting the metabolism of acetylcholine by acetylcholinesterase. However, since the ratio of choline/acetylcholine was not significantly changed by a repeated treatment of either dieckol or PFF (Table II), the constant supply of choline was not the main reason for the increase in the brain level of acetylcholine. Thus, to address the second possibility, the ability of both dieckol and PFF to regulate the biochemical activity of acetylcholinesterase was measured.

Effect of dieckol and PFF on the activity of acetylcholinesterase

Fig. 5 shows that both dieckol and PFF at the given concentrations inhibited the activity of acetylcholinesterase. Fitting the data to rectangular hyperbolas estimated that the IC_{50} values of dieckol and PFF on the inhibition of acetylcholinesterase were about 17.5 μM and 27.4 μM, respectively. The ability of dieckol to inhibit this enzyme was about 1.6-fold greater than that of PFF. Therefore, these results indicate that both dieckol and PFF completely block the activity of acetylcholinesterase.

In conclusion, memory was significantly enhanced by both dieckol and PFF. Both dieckol and PFF increased the glutamate and 5-HT levels in the hippocampus, which were reduced by an ethanol treatment. In contrast, the GABA level in the hippocampus was decreased by the treatment of both dieckol and PFF. The acetylcholine level was increased in all three regions of the average mouse brain. Both dieckol and PFF have an ability to inhibit the activity of acetylcholinesterase. Overall, the results suggest that the phlorotannin compounds of *Ecklonia cava*, dieckol and PFF, regulate the levels of major central neurotransmitters in the brain, especially the increment of the acetylcholine level in the brain by the inhibition of the activity of acetylcholinesterase. This may be, at least in

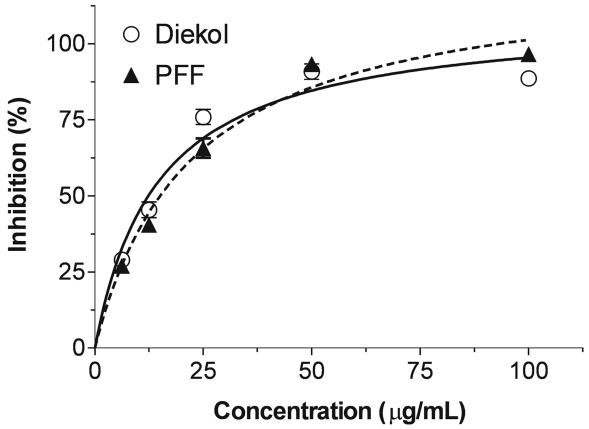

Fig. 5. Ability of dieckol or phlorofucofuroeckol (PFF) to inhibit the activity of acetylcholinesterase. Each data point is an average of three independent experiments, each performed in duplicate.

Table II. Comparison of the ability of either dieckol or phlorofucofuroeckol (PFF) to affect the levels of neurotransmitters and metabolites in three different brain regions of ethanol-treated mice

	HVA/Dopamine			DOPAC/Dopamine			Glutamate/GABA			5-HIAA/5-HT			Choline/Acetylcholine		
	Str	Hipp	Cor	Str	Hipp	Cor	Str	Hipp	Cor	Str	Hipp	Cor	Str	Hipp	Cor
Vehicle	1.27 ± 0.55	1.23 ± 0.29	0.83 ± 0.15	0.94 ± 0.43	0.31 ± 0.07	0.51 ± 0.18	4.87 ± 0.63	4.24 ± 0.57	6.33 ± 0.54	0.89 ± 0.06	0.64 ± 0.07	0.34 ± 0.04	1.69 ± 0.42	2.22 ± 0.74	1.65 ± 0.18
Dieckol	1.96 ± 0.39	1.40 ± 0.20	1.09 ± 0.27	1.14 ± 0.25	0.27 ± 0.03	0.39 ± 0.05	5.45 ± 0.39	[a]8.44 ± 0.40	6.37 ± 0.53	0.86 ± 0.07	0.73 ± 0.07	0.37 ± 0.02	0.89 ± 0.04	1.52 ± 0.17	1.61 ± 0.13
PFF	0.93 ± 0.36	0.95 ± 0.14	0.74 ± 0.13	0.52 ± 0.20	0.26 ± 0.03	0.32 ± 0.05	6.78 ± 0.60	[a]7.91 ± 0.28	5.22 ± 0.41	0.88 ± 0.06	0.69 ± 0.02	0.39 ± 0.03	1.04 ± 0.10	1.39 ± 0.14	1.55 ± 0.14

Significant differences in response to dieckol or PFF in comparison with corresponding ethanol, [a]$p<0.01$ and [b]$p<0.05$. GABA; γ-aminobutyric acid, 5-HT; 5-hydroxytryptamine, DOPAC; 3,4-dihydroxyphenyl acetic acid, 5-HIAA; 5-hydroxyindole-3-acetic acid, HVA; homovanillic acid, Str; striatum, Hipp; hippocampus, Cor; frontal cortex.

part, associated with the memory-enhancing abilities of dieckol and PFF.

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