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Evaluation of the Inhibitory Effects of Quercetin-Related Flavonoids and Tea Catechins on the Monoamine Oxidase-A Reaction in Mouse Brain Mitochondria

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ABSTRACT: Quercetin, a typical dietary flavonoid, is thought to exert antidepressant effects by inhibiting the monoamine oxidase-A (MAO-A) reaction, which is responsible for regulation of the metabolism of the neurotransmitter 5-hydroxytryptamine (5-HT) in the brain. This study compared the MAO-A inhibitory activity of quercetin with those of *O*-methylated quercetin (isorhamnetin, tamarixetin), luteolin, and green tea catechins ((−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate) by measuring the formation of the oxidative deamination product of 5-HT, 5-hydroxyindole aldehyde (5-HIAL), in mouse brain mitochondria. Quercetin was inferior to luteolin in the inhibition of MAO-A activity, whereas isorhamnetin, tamarixetin, and tea catechins scarcely exerted inhibitory activity. Quercetin did not affect MAO-A activity in mouse intestinal mitochondria, indicating that it *does not* evoke side effects on the metabolism of dietary monoamines in the gut. These data suggest that quercetin is a weak (but safe) MAO-A inhibitor in the modulation of 5-HT levels in the brain.

KEYWORDS: *quercetin, flavonoid, tea catechin, monoamine oxidase-A, inhibition, serotonin*

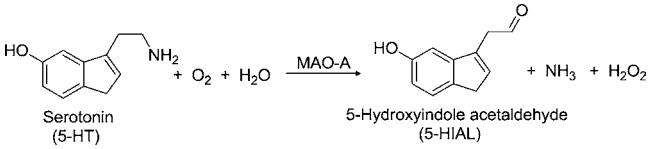
INTRODUCTION

Monoamine oxidase (MAO) is a flavin enzyme (EC 1.4.3.4) located in the outer membrane of mitochondria in all cells in the body. It catalyzes the oxidative deamination of biogenic and xenobiotic amines. MAO plays a crucial part in the metabolism of neuroactive and vasoactive amines in the central nervous system (CNS) and peripheral tissues.

There are two forms of MAO: MAO-A and MAO-B. MAO-A preferentially oxidizes serotonin (5-hydroxytryptamine; 5-HT), norepinephrine, and epinephrine; MAO-B metabolizes dopamine. Depression is one of the most frequently occurring psychiatric disorders. There is some evidence that the activity of mitochondrial MAO-A is elevated throughout the brain during major depression.[1,2] Selective MAO-A inhibitors have been used as antidepressants and for treatment of other common neurodegenerative diseases.[3]

Several authors have suggested an essential role of flavonoids in the antidepressant effects of herbal medicines.[4−6] It has been suggested that 3,3′,4′,5,7-pentahydroxyflavone (quercetin) and its structurally related flavonoids exert antidepressant-like effects in rodent models of depression.[7−10] Several studies also reported the possibility of dietary quercetin and its metabolites crossing the blood−brain barrier and accumulating in the brain.[11−13] Our research team confirmed that quercetin accumulates in brain tissue as conjugated metabolites of quercetin and *O*-methylated quercetin (isorhamnetin and tamarixetin) by dietary intake in rats.[14] Furthermore, in vitro studies have shown that quercetin and its structurally related flavonoids exert considerable inhibition on the MAO-A reaction.[15−17] Therefore, it is rational to hypothesize that dietary quercetin exerts its antidepressant-like effect by direct attenuation of MAO-A activity in the brain.[18] Nevertheless, the mechanism of inhibition of MAO-A by quercetin and related flavonoids is incompletely understood because authors have used diverse methods for the measurement of MAO-A activity. In our previous study,[19] a novel ultraviolet−high-performance liquid chromatography (UV-HPLC) method was developed for the direct assay of the deamination product of 5-HT, that is, 5-hydroxyindole acetaldehyde (5-HIAL), through the MAO-A reaction (Scheme 1). Using this method, we found that quercetin could attenuate the activity of mitochondrial MAO-A in the mouse brain, whereas metabolic conversion to glucuronide conjugates abolished this attenuating effect.[19]

Scheme 1. MAO-A Reaction



The aim of the present study was to evaluate the importance of quercetin and structurally related flavonoids on the modulation of MAO-A activity in the mitochondria of brain cells in relation to their antidepressant effects. We selected luteolin, isorhamnetin, and tamarixetin as quercetin-related flavonoids (Figure 1). Their MAO-inhibitory activities were evaluated using HPLC for the detection of 5-HIAL together with a conventional method for the detection of an alternative reaction product, hydrogen peroxide (H_2O_2). Catechins found in green tea include (−)-epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate), which

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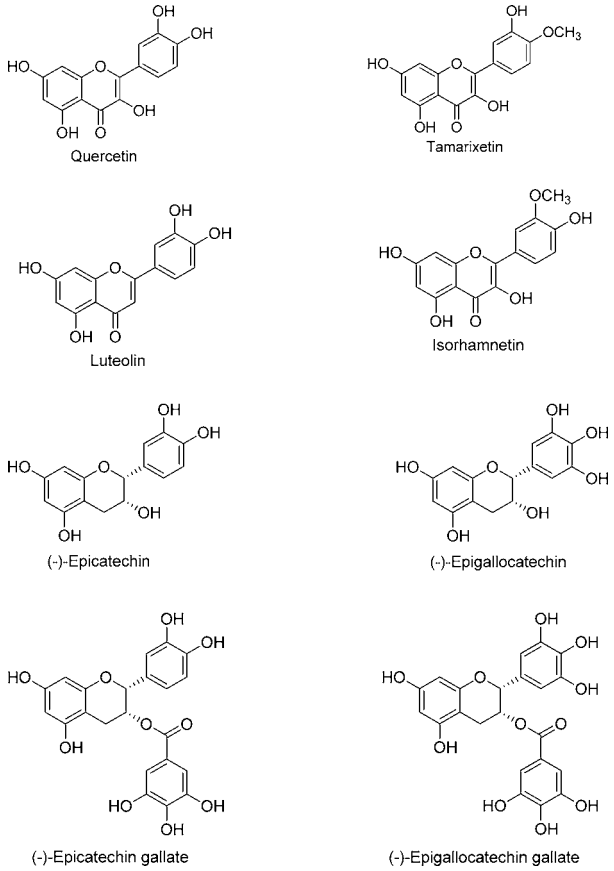

Figure 1. Structures of flavonols, flavone, and tea catechins used in the present study.

are flavan-3-ol-type flavonoids (Figure 1). These were also examined because (−)-epicatechin and (−)-epigallocatechin gallate were recently reported to attenuate the activity of MAO-B enzymes in rats.[20,21] The effectiveness of quercetin in the MAO-A reaction in the intestinal mitochondria of mice was also examined to evaluate the possibility of dietary quercetin interfering with the metabolism of dietary monoamines in the gut.[22]

MATERIALS AND METHODS

Ethical Approval of the Study Protocol. This study was undertaken according to the guidelines for the care and use of laboratory animals of the University of Tokushima Graduate School (Tokushima, Japan).

Chemicals and Reagents. Quercetin, isorhamnetin (3′-*O*-methylquercetin), tamarixetin (4′-*O*-methylquercetin), and luteolin were purchased from Extrasynthese (Genay Cedex, France). (−)-Epicatechin, (−)-epicatechin gallate, (−)-epigallocatechin, and (−)-epigallocatechin gallate were obtained from Kurita (Tokyo, Japan). Flavanone, clorgyline, pargyline, and MAO-A human recombinant were from Sigma-Aldrich (St. Louis, MO, USA). 5-HT and flavone were obtained from Nacalai Tesque (Kyoto, Japan). All other reagents were of analytical grade and used without further purification.

Isolation of Mouse Brain Mitochondria Fractions. Brain mitochondria fractions were obtained from male C57BL/6j mice (age, 6 weeks; Japan SLC, Shizuoka, Japan). After decapitation, the entire brain was washed with ice-cold 0.85 M NaCl solution. Homogenization of brain tissue was done using a Potter−Elvehjem homogenizer (Wheaton, Millville, NJ, USA) in the presence of 9 volumes of 10 mM potassium phosphate-buffered saline (PBS) (pH 7.4) containing 320 mM sucrose. This was followed by centrifugation at 1000*g* for 10 min at 4 °C. Supernatants containing mitochondrial fractions were subjected to further centrifugation at 15000*g* for 30 min at 4 °C. The second supernatants were discarded and mitochondrial pellets resuspended with the same buffer and stored at −28 °C until use. Mitochondrial protein concentration was determined using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA). Mitochondrial fractions were also obtained from the small intestine of the same mouse simultaneously.

Determination of the Activity of MAO-A by Measuring H_2O_2 Formation. The amount of H_2O_2 in the reaction mixture was measured using an Amplex Red Monoamine Oxidase Assay Kit (Invitrogen, Paisley, UK) with slight modification. The reaction mixture contained 50 mM PBS (pH 7.4), 100 μM Amplex Red reagent, 1 U/mL horseradish peroxidase (HRP), 1 mM 5-HT as substrate, and 0.5 U/mL standard MAO-A (human recombinant) or 0.5 mg/mL brain mitochondrial protein. Stock solutions of flavonoids in dimethyl sulfoxide (DMSO) were added in the reaction mixture to reach the desired concentration and preincubated for 5 min at 37 °C before substrate addition. The final content of DMSO was <5%. The reaction was done in 96-well black plates at 37 °C for 20 min. To stop the reaction, 40 μL of Amplex Red/Ultra Red Stop Reagent solution was added to 200 μL of the reaction mixture. Fluorescence intensity was measured with λ_{Ex} = 550 nm (split = 5 nm) and λ_{Em} = 590 nm (split = 5 nm) by use of a plate spectrophotometer (Varioskan Flash, Thermo Scientific, Waltham, MA, USA). The remaining MAO-A activity of the reaction mixture was calculated as a percent of the control without addition of the inhibitor.

MAO-A Activity Determination by Measuring 5-HIAL Formation. The oxidative deamination product of 5-HT is 5-HIAL. The level of 5-HIAL was measured by the UV-HPLC method according to a procedure developed by our research group.[19] Briefly, the reaction mixture contained 10 mM PBS (pH 7.4), 4 mM 5-HT (as substrate), 0.5 U/mL standard MAO-A (human recombinant), or 0.5 mg/mL brain mitochondrial protein. Stock solutions of flavonoids in DMSO were added to the reaction mixture to reach the desired concentration and preincubated at 37 °C for 5 min before the addition of substrate. The final content of DMSO was <5%. The reaction was carried out at 37 °C for 20 min. To stop the reaction, 100 μL of 1 M HCl was added to 0.5 mL of the reaction mixture. 5-HIAL was extracted with 1 mL of butyl acetate, evaporated under a vacuum, and dissolved in 50 μL of methanol. Samples were stored at −28 °C until injection into the HPLC column (capcell pak C18 MG II (4.6 × 250 mm; Shiseido, Tokyo, Japan). The mobile phase was 10% acetonitrile and 90% buffer containing 50 mM tartaric acid, 40 mM sodium acetate, and 0.65 mM 1-octanesulfonic acid sodium acetate. Isocratic elution was used with a flow rate of 1.0 mL/min. Elution was monitored with a SPD-20A UV detector (Shimadzu, Kyoto, Japan) at 280 nm. Remaining MAO-A activity was calculated as a percent of the peak area of 5-HIAL in the presence of the inhibitor to that of control without the inhibitor (accepted as 100%).[19]

Calculation of clog*P*. The clog*P* values of selected flavonoids were calculated using ChemBioDraw Ultra ver11.0 (PerkinElmer, Waltham, MA, USA).

RESULTS

Improvement of the Methodology for Evaluating Mitochondrial MAO-A Activity. Initially, the method for measuring H_2O_2 formation was improved for selective evaluation of mitochondrial MAO-A activity without the addition of MAO-B inhibitor. Figure 2A shows the fluorescence response of resorufin that was dependent upon the formation of H_2O_2 during the MAO reaction in the mitochondria of brain cells in the presence of specific and nonspecific substrates and inhibitors for MAO-A and MAO-B. *p*-Tyramine is a nonspecific substrate for MAO-A and MAO-B.[23] *p*-Tyramine showed the strongest response, whereas the MAO-A-specific substrate 5-HT[24] had the weakest response. The response for the MAO-B-specific substrate benzylamine was similar to that for *p*-

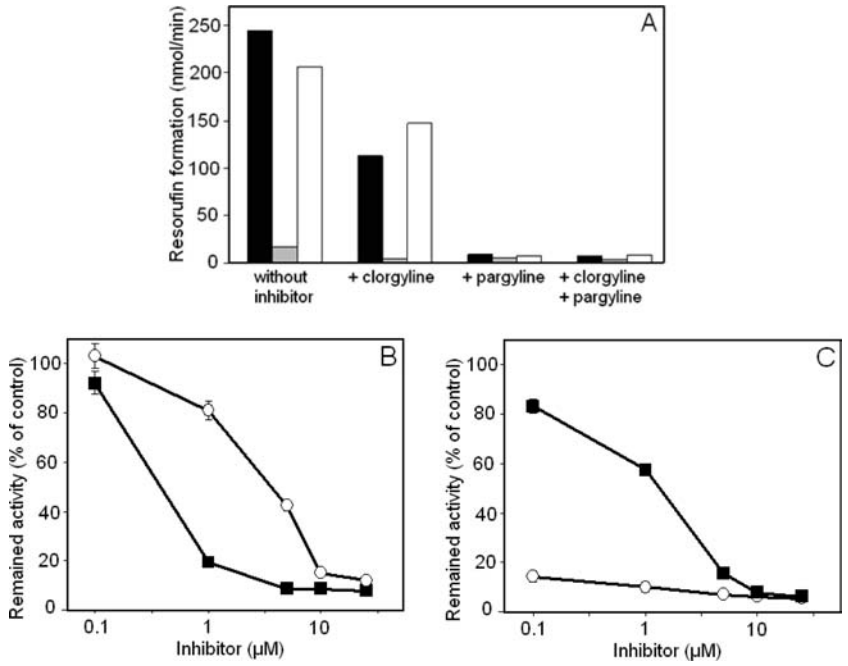

Figure 2. Effect of MAO inhibitors on the MAO reaction in the mitochondria of mouse brain cells. (A) Effect of MAO inhibitors on the MAO-A and MAO-B reaction with different substrates. The reaction mixture contained 0.1 mg/mL mouse brain mitochondrial protein in 50 mM PBS (pH 7.4); 200 μM Amplex Red reagent; 1 U/mL HRP; 1 mM *p*-tyramine (black), 5-HT (gray), or benzylamine (white) as substrate; and 5 μM clorgyline or pargyline as MAO-A and MAO-B inhibitors, respectively. Values are the means from one experiment undertaken with three replicates of each sample. (B, C) Dependence of the remaining activity of mitochondrial MAO-A on the concentration of the MAO-A inhibitor clorgyline (open circle) and MAO-B inhibitor pargyline (black square). The reaction mixture contained 0.05 mg/mL mouse brain mitochondrial protein in 50 mM PBS (pH 7.4); 200 μM Amplex Red reagent; 1 U/mL HRP; and 1 mM *p*-tyramine (B) or 5-HT (C) as substrate. Values are the percent of control without inhibitors and means ± SD from independent triplicate experiments.

tyramine. Addition of the MAO-B inhibitor pargyline[25] led to almost complete disappearance of the fluorescence of resorufin independent of MAO-A/B substrate type, whereas addition of the MAO-A inhibitor clorgyline resulted in complete disappearance of the fluorescence for 5-HT and only a partial decrease in fluorescence for *p*-tyramine and benzylamine. High concentrations (>10 μM) of the MAO inhibitors clorgyline and pargyline inhibited the activities of MAO-A and MAO-B in a nonselective manner (Figures 2B,C). Pargyline was better at inhibiting MAO than clorgyline when *p*-tyramine was used as the substrate (Figure 2B). When 5-HT was used as a specific MAO-A substrate, clorgyline inhibited the reaction effectively at 0.1 μM, whereas pargyline inhibited the reaction effectively at >5 μM (Figure 2C). However, the weak inhibitory effect of pargyline was shown at even 0.1 μM and reached 40% inhibition at 1.0 μM. These findings suggested that the MAO-B inhibitor pargyline affected MAO-A activity if it was used to exclude MAO-B activity in the mitochondria of brain cells. Therefore, we decided to use 5-HT as the MAO-A-specific substrate without the addition of pargyline for measuring mitochondrial MAO-A activity.

Inhibitory Effect of Selected Flavonoids on the Activity of MAO-A of Mitochondria in Mouse Brain Cells. In the evaluation of MAO-A inhibitory activity of selected flavonoids, the method for measuring 5-HIAL and that for measuring H_2O_2 formation were applied. Also, the relationship between the remaining MAO-A activity and the concentration of each flavonoid was obtained. In both methods, 5-HT was used as the MAO-A substrate without the addition of a MAO-B inhibitor on the basis of the results described above. Figure 3 shows a typical HPLC chromatogram for a reaction

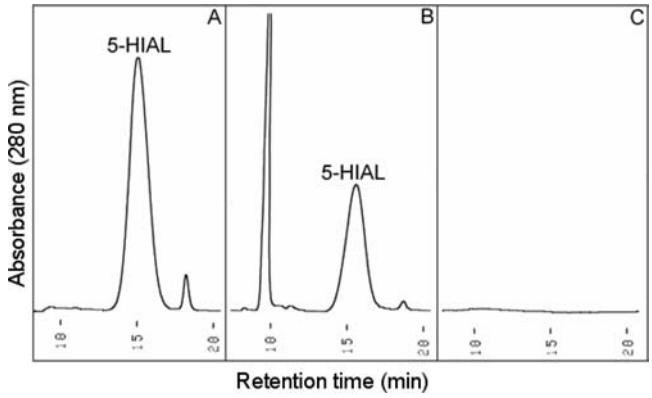

Figure 3. HPLC chromatogram of the product of oxidative deamination of 5-HT by mouse brain mitochondrial MAO-A: (A) in the absence of quercetin; (B) in the presence of 500 μM quercetin; (C) in the absence of mitochondria fractions. The reaction mixture (final volume, 0.5 mL) contained 0.5 mg/mL mouse brain mitochondrial protein in 10 mM PBS (pH 7.4) and 4 mM 5-HT as MAO-A substrate. The reaction was carried out for 20 min at 37 °C and terminated by the addition of 100 μL of 1 M HCl. The product of deamination of 5-HT was extracted and injected into the HPLC system. Elution of the 5-HIAL peak was monitored at 280 nm.

mixture of 5-HT with mouse mitochondria in the presence and absence of quercetin. In accordance with our previous work,[19] the peak that appeared at a retention time of 15 min was attributed to 5-HIAL. Panels A and B of Figure 4 show the result of inhibition of mitochondrial MAO-A activity by quercetin, luteolin, isorhamnetin, tamarixetin, and the positive control clorgyline. With respect to the method of measuring 5-

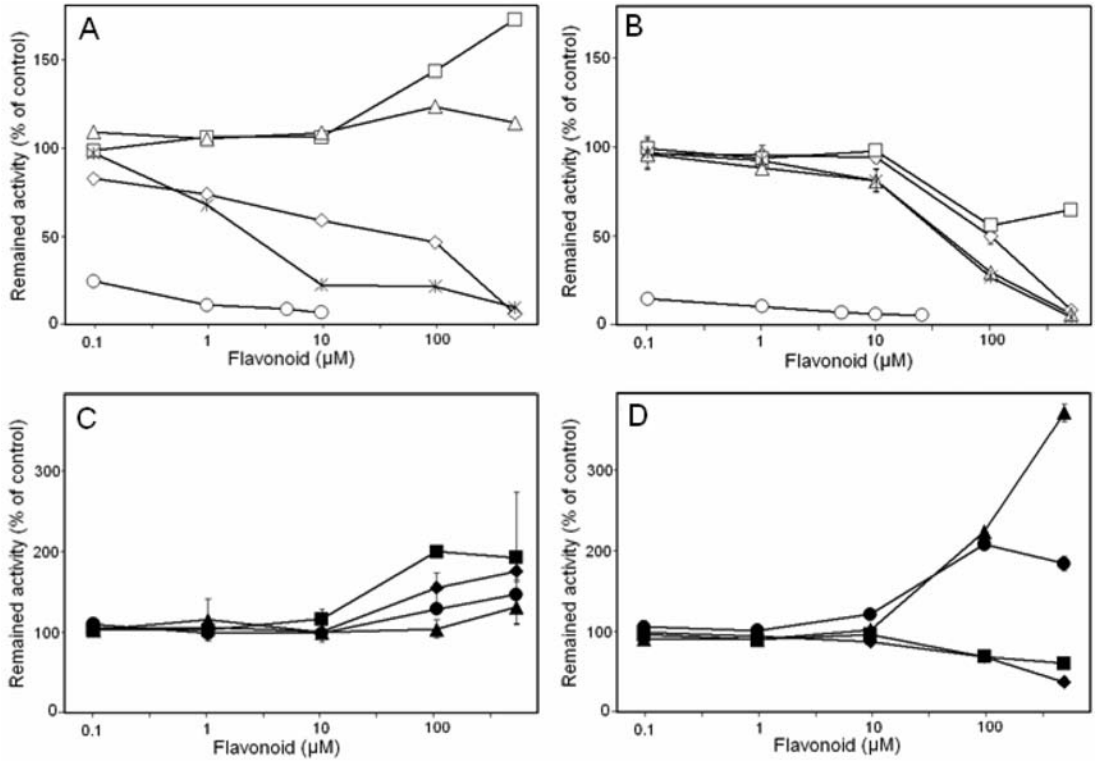

Figure 4. Effect of selected flavonoids on the MAO-A activity of mouse brain mitochondria: (A, C) Measurement of 5-HIAL formation by HPLC. The reaction mixture (final volume, 0.5 mL) contained 0.5 mg/mL mouse brain mitochondrial protein in 10 mM PBS (pH 7.4) and 4 mM 5-HT as substrate. The reaction was carried out for 20 min at 37 °C, and terminated by the addition of 100 μL of 1 M HCl. (B, D) Measurement of H_2O_2 formation using the Amplex Red assay. The reaction mixture contained 0.5 mg/mL mouse brain mitochondrial protein in 50 mM PBS (pH 7.4); 100 μM Amplex Red reagent; 1 U/mL HRP; and 1 mM 5-HT as substrate. The reaction was carried out in black 96-well plates (volume of reaction mixture, 200 μL) for 20 min at at 37 °C. Open circle, clorgyline; open diamond, quercetin; open square, isorhamnetin; open triangle, tamarixetin; asterisk, luteolin; solid diamond, (−)-epicatechin; solid square, (−)-epicatechin gallate; solid triangle, (−)-epigallocatechin; solid circle, (−)-epigallocatechin gallate. Values are the percent of control without inhibitors and means ± SD from independent triplicate experiments.

HIAL formation, quercetin and luteolin were effective MAO-A inhibitors at >1 μM, and the half-maximal inhibitory concentrations (IC_{50}) for quercetin and luteolin were estimated to be 40 and 2 μM, respectively (Figure 4A). Isorhamnetin and tamarixetin did not show inhibitory effects on the production of 5-HIAL. In the conventional method for the measurement of H_2O_2, all flavonoids lowered the remaining activity at >10 μM (Figure 4B). Panels C and D of Figure 4 show the effects of catechins found in green tea on MAO-A activity in mouse mitochondria. None of the tea catechins inhibited MAO-A activity even at >100 μM. Conversely, (−)-epigallocatechin and (−)-epigallocatechin gallate at higher concentrations elevated the production of H_2O_2 (Figure 4D).

Hydrophobicity of Selected Flavonoids As Determined by *clogP*. Hydrophobic interactions may have a critical role in the effectiveness of flavonoids on the inhibition of mitochondrial MAO-A activity because this enzyme should be located at the hydrophobic phospholipid bilayers that constitute the outer membranes. Table 1 shows the calculated *clogP* values (which are frequently used as a reliable index of hydrophobicity of organic compounds). The hydrophobicity of quercetin, luteolin, isorhamnetin, and tamarixetin were not particularly different because they gave similar *clogP* values. Each tea catechin possesses different hydrophobicities depending on its structure. (−)-Epicatechin gallate was found to have the highest hydrophobicity among the four catechins. Little difference was observed between flavone and flavanone with regard to hydrophobicity because their *clogP* values were almost identical.

Table 1. Calculated *clogP* Values of Selected Flavonoids

flavonoid	*clogP*
quercetin	1.50
isorhamnetin	1.95
tamarixetin	1.95
luteolin	2.31
(−)-epicatechin	0.53
(−)-epicatechin gallate	2.15
(−)-epigallocatechin	−0.13
(−)-epigallocatechin gallate	1.49
flavone	3.48
flavanone	3.47

Effect of Flavone and Flavanone on the MAO-A Activity of Human Recombinant Enzyme Preparations. To clarify the role of the steric factors of the diphenylpropane ($C_6-C_3-C_6$) skeleton of flavonoids on their MAO-A inhibitory activity, we compared the inhibitory effect of flavone with that of flavanone using MAO-A human recombinant enzyme preparations. The inhibitory effect of flavone was appreciably higher than that of flavanone (Figure 5). Flavone possesses a planar structure because of a double bond at the 2−3-position in the C-ring, which enables the delocalization of π electrons between the B- and C-rings.[26] The steric structure of flavanone is nonplanar because it lacks a double bond at the 2−3-

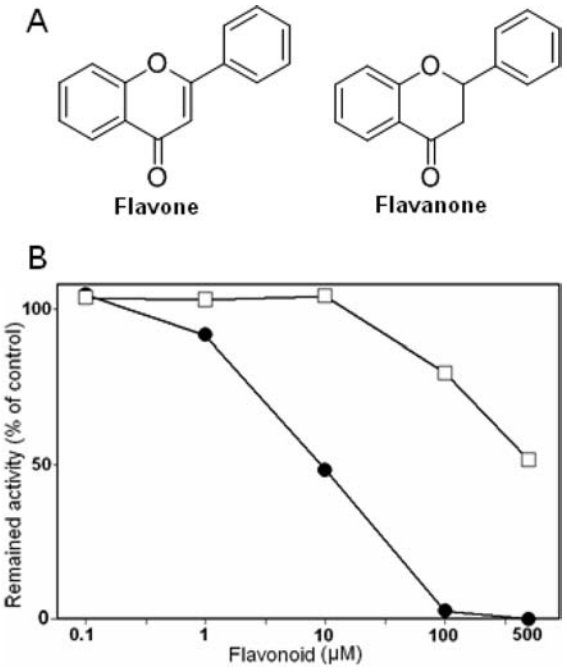

Figure 5. Inhibitory effect of flavone and flavanone on the activity of standard MAO-A: (A) structures of flavone and flavanone; (B) MAO-A activity in the presence of flavone or flavanone at different concentrations. MAO-A activity was measured as 5-HIAL formation by HPLC. The reaction mixture (final volume, 0.5 mL) contained 0.5 U/mL of standard MAO-A in 10 mM PBS (pH 7.4) and 4 mM 5-HT as substrate. The reaction was carried out for 20 min at 37 °C and terminated by the addition of 100 μL of 1 M HCl. The product of deamination of 5-HT was extracted and injected into the HPLC system. Elution of the 5-HIAL peak was monitored at 280 nm.

position.[26] It is therefore likely that a planar structure is required for the effective inhibition of mitochondrial MAO-A activity by dietary flavonoids.

Lineweaver–Burk Plot of the Inhibition of MAO-A Activity of Human Recombinant Enzyme Preparations by Luteolin. Figure 6 shows the Lineweaver–Burk plot for the inhibition of MAO-A activity by different concentrations of luteolin using human recombinant enzyme preparations. Inhibition of MAO-A activity by luteolin was in accordance with a noncompetitive mechanism, indicating that this flavonoid binds with the enzyme molecule in a location that is not the active center.

Effect of Quercetin on Mouse Mitochondrial MAO-A Activities in the Small Intestine. We compared mouse mitochondrial MAO-A activity from the small intestine with that from the brain. MAO-A activity from the small intestine was about 3-fold higher than that from the brain (Figure 7A). Mitochondrial MAO-A activities from the brain and small intestine were inhibited effectively by the MAO-A inhibitor clorgyline at 0.1 μM (Figure 7B). The inhibitory effect of quercetin on mitochondrial MAO-A activity from the small intestine was not observed in the range of 0.1–100 μM, an effect that was different from that observed in the brain.

DISCUSSION

A wide variety of methods have been applied to the measurement of MAO activity in biological samples. A colorimetric assay based on the formation of H_2O_2 is frequently considered to be the conventional method.[27] To measure the

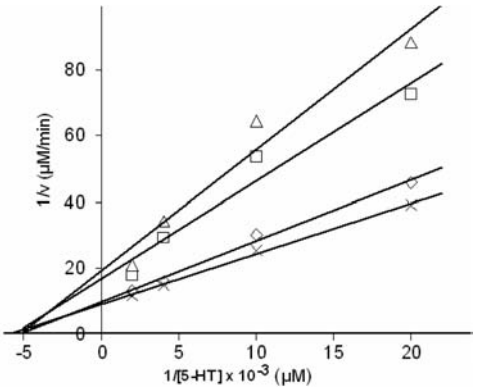

Figure 6. Lineweaver–Burk plot of the inhibition of the activity of standard MAO-A by different concentrations of luteolin. H_2O_2 formation was measured using the Amplex Red assay. The reaction mixture contained 0.5 U/mL of standard MAO-A (human recombinant) in 50 mM PBS (pH 7.4); 100 μM Amplex Red reagent; 1 U/mL HRP; 10–1000 μM 5-HT as substrate; and 0 (times sign), 1 (open diamond), 10 (open square), or 20 (open triangle) μM luteolin. The reaction was carried out in black 96-well plates (volume of reaction mixture, 200 μL) for 20 min at at 37 °C. Values are the percent of control without inhibitors and means ± SD from independent triplicate experiments.

specific activity of MAO-A or MAO-B in biological samples, specific inhibitors of MAO-B and MAO-A (i.e., pargyline and clorgyline, respectively) are occasionally added to the reaction mixture[25,28] and *p*-tyramine is used as the substrate for MAO-A and MAO-B.[23] However, both inhibitors inhibited MAO activities nonspecifically at higher concentrations (Figure 2). To focus on MAO-A activity in brain mitochondria fractions, we used 5-HT as a specific MAO-A substrate without the addition of the MAO-B inhibitor pargyline for the fluorescence method based on the measurement of H_2O_2 formation (Amplex Red assay) together with a HPLC method based on the conversion of 5-HT to 5-HIAL.

Several studies have been published on the inhibition of MAO-A activity by quercetin and its structurally related flavonoids using different assay methods and different substrates.[15−17] Our previous study using HPLC based on the measurement of 5-HIAL demonstrated that quercetin is effective in the inhibition of brain mitochondrial MAO-A activity, whereas its conjugated metabolite, quercetin 3-*O*-β-glucuronide, scarcely acts as a MAO-A inhibitor.[19] The HPLC method in the present study revealed the IC_{50} value for quercetin to be ≈40 μM (Figure 4A), whereas its IC_{50} value obtained from previous works using different methods ranged between 0.01 and 18 μM.[15−18] Although calculation of the IC_{50} value is dependent upon experimental conditions, the MAO-A inhibitory effect of quercetin was much weaker than that of the antidepressant MAO-A inhibitor clorgyline. Interestingly, luteolin (which is a flavone-type flavonoid lacking a hydroxyl group at the 3-position of the C-ring) was a more effective MAO-A inhibitor than quercetin in our experimental system (IC_{50} of luteolin ≈ 2 μM) (Figure 4A). Many flavonoids can act as enzyme regulators, activating or attenuating their activity by interacting with active centers or other parts of protein molecules. However, information about the interactions of flavonoids with MAO-A is scarce. The results of docking simulation and dialysis studies suggested that quercetin interacted with MAO-A in a reversible manner in a place different from the active center.[15] Our results clarified that

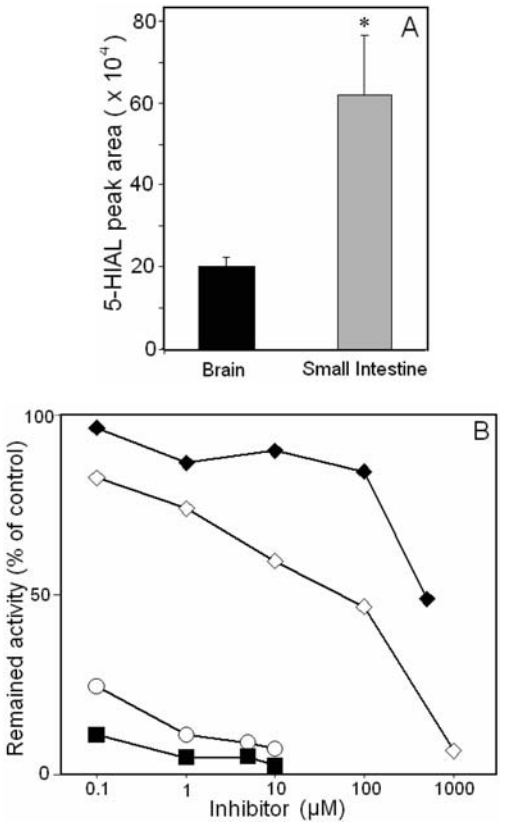

Figure 7. Comparison of the inhibitory effect of quercetin and the MAO-A inhibitor clorgyline on the activity of mitochondrial MAO-A from the brain and small intestine of mice. (A) Comparison of MAO-A activity from brain (black) and small intestine (gray). Values are means ± SD from independent triplicate experiments. The asterisk indicates significant difference ($p < 0.05$; Turkey–Kramer method). (B) Comparison of the inhibitory effect of quercetin and clorgyline on MAO-A activity from brain and small intestine. Remaining activity was calculated as percent of control without an inhibitor. Open diamond, effect of quercetin on MAO-A activity in the brain; solid diamond, effect of quercetin on MAO-A activity in the small intestine; open circle, effect of clorgyline on MAO-A activity in the brain; solid circle, effect of clorgyline on MAO-A activity in the small intestine. MAO-A activity was measured as 5-HIAL formation by HPLC. The reaction mixture (final volume, 0.5 mL) contained 0.5 mg/mL of brain or small intestine mitochondrial protein in 10 mM PBS (pH 7.4) and 4 mM 5-HT as substrate. The reaction was carried out for 20 min at 37 °C and terminated by the addition of 100 μL of 1 M HCl. The product of deamination of 5-HT was extracted and injected into the HPLC system.

inhibition of MAO-A by luteolin was noncompetitive, suggesting the interaction of luteolin with proteins at a site different from the active center. In contrast, inhibition was not observed by *O*-methylated quercetin (isorhamnetin and tamarixetin). This finding suggested that the *o*-dihydroxy group in the B-ring (catechol structure) is required for quercetin to exert its inhibitory action on mitochondrial MAO-A activity. Correlating differences in inhibitory effects among quercetin and its structurally related flavonoids with their hydrophobic properties is difficult because their *clogP* values are not very different from each other (Table 1). Taken together, the 3-hydroxy group in the C-ring lowers the inhibitory effect of quercetin considerably, and *O*-methylation of the catechol structure extinguishes its inhibitory effect on mitochondrial MAO-A activity in the mouse brain. The microenvironment of MAO-A located in the outer membranes of mitochondria may affect the effectiveness of the inhibitory effect of such flavonoids, in addition to their intrinsic inhibitory effect on the MAO-A enzyme.

Quercetin is hydrophobic, as suggested by its *clogP* value (Table 1). Therefore, it seems to be able to cross mitochondrial membranes and accumulate within mitochondria, binding to inner proteins.[29] However, our previous study using rats clarified that a low (but detectable) level of dietary quercetin accumulated largely as its conjugated derivatives and its *O*-methylated derivatives in brain tissues.[14] Conversely, several research groups highlighted the possibility of generation of the quercetin aglycone from its conjugated derivatives due to β-glucuronidase activity during inflammation.[30−32] Microglial cells can deliver the aglycone to nerve cells by deconjugation of its conjugated derivatives.[33] In this way, the quercetin aglycone can be generated and act as a weak MAO-A inhibitor by penetrating into the mitochondrial membranes of nerve cells if inflammation in the CNS occurs. Dietary quercetin is also subject to ring scission to decomposition products by the action of intestinal mictoflora.[34] Vissiennon et al.[35] recently reported these decomposition products were responsible for the anxiolytic effect of quercetin in mice under elevated plus maze test. Microflora-dependent metabolic conversion may also participate in the regulation of MAO-A activity when the decomposition products are introduced into the brain tissue through blood circulation.

The fluorescence assay used for the detection of H_2O_2 indicated that quercetin, luteolin, isorhamnetin, and tamarixetin inhibited H_2O_2 generation at only high concentrations (Figure 4B). This may result from the scavenging activity of the superoxide anion radical ($O_2^{\bullet-}$) because the MAO-A reaction accompanies the formation of $O_2^{\bullet-}$, which is then converted to H_2O_2 by a disproportionation reaction.[36] The potential for H_2O_2 generation during the MAO-A reaction may far exceed that of other mitochondrial sources. Therefore, MAO-A may be a major source of H_2O_2 in tissues in ischemia[37] and aging[38] and during oxidation of exogenous amines.[39] Up-regulation of the expression of MAO-A and the resulting increased production of H_2O_2 might be responsible for the mitochondrial damage observed in mental disorders.[40] However, mammalian mitochondria possess a complex multilevel defense network against reactive oxygen species (including antioxidative enzymes and small molecule antioxidants). Therefore, suppression of H_2O_2 formation by flavonoids through $O_2^{\bullet-}$ scavenging may have a limited role in the prevention of the oxidative stress derived from increased MAO-A activity in depression.

The present study using HPLC clarified that tea catechins did not possess inhibitory effects on MAO-A activity in mouse brain mitochondria (Figure 4C). This result is interesting because (−)-epigallocatechin gallate has been reported to be an effective MAO-B inhibitor[20] and suggested to accumulate readily in mitochondrial particles if added into cultured cells.[41] Tea catechins are the derivatives of flavan-3-ol, which has a saturated bond at the 2- and 3-position in the C-ring, and is thus nonplanar (similar to flavanone). We found that planar flavone but *not* nonplanar flavanone was effective in MAO-A inhibition (Figure 5). Hydrophobicity is unlikely to be the determinant of the lack of MAO-A inhibitory effects in tea catechins because their *clogP* values varied independently of their effect on MAO-A activity. Therefore, a nonplanar structure may be crucial for the lack of MAO-A inhibition

activity by tea catechins. That is, a planar structure may be required for flavonoids to exert MAO-A inhibitory effects. Further study on structure−activity relationships are needed to clarify the contribution of the steric factors of flavonoids on MAO-A inhibition.

The galloyl-type catechins (−)-epigallocatechin and (−)-epigallocatechin gallate at higher concentrations increased H_2O_2 formation during incubation of brain mitochondria with 5-HT (Figure 4B). This result is indicative of a pro-oxidant activity of galloyl-type catechins, through which $O_2^{\bullet-}$ can be generated by their autoxidation. (−)-Epigallocatechin gallate is known to act as a pro-oxidant in cellular systems by generating H_2O_2 through its autoxidation.[42,43] Therefore, the measurement of H_2O_2 production for the MAO-A reaction may reflect the pro-oxidant activity of tested flavonoids at higher concentration.

Drugs that inhibit MAO-A have been used for the treatment of depression.[3] However, prolonged intake of MAO-A inhibitors may lead to undesirable consequences. MAO-A located in the digestive system has a crucial role in the detoxification of exogenous amines such as tyramine present in fermented dairy products. Use of some MAO-A inhibitors increases the risk of poisoning by exogenous amines. The mechanism of action of a well-known side effect of MAO inhibitors, the "cheese reaction", is based on the inhibition of intestinal MAO activity during the intake of antidepressants acting as MAO inhibitors.[22] Dietary quercetin is unlikely to exert this side effect because little inhibition was observed in the MAO-A reaction in the small intestine (Figure 7). Thus, quercetin is a weak (but safe) MAO-A inhibitor as compared with antidepressant drugs. Mild attenuation of MAO-A activity by dietary quercetin and other flavonoids may be preferable for the prevention of depression and other elevated MAO activity-related diseases because daily consumption of fruits and vegetables rich in flavonoids seems to accelerate the accumulation of their metabolites in various tissues (including the brain). However, there are three requirements for the exhibition of antidepressant activity by dietary flavonoids as MAO-A inhibitors: (i) high concentrations in the brain or accumulation in synaptosomal mitochondria; (ii) direct encounter with MAO-A (i.e., exposure to the outer membranes of mitochondria); and (iii) release of the aglycone from conjugated metabolites. Furthermore, there is only limited evidence on the in vivo effects of dietary flavonoids in relation to the inhibition of mitochondrial MAO-A in the CNS and the resulting antidepression effects.[44] The results of the present study warrant further investigation of the relationship between the MAO-inhibitory activity of dietary flavonoids and their potential as antidepressant remedies, although this study was limited to in vitro assay using mouse brain mitochondria.

In conclusion, quercetin and its structurally related flavonoid luteolin can act as weak MAO-A inhibitors if mouse brain mitochondria are used as enzyme sources. A planar structure may be essential for flavonoids to inhibit MAO-A activity. Quercetin can modulate mitochondrial MAO-A activity in brain cells without side effects in the digestive tract. These data suggest that dietary quercetin and its related flavonoids are weak (but safe) MAO-A modulators in the CNS.

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Notes

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ABBREVIATIONS USED

CNS, central nervous system; 5-HIAL, 5-hydroxyindole acetaldehyde; 5-HT, 5-hydroxytryptamine; DMSO, dimethyl sulfoxide; HPLC, high-performance liquid chromatography; MAO, monoamine oxidase.

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