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Inhibition of MAO A and B by some plant-derived alkaloids, phenols and anthraquinones

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Abstract

A total of seventeen phytochemicals including seven alkaloids (piperine, strychnine, brucine, stachydrine, tetrandrine, frangchinoline and sinomenine), four phenols (paeonol, honokiol, magnolol and eugenol) and six anthraquinones (emodin, rhein, chrysorphanol, aloe-emodin, physcion and 1,8-dihydroxyanthraquinone) was examined for inhibitory activity of monoamine oxidase (MAO) A and B from rat brain mitochondrial. Among these compounds, piperine and paeonol were found to be inhibitory against MAO A in a dose-dependent manner with IC_{50} values of 49.3 and 54.6 μM, respectively. Piperine, paeonol and emodin were shown to inhibit MAO B in a dose-dependent manner with the IC_{50} data of 91.3, 42.5 and 35.4 μM, respectively. Lineweaver–Burk transformation of the inhibition data indicated that the inhibitory action of piperine on MAO A was of mixed type, and that of paeonol on the same type of the enzyme was of non-competitive type. For piperine, the K_i and K_I were determined to be 35.8 and 25.7 μM, respectively. For paeonol, the K_i was estimated to be 51.1 μM. The inhibition of piperine and paeonol on MAO B was of competitive type with K_i values of 79.9 and 38.2 μM, respectively. The inhibition of emodin on MAO B was of mixed type with the K_i and K_I data of 15.1 and 22.9 μM, respectively. The present investigation showed that the phytochemicals piperine, paeonol and emodin are potent MAO inhibitors whereas other compounds were inactive against any type of MAO at 100 μM in the present assay.

Keywords: Piperine; Paeonol; Emodin; Monoamine oxidase

1. Introduction

Monoamine oxidase (MAO, EC.1.4.3.4) is an important enzyme in the metabolism of a wide range of endogenous monoamine neurotrasmitters such as noradrenaline, dopamine, and serotonin (5-HT). This enzyme catalyzes as well the removal of exogenous amines. Some MAO A inhibitors are efficacious for treating anxiety and depression while the inhibition of MAO B appears to be effective to prevent and treat Parkinson's disease (Silverman et al., 1993; Kanazawa, 1994). However, severe adverse effects such as cytotoxic (Kohda et al., 1998), hyperpyrexia, disseminated intravascular coagulation, convulsions, coma and muscle rigidity (Power et al., 1995) have been observed with some classical MAO-A and/or -B inhibitors mainly owing to the interactions with other drugs and foodstuffs (Dingemanse, 1993). Thus, there is an urgent need to find new MAO inhibitors devoid desirably of these severe adverse effects. As a follow-up to our previous investigation of plant-derived inhibitors of both types of MAO (Kong et al., 2000, 2001; Pan et al., 2000; Zhou et al., 2001), we here with wish to report the pharmacological results with the inhibition on MAO A and B (from rat brain mitochondrial) of seventeen phytochemicals originated from the traditional Chinese medicine, which have long been used for the treatment of some mental diseases and anti-aging (Jiangsu College of New Medicine, 1977).

2. Materials and methods

2.1. Reagents

The phytochemicals (seven alkaloids piperine (Dwuma-Badu et al., 1976), strychnine (Akopian and Shcherbina, 1970), brucine (Yang and Yan, 1993), stachydrine (Singh et al., 1975), tetrandrine (Lin et al., 1993), frangchinoline and sinomenine (Yamasaki, 1976), four phenols paeonol

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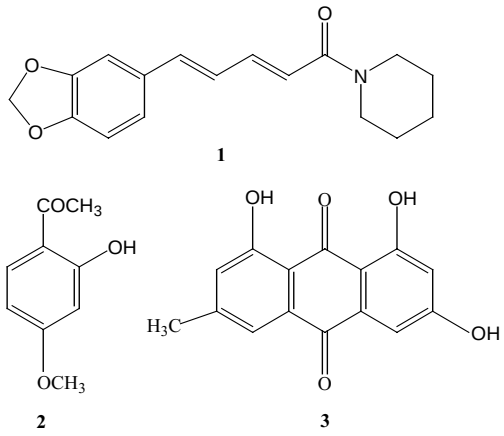

Fig. 1. Structures of piperine (1), paeonol (2) and emodin (3).

(Di et al., 1998), honokiol, magnolol (Maruyama et al., 1998) and eugenol (Zheng et al., 1992), and six anthraquinones emodin (Huang et al., 1991), rhein, chrysophanol, aloe-emodin, physcion (Min et al., 1998; Agarwal et al., 2000) and 1,8-dihydroxyanthraquinone (Eckardt et al., 1985) were provided by the National Institute for Control of Pharmaceutical and Biological Products, Beijing, China. And the purity of each product was ascertained by TLC and HPLC analyses. The structures of bioactive compounds were given in Fig. 1. [^{14}C]-serotonin (5-HT) and [^{14}C]β-phenylethylamine (β-PEA) were products of DuPont NEN (USA), and clorgyline, *l*-deprenyl and dimethyl sulfoxide (DMSO) were purchased from Sigma (USA). All other chemicals used in the study were of analytical grade.

2.2. MAO A and B assay

Rat brain mitochondrial fraction was prepared as a source of MAO activity following the procedure described previously (Schurr and Livne, 1976). Briefly, the mitochondrial fraction and sodium phosphate buffer (50 mM, pH 7.4) were mingled in a proportion of 1:20 with gentle agitation at 4° for 60 min. The mixture was centrifuged immediately at 16,000 × *g* for 30 min at 0° and the pellets were resuspended in the same buffer containing additional sucrose at a concentration of 250 mM. MAO activity was assessed radiochemically by slightly modifying the procedure outlined previously (Fowler et al., 1979; Pizzinat et al., 1999). Thus, the assay mixtures contained 50 μM [^{14}C]5-HT or 10 μM [^{14}C]β-PEA as specific substrates for MAO A and B, respectively, 10 μl solution of isolated compounds in DMSO at different concentrations, and 100 mM sodium phosphate buffer (pH 7.4) up to a final volume of 200 μl. After a 20 min preincubation at 37 °C, the reaction was started by adding 50 μg of the mitochondrial fraction. The reaction was allowed to proceed at 37 °C for 20 min, and terminated by addition of 2 M HCl (1 ml), the radioactive product was extracted with 2 ml of toluene/ethyl acetate (v/v, 1:1). The radioactivity of the organic phase was counted in a liquid scintillation spectrometer. Blank samples were prepared by adding 2 M HCl (1 ml) prior to reaction, and worked up subsequently in the same manner. Enzyme activity was expressed as nmol product formed per mg protein per min. In the kinetic analyses, the reaction mixture consisting of different concentrations of [^{14}C]5-HT (20–200 μM) or [^{14}C]β-PEA (3.3–20 μM) were used as MAO A or B substrates, respectively, in the absence and presence of inhibitors.

2.3. Estimation of protein

Protein concentration was estimated by the Lowry method (Lowry et al., 1951) using bovine serum albumin as the standard.

2.4. Data analysis

The data were presented as $\bar{x} \pm s$. The IC_{50} value was calculated using computer software 'GraphPad InPlot'. The K_i and K_I values were determined by consulting Lineweaver–Burk's plot using linear regression analysis. Specifically, K_i was calculated from the slope of the inhibition curve by the equation (slope $= K_m/V_{max}(1+[I_0]/K_i)$) ($[I_0]$, K_m and V_{max} representing inhibitor's initial concentration, Michaelis constant and maximum initial velocity, respectively), and K_I was calculated from the *y*-intercept of the inhibition curve using the equation *y*-intercept $= 1/V_{max}(1 + [I_0]/K_I)$.

3. Results

3.1. Inhibition of phytochemicals on MAO A

Among the seventeen test compounds, piperine and paeonol (Fig. 1) inhibited the activity of MAO A in a dose-dependent manner with IC_{50} values of 49.3 and 54.6 μM, respectively (Fig. 2). However, others exhibited no inhibition on this type of MAO (IC_{50} value > 100 μM). In the study, the IC_{50} value of clorgyline, a MAO A inhibitor used as a positive control, was estimated to be 0.2 μM. The Lineweaver–Burk plots of piperine and paeonol for 5-HT (as a substrate) were shown in Figs. 3 and 4. The mode of inhibition of MAO A by piperine was shown to be of mixed type with K_i and K_I data of 35.8 and 25.7 μM, respectively. The mode of inhibition of MAO A by paeonol was non-competitive with the K_i value of 51.8 μM.

3.2. Inhibition of phytochemicals on MAO B

Piperine, paeonol and emodin (Fig. 1) among the assayed compounds inhibited the activity of MAO B in a dose-dependent manner with IC_{50} values of 91.3, 42.5 and 35.4 μM, respectively (Fig. 5). In our study, the IC_{50} value of deprenyl, a MAO B inhibitor used as a positive control, was 0.3 μM. The modes of inhibition towards β-PEA as a

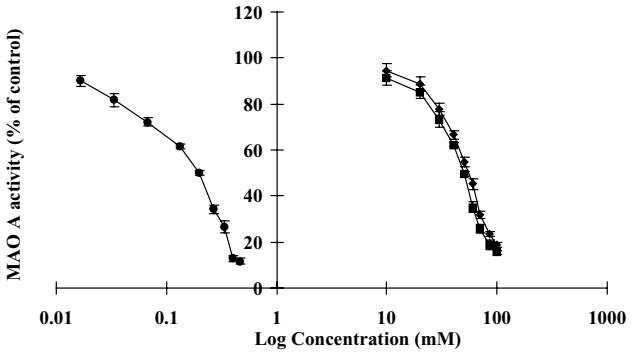

Fig. 2. Dose-dependent inhibitory actions of piperine and paeonol on MAO A. MAO A assays were performed as described in Section 2. Different concentrations of piperine (■), paeonol (◆) and clorgyline (●)were incorporated in the assays. Results are expressed as percentage of control where no inhibitor was added. Data are the average of five independent experiments and error bars indicate standard deviations.

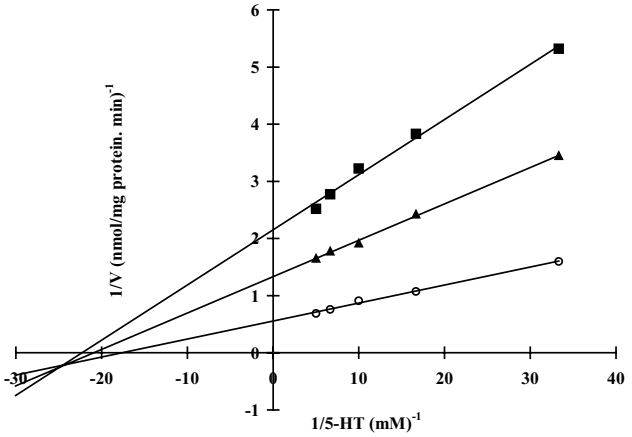

Fig. 3. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO A by piperine. MAO assay was performed at different concentrations of the substrate [^{14}C]5-HT. Control without any inhibitor (○), in the presence of 25 (▲) and 50 μM (■) piperine. The values are expressed as the average of triplicates.

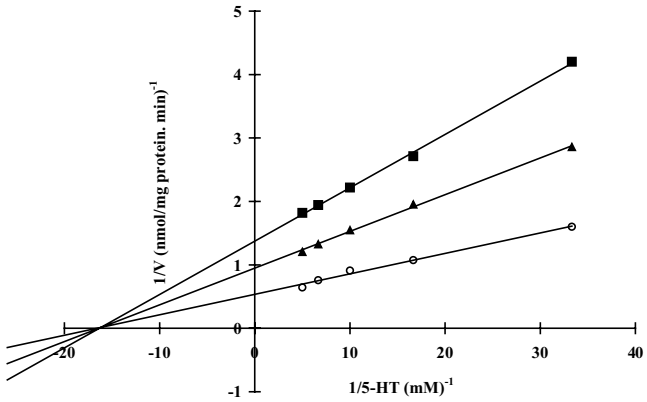

Fig. 4. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO A by paeonol. MAO assay was performed at different concentrations of the substrate [^{14}C]5-HT. Control without any inhibitor (○), in the presence of 27 (▲) and 54 μM (■) paeonol. The values are expressed as the average of triplicates.

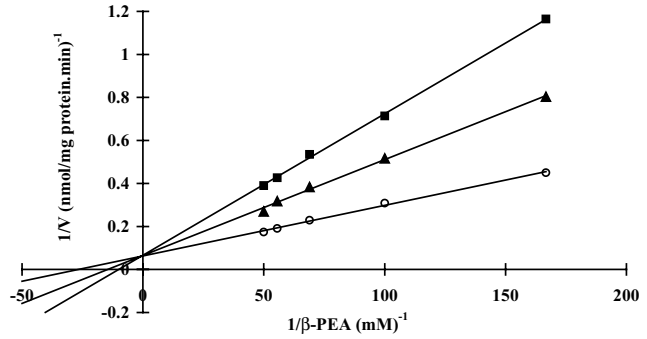

Fig. 6. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO B by piperine. MAO assay was performed at different concentrations of the substrate [^{14}C]β-PEA. Control without any inhibitor (○), in the presence of 45 (▲) and 90 μM (■) piperine. The values are expressed as the average of triplicates.

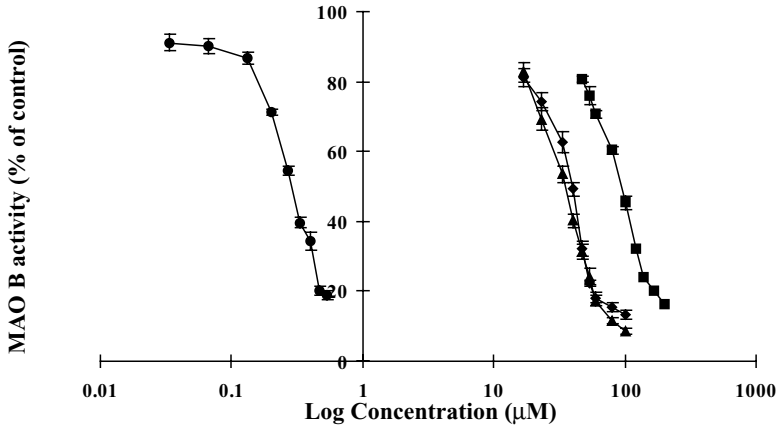

Fig. 5. Dose-dependent inhibitory actions of piperine, paeonol and emodin on MAO B. MAO B assays were performed as described in Section 2. Different concentrations of piperine (■), paeonol (◆), emodin (▲) and deprenyl (●) were incorporated in the assays. Results are expressed as percentage of control where no inhibitor was added. Data are the average of five independent experiments and error bars indicate standard deviations.

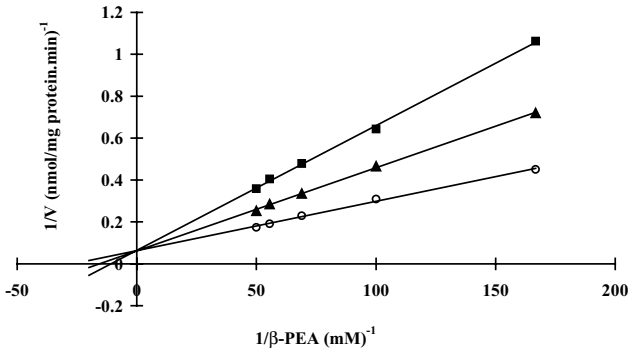

Fig. 7. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO B by paeonol. MAO assay was performed at different concentrations of the substrate [^{14}C]β-PEA. Control without any inhibitor (○), in the presence of 21 (▲) and 42 μM (■) paeonol. The values are expressed as the average of triplicates.

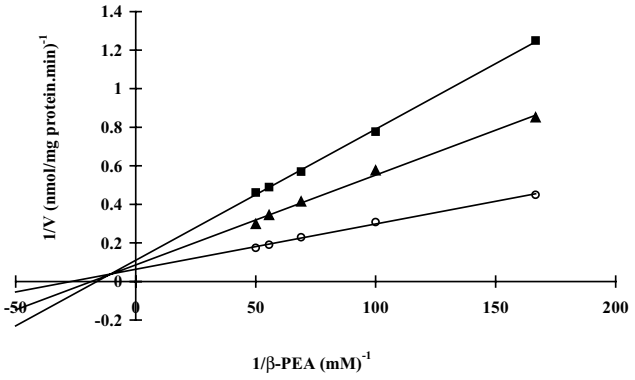

Fig. 8. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO B by emodin. MAO assay was performed at different concentrations of the substrate [^{14}C]β-PEA. Control without any inhibitor (○), in the presence of 17 (▲) and 35 μM (■) emodin. The values are expressed as the average of triplicates.

substrate by both piperine and paeonol were of competitive type with K_i values of 79.9 and 38.2 μM, respectively (Figs. 6–8). However, emodin was of mixed type for β-PEA as a substrate with the K_i and K_I values of 15.1 and 22.9 μM.

4. Discussion

Among seven alkaloids, only the piperidine derivative piperine (1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine) showed inhibitory activities towards MAO A and B. And, inhibition of piperine on MAO A was more potent than that on MAO B. Others such as the benzylisoquinoline alkaloids sinomenine and fangchinoline, the pyrroline derivative stachydrine and monoterpenic indole base strychnine had no inhibitory activities towards any type of MAO in the present assay. In our previous communication (Kong et al., 2001), we mentioned that jatrorrhizine with a phenolic hydroxyl have a stronger inhibitory activity against MAO A and B than that of berberine of the type but without phenolic hydroxyl which could be necessary for the initiation of the enzyme inhibitory activity. Regarding piperine which is free of phenolic hydroxyl, we postulated that the discerned inhibition could be presumably initiated by the hydrogen bonding of its naked amide with active protons such as –NH–, –OH and –SH in the active sites of both type of MAO. Piperine is best known as the pungent principle of the black pepper (*Piper nigrum*) and found to have a wide spectrum of pharmacological activities such as being anticonvulsant and stimulating serotonin biosynthesis in the rat brain (Eldershaw et al., 1994). The present investigation ascertained for the first time that piperine is also an MAO inhibitor, and the finding may be of importance to obtain a better understanding of the traditional application of *Piper nigrum*.

Among the four assayed plant phenols, paeonol (2-hydroxy-4-methoxyacetophenone) showed exclusively inhibitory activities towards MAO A and B. However, its inhibition on MAO A is a bit less than that on type B of the enzyme. This observation could rationalize to some extend the traditional application of root bark of *Paeonia suffruticosa* (the main source plant of paeonol) as a sedative agent to treat central stress (Jiangsu College of New Medicine, 1977). Surprisingly, the other three phenols eugenol, honokiol and magnolol exhibited no inhibition on any type of MAO in the study. The striking difference in the enzyme inhibition among these plant phenols could be due to the deviation of the structure type, and of the feasibility for the functions (say, phenolic hydroxyl and ketone) to interact with the active site of MAO via hydrogen bonding. However, the anxiolytic effect of honokiol and magnolol, which also were the main principals of a famous formula Banxia Houpu Decoction, often used to treat depression and anxiety (Maruyama et al., 1998; Luo et al., 2000), is most probably based on other mechanism(s).

Among six anthraquinones, only emodin (3-methyl-1,6,8-trihydroxyanthraquinone) showed an inhibition on MAO B. Structurally, emodin is closely related to 1,8-dihydroxyanthraquinone, physcion (3-*O*-methyl ether of emodin) chrysophanol (3-dehydroxy-emodin). Both hydroxyls on C-1 and C-8 are equally hydrogen-bonded with the 9-carbonyl group limiting presumably their interaction with the active sites of MAO B. Furthermore, the quinones with 3-hydroxymethyl group and H-6 as in aloe-emodin and rhein, or without any substituent on C_3 and C_6 as in 1,8-dihydroanthraquinone, did not show any inhibition on both type of MAO. The observation indicated that the 'free phenolic hydroxyl', as emodin bears, is necessary for inhibiting MAO B. Phytochemically, emodin happens to be the main constituent of rhizomes of *Polygonum multiflorum* that has been used for anti-aging purpose in China since ancient times. Previously, the extract of the plant was also found to be inhibitory against MAO B without ascertaining the corresponding active constituents (Jiangsu College of New Medicine, 1977; Cheng et al., 1991). Our findings indicate that emodin could be the main MAO B inhibitory principle in the herb, and presumably in the extract as well.

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