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 IC50 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 IC50 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 Ki and Ki were determined to be 35.8 and 25.7 μM, respectively. For paeonol, the Ki was estimated to be 51.1 μM. The inhibition of piperine and paeonol on MAO B was of competitive type with Ki values of 79.9 and 38.2 μM, respectively. The inhibition of emodin on MAO B was of mixed type with the Ki and Ki 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.

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1. Introduction

Monoamine oxidase (MAO, EC 1.4.3.4) is an important enzyme in the metabolism of a wide range of endogenous monoamine neurotransmitters 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 -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 (Yung and Yan, 1993), stachydrine (Singh et al., 1975), tetrandrine (Lin et al., 1993), frangchinoline and sinomenine (Yamasaki, 1976), four phenols paeonol
Mitochondrial fraction and sodium phosphate buffer (50 mM, pH 7.4) were used previously (Schurr and Livne, 1976). Briefly, the mitochondrial fraction of MAO activity following the procedure described previously (Fowler et al., 1979; Pizzinat et al., 1999). Thus, MAO A and B assay other chemicals used in the study were of analytical grade. 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 using the equation (slope = \( K_i/V_{max} (1+[I]/K_i) \)) \( \bar{[I]} \), \( K_{ii} \) 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 \( y\text{-intercept} = 1/V_{max}(1+\bar{[I]}/K_i) \).

2.1. 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 fractionation conditions. Enzyme activity was assayed as nmoles product formed per mg protein per min. In the kinetic analyses, the reaction mixture consisting of different concentrations of [14C]-5-HT (20–200 \( \mu \)M) or [14C]-β-PEA (3.3–20 \( \mu \)M) were used as MAO A or B substrates, respectively, in the absence and presence of inhibitors.

2.2. MAO A and B assay

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 \( \mu \)M, respectively (Fig. 2). However, others exhibited no inhibition on this type of MAO (IC(50) value > 100 \( \mu \)M). In the study, the IC(50) value of clorgyline, a MAO A inhibitor used as a positive control, was estimated to be 0.2 \( \mu \)M. 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 non-competitive with the \( K_i \) value of 51.8 \( \mu \)M.

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 \( \mu \)M, respectively (Fig. 2). However, others exhibited no inhibition on this type of MAO (IC(50) value > 100 \( \mu \)M). In the study, the IC(50) value of clorgyline, a MAO A inhibitor used as a positive control, was estimated to be 0.2 \( \mu \)M. 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 non-competitive with the \( K_i \) value of 51.8 \( \mu \)M.

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 \( \mu \)M, respectively (Fig. 5). In our study, the IC(50) value of deprenyl, a MAO B inhibitor used as a positive control, was 0.3 \( \mu \)M. 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 paeonol. MAO assay was performed at different concentrations of the substrate [14C]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. 4. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO A by paeonol. MAO assay was performed at different concentrations of the substrate [14C]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. 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. 6. Lineweaver–Burk plot of inhibition on rat brain mitochondrial MAO B by piperine. MAO assay was performed at different concentrations of the substrate [14C]β-PEA. Control without any inhibitor (○), in the presence of 45 (▲) and 90 μM (●) piperine. The values are expressed as the average of triplicates.
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presence of 17 (H17009/H17009/K substrate by both piperine and paeonol were of competitive inhibition on rat brain mitochondrial MAO B by emodin. MAO assay was performed at different concentrations of the substrate [14C]-PEA. Control without any inhibitor (C), in the presence of 21 (A) and 42 μM (B) paeonol. The values are expressed as the average of triplicates.

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

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 B by emodin. MAO assay was performed at different concentrations of the substrate [14C]-PEA. Control without any inhibitor (C), in the presence of 21 (A) and 42 μM (B) paeonol. The values are expressed as the average of triplicates.

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 extent 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, phsycion (3-O-methyl ether of emodin) chrysophanol (3-dehydroxy-emodin). Both quinones 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 C7 and C8 as in 1,8-dihydroxanthraquinone, 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 multimflorum 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.