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Inhibition of Monoamine Oxidase by Palmatine

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Monoamine oxidase (EC 1.4.3.4; MAO), present in the outer mitochondrial membrane, plays an important role in the catabolism of catecholamines and serotonin, and regulation of their levels in the brain (Tipton *et al.*, 1987). In addition, MAO confers protection by inactiva-ting potentially toxic exogenous amines.

Many MAO inhibitors have beem reported belonging to various chemical classes: isoquinolines and tetrahydroisoquinolines (Bembenek *et al.*, 1990), 4-(2-benzofuranyl)piperidines (Strolin-Benedetti and Dostert, 1987), oxadiazoles (Mazouz *et al.*, 1990), phenoxathin-10,10-dioxides (White and Scates, 1992), and natural xanthones (Suzuki *et al.*, 1981). In addition, oxygen-containing compounds such as coumarin, dibenzofuran, flavone, xanthene, thioxanthone and acridone also exert an inhibitory action against MAO (Thull and Testa, 1994). Among the isoquinoline derivatives, salsolinol, tetrahydropapaveroline, 1-methylisoquinoline, N-methylisoquinolinium ion and tetrahydroisoquinolines inhibit MAO activity (Bembenek *et al.*, 1990; Giovine *et al.*, 1976; Naoi *et al.*, 1988, 1989).

Recently, we investigated the inhibitory effects of bioactive fractions from *Coptis japonica* on MAO activity and were able to prove that their bioactive fractions mainly contained the protoberberine isoquinoline alkaloids (data not shown). Palmatine belongs to a group of the protoberberine alkaloids and has been demonstrated to carry antiarrhythmic (Ma *et al.*, 1985), analgesic (Chen *et al.*, 1984), antimalarial (Vennerstrom and Klayman, 1988) and sedative (Hsieh *et al.*, 1993) effects. In this study, the inhibitory effects of palmatine on MAO activity were examined using kynuramine as a substrate.

Palmatine chloride, kynuramine, 4-hydroxyquinoline, zinc sulfate, iproniazid and sucrose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade.

Mice (ICR, male, 20-25 g) were obtained from Samyook Animal Center (Soowon, Korea). Mouse brain mitochondria was isolated by the method of Thull and Testa (1994) with a slight modification (Lee *et al.*, 1999). MAO activity was adjusted to 0.320 ± 0.012 nmol/min/mg protein for the experiments. Protein amounts were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

MAO activity was measured fluorimetrically using kynuramine as a substrate according to Kraml's method (1965). Level of 5-hydroxyquinoline formed by enzyme reaction for 30 min at 37°C was measured using fluorospectrophotometer (λex., 315 nm/λem., 380 nm, Hitachi Model F-3000, Tokyo, Japan).

The values of the Michaelis constant (K_m) and the maximum velocity (V_{max}) were obtained by Lineweaver-Burk's plot using various concentrations of kynuramine. Results were presented means ± SEM from five experiments. Statistical significances were determined at the $P<0.05$ using Student's t-test.

Previously, the bioactive fractions containing the protoberberine isoquinoline alkaloids were found to inhibit MAO activity (data not shown). In addition, isoquinoline compounds such as salsolinol, tetrahydropapaveroline, N-methylisoquinoline, 1-methylisoquinoline, tetrahydroisoquinolines and higenamine have been found to inhibit MAO activity (Bembenek *et al.*, 1990; Giovine *et al.*, 1976; Naoi *et al.*, 1988, 1989; Lee *et al.*, 1999). Hence, we investigated the effects of palmatine on MAO activity.

In this experiment, palmatine displayed a mild inhibitory effect on MAO (40.0% inhibition at concentration of 50 μM) (Table I). The IC_{50} value of palmatine was 90.6 μM. However, pamatine's activity against MAO was less than iproniazid, a selective MAO inhibitor (Table I).

The values of K_m and V_{max} of the mouse brain MAO using kynuramine as a substrate were 78.2 ± 4.0 μM

Table I. Inhibitory effects of palmatine on mouse brain monoamine oxidase (MAO).

Inhibitor	MAO activity (nmol/min/mg protein) (% of control)
Control	0.320±0.012 (100)
Iproniazid, 10 μM	0.163±0.031 (50.9)**
Palmatine, 25 μM	0.241±0.016 (75.3)*
50 μM	0.192±0.015 (60.0)**
100 μM	0.152±0.007 (47.5)***

Control level of MAO activity was taken as 0.320 nmol/min/mg protein. Iproniazid was used as a positive control. The data are presented as means ± SEM for five experiments. Significantly differenct from the control value: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (Student's t-test).

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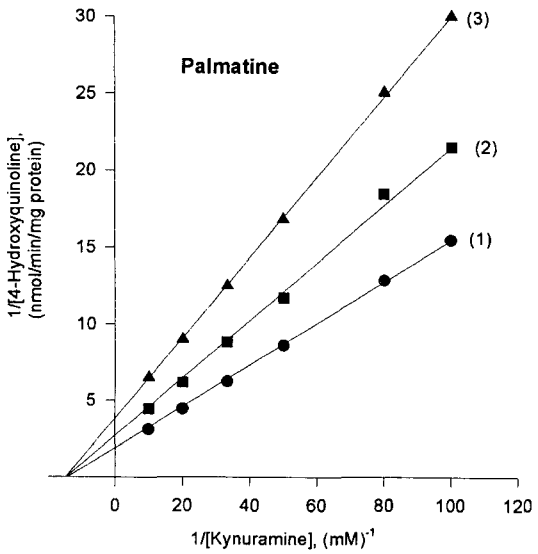

Fig. 1. Inhibition of monoamine oxidase (MAO) by palmatine added in the enzyme reaction mixture. The reciprocal of MAO activity was plotted against the reciprocal of substrate concentration. Palmatine concentration: 1, 0 μM; 2, 25 μM; 3, 80 μM.

(n=5) and 0.65 ± 0.05 nmol/min/mg protein (n=5), respectively. Furthermore, the kinetic analysis by a Lineweaver-Burk reciprocal plot indicates that palmatine is a noncompetitive inhibitor of MAO (Fig. 1).

Palmatine exerts a sedative effect. The mechanism of action may be related to decrease in the levels of catecholamine and serotonin in the cortex (Hsieh *et al.*, 1993). Palmatine was found to decrease dopamine content by reducing tyrosine hydroxylase activity in PC12 cells (Lee and Kim, 1996). Palmatine also inhibits competitively bovine adrenal tyrosine hydroxylase (Lee *et al.*, 1997). Our present study indicates that palmatine noncompetitively inhibits MAO activity. Palmatine interacts with DNA, but does not induce apoptosis in human leukemic HL-60 cells (Kuo *et al.*, 1995). Palmatine also interacts with the HIV-I reverse transcriptase and inhibits the enzyme activity (Gudima *et al.*, 1994). Despite palmatine's interaction with DNA, above results suggest that palmatine may be involved in the regulation of catecholamine levels at the biologically active sites. The *In vivo* physiological functions of palmatine need to be studied further.

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