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Inhibitory Effects of Coptisine on Monoamine Oxidase Activity

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Abstract – The effects of coptisine on monoamine oxidase (EC 1.4.3.4; MAO) activity in mouse brain were investigated. Coptisine showed an inhibitory effect on MAO activity with a concentration-dependent manner. Coptisine exhibited 51.0% inhibition of MAO activity at 9 μM. The IC_{50} value of coptisine was 8.7 μM. Coptisine inhibited MAO activity competitively with kynuramine as a substrate. The K_i value of coptisine was 4.1 μM. These results indicate that coptisine functions to regulate the catecholamine content at biologically active sites.

Key words – monoamine oxidase, coptisine, mouse brain, kynuramine, competitive inhibition

Introduction

Monoamine oxidase (EC 1.4.3.4; MAO) plays an important role in the catabolism of catecholamines and serotonin, and participates in regulating their levels in the brain (Nagatsu *et al.*, 1970). MAO also plays a protective role by inactivating potentially toxic exogenous amines (Tipton *et al.*, 1987).

Among the many MAO inhibitors, isoquinolines and tetrahydroisoquinolines show an inhibition of MAO activity (Naoi *et al.*, 1989; Bembenek *at al.*, 1990). Recently, we have proved that the protoberberine isoquinoline alkaloids such as berberine and palmatine inhibit MAO activity (Lee *et al.*, 1999a). Coptisine is also a protoberberine alkaloid and its chemical structure is very similar with berberine and palmatine.

In this study, therefore, the inhibitory effects of coptisine on MAO activity in mouse brain were examined using kynuramine as a substrate.

Experimental

Materials – Coptisine was prepared by Dr J. S. Ro, College of Pharmacy, Chungbuk National University (Korea). Kynuramine, 4-hydroxyquinoline, zinc sulfate, iproniazid and sucrose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade.

Enzyme preparation – Mice (ICR, male, 20-25 g) were obtained from Samyook Animal Laboratory Inc. (Osan, Korea). A crude mitochondrial fraction from mouse brain was isolated by the method of Thull and Testa (1994) with a slight modification (Lee *et al.*, 1999a). MAO activity was adjusted to 0.308±0.017 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.

Assay for MAO activity – MAO activity was measured fluorimetrically using kynuramine as a substrate according to the method of Kraml (1965). 5-Hydroxyquinoline formed by enzyme reaction for 30 min at 37°C was measured using a fluorescence spectrophotometer (λex., 315 nm; λem., 380 nm, Hitachi Model F-3000, Tokyo, Japan).

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

Results and Discussion

Previously, natural isoquinoline alkaloids such as berberine, palmatine, higenamine and sanguinarine have been found to inhibit MAO activity (Lee *et al.*, 1999a; 1999b; 2000). We, therefore, investigated the effects of coptisine, one of the protoberberine isoquinoline alkaloids, on MAO activity.

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Table 1. Inhibitory effects of coptisine on monoamine oxidase (MAO) in mouse brain.

Inhibitors		MAO activity (% of control) (nmol/min/mg protein)	IC_{50} value (μM)
Control		0.308±0.017(100)	
Iproniazid			12.1
	10 μM	0.155±0.031(50.3)**	
Coptisine			8.7
	3 μM	0.220±0.006(71.4)*	
	9 μM	0.151±0.010(49.0)***	
	15 μM	0.112±0.009(36.4)***	

The control of MAO activity was taken as 0.308 nmol/min/mg protein. Iproniazid was used for the positive control. Results represent the means±SEM of five dishes. Significantly different from the control value: *P<0.05; **P<0.01; ***P<0.001 (Student's t test).

Coptisine inhibited MAO activity with a concentration-dependent manner (51.0% inhibition at 9 μM) (Table 1). The IC_{50} value of coptisine was 8.7 μM. Coptisine showed slightly stronger inhibition of MAO activity than iproniazid, a selective MAO inhibitor (IC_{50} value of iproniazid, 12.1 μM) (Table 1).

According to the kinetic properties of MAO from mouse brain, the values of K_m and V_{max} by using a substrate kynuramine were 78.2±4.0 μM (n = 5) and 0.65±0.05 nmol/min/mg protein (n = 5), respectively. The kinetic analysis by a Lineweaver-Burk reciprocal plot indicates that coptisine is a competitive inhibitor of MAO with respect to kynuramine. The K_i value for coptisine was 4.1±0.8 μM (n = 3).

Coptisine has a protoberberine ring and its chemical structure is very similar with berberine and palmatine. Coptisine provided a greater inhibitory effect on MAO activity than berberine and palmatine (IC_{50} values of berberine and palmatine, 98.2 μM and 90.6 μM) (Lee *et al.*, 1999a). In addition, berberine and palmatine exhibit non-competitive inhibition of MAO (K_i values of berberine and palmatine, 44.2 μM and 58.9 μM) (Lee *et al.*, 1999a). These results suggest that the inhibitory effects and kinetic pattern between coptisine, berberine and palmatine are quite different.

Recently, berberine and palmatine are found to decrease dopamine content by reducing the tyrosine hydroxylase activity in PC12 cells (Shin *et al.*, 2000). Berberine and palmatine also inhibit competitively bovine adrenal tyrosine hydroxylase (Lee and Zhang, 1996; Lee *et al.*, 1995). However, treatment of coptisine does not alter dopamine content in PC12 cells (Shin *et al.*, 2000).

Taken together, these results suggest that coptisine is a new, selective and strong inhibitor of MAO. The kinetic mechanisms of coptisine including structure-activity relationship, and type A and B of MAO need further studies.

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