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Inhibition of Tyrosine Hydroxylase by Palmatine

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Palmatine, an protoberberine isoquinoline alkaloid, has been found to inhibit dopamine biosynthesis by reducing tyrosine hydroxylase (TH) activity in PC12 cells (Lee and Kim, 1996). We have therefore investigated the effects of palmatine on bovine adrenal TH. Palmatine showed a mild inhibition on bovine adrenal TH (36.4% inhibition at concentration of 200 μM). Bovine adrenal TH was inhibited competitively by palmatine with a substrate L-tyrosine. The Ki value was found to be 0.67 mM. This result suggests that the inhibition of TH activity by palmatine may be partially involved in the reduction of dopamine biosynthesis in PC12 cells.

Key Words : Palmatine, Bovine adrenal, Tyrosine hydroxylase, L-tyrosine, DL-6-methyl-5,6,7,8-tetrahydropterine, Competitive inhibition

INTRODUCTION

Palmatine is an isoquinoline alkaloid and has been demonstrated as antiarrhythmic (Ma *et al.*, 1985), analgesic (Chen *et al.*, 1984), antimalarial (Vennerstrom and Klayman, 1988) and sedative (Hsieh *et al.*, 1993) effects.

Isoquinoline derivatives have many biological functions. Salsolinol and N-methylisoquinolinium ion inhibit tyrosine hydroxylase (TH) and monoamine oxidase (MAO) activities (Minami *et al.*, 1992; Naoi *et al.*, 1989). Tetrahydroisoquinolines and 1-methylisoquinoline have been found to inhibit MAO (Minami *et al.*, 1992; Naoi *et al.*, 1988; Bembeneck *et al.*, 1990). TH (EC 1.14.16.2), the rate-limiting enzyme in the catecholamine biosynthesis, catalyzes the formation of L-DOPA from L-tyrosine (Nagatsu *et al.*, 1964).

Previously, we have reported that palmatine exhibits the ability to decrease dopamine content in rat pheochromocytoma PC12 cells (Lee and Kim, 1996). TH activity has also been reduced by the addition of palmatine in PC12 cells (Lee and Kim, 1996). It was thought that palmatine might be able to inhibit the activity of TH. Therefore, we investigated this hypothesis in bovine adrenal TH using L-tyrosine as a substrate.

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MATERIALS AND METHODS

Materials

Palmatine chloride, L-tyrosine, DL-6-methyl-5,6,7,8-tetrahydropterine, catalase, 3,4-dihydroxybenzylamine and alumina were purchased from the Sigma Chemical Company (St. Louis, MO, USA). All other reagents were of reagent grade.

Bovine adrenal TH

Bovine adrenal TH was purified with minor modification according to the method of Joh and Ross (1983). The bovine adrenal medulla (50 g) was homogenized with 10 mM potassium phosphate buffer (pH 7.0) and filtered through cheese cloth. The filtrate was then centrifuged at 1,000×g for 5 min. The supernatent was further centrifuged at 105,000×g for 30 min. The sediment was dissolved in 20 mM potassium phosphate buffer (pH 7.0) and the solution was subjected ammonium sulfate precipitation at 80% saturation. The 80% ammonium sulfate precipitate was dialyzed against 10 mM potassium phosphate buffer (pH 7.0) and the protein was fractionated, taking the protein which precipitated between 30 and 60% saturation in ammonium sulfate. This fraction was dialyzed against 10 mM the buffer and the protein amount was determined according to the method of Lowry *et al.* (1951) using bovine albumin as standard. TH activity obtained from the final enzyme preparations was adjusted to 1.10 nmol/min/mg protein for the experiments.

Assay for TH

TH activity was determined using L-tyrosine as substrate according to a slightly modified method of Nagatsu *et al.* (1979) as described previously (Lee *et al.*, 1994). The reaction mixture contained 1.5 M NaOAc (pH 5.8, 20 μl), 10 mM tyrosine (10 μl), 10 mM 6-methyltetrahydropterine (10 μl), 2 mg/ml catalase (10 μl) and enzyme preparation (50 μl). The enzyme reaction was performed at 37°C for 10 min, and the reaction was stopped with 600 μl of 0.5 M perchloric acid containing 100 pmol of 3,4-dihydroxybenzylamine (internal standard). After addition of 5 ml of EDTA (2%) and 1.5 ml of KH_2PO_4 (0.35 M), an aliquot of N-NaOH were added to adust the pH to 8.4-8.6, and then the reaction mixture was passed through the alumina cartridge (100 mg). L-DOPA and 3,4-dihydroxybenzylamine absorbed were eluted with 300 μl of 0.5 M HCl. Twenty μl of the eluate were injected into the high-performance liquid chromatograph with a CM8010 electrochemical detector (Toso, Japan) and a TSK-gel ODS 120T (5 μm, 25×0.45 cm, Toso). The mobile phase was a 0.1 M potassium phosphate buffer (pH 3.5)-1% methanol with a flow rate of 1 ml/min. The detector potential was set at 0.8 V against the Ag/AgCl electrode.

Data analysis

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 L-tyrosine.

RESULTS AND DISCUSSION

Palmatine can be grouped the protoberberine isoquinoline alkaloids and has many biological functions such as analgesic and sedative effects (Chen *et al.*, 1984; Hsieh *et al.*, 1993).

In this experiment, palmatine showed an inhibitory effect on bovine adrenal TH (36.4% inhibition at concentration of 200 μM) (Table I). The values of K_m and V_{max} on bovine adrenal TH using L-tyrosine were 77±5 μM (n=4) and 0.78 nmol/min/mg protein, respectively. The effects of palmatine on TH kinetics were shown in Fig. 1. This plot indicated competitive inhibition with respect to L-tyrosine according to the definition of Lineweaver-Burk. The Ki value for palmatine was found to be 0.67 mM.

Table I. Inhibitory effects of palmatine on bovine adrenal TH

Inhibitor	TH activity (nmol/min/mg protein) (% of control)
Control	1.10±0.14 (100)
Palmatine, 50 μM	9.96±0.24 (87.3)
100 μM	0.83±0.11 (75.5)
200 μM	0.70±0.09 (63.6)*

The control of TH activity, 1.10 nmol/min/mg protein, was taken as 100. The data were expressed as means±SEM for 4 experiments. Significantly different from the control value : *, $p<0.05$ (Student's t test)

(±)Tetrahydropalmatine, which posseses analgesic as well as sedative-tranquilizing and hypnotic activity, has been found to have an antagonistic action at the level of D_1 and D_2 receptors in human putamen (Vauquelin *et al.*, 1989). As reported previously, protoberberine alkaloids such as berberine and palmatine have an inhibitory effect on dopamine biosynthesis by reducing TH activity in PC12 cells (Lee *et al.*, 1994; Lee and Kim, 1996). Palmatine, up to 20 μg/ml medium, does not show cytotoxicity towards PC12 cells (Lee and Kim, 1996).

Palmatine has a sedative effect and its mechanism may be related to decrease the catecholamine and serotonin content in the cortex (Hsieh *et al.*, 1993). Berberine, an analog of palmatine, interacts with DNA and induces the apoptosis in human leukemic HL cells (berberine treatment; 25 μg/ml for 6-48 hr) (Kuo *et al.*, 1995). But palmatine (25 μg/ml for 2-24 hr), which complexes with DNA in the cells, is not able to induce the apoptosis (Kuo *et al.*, 1995). Palmatine also interacts with the HIV-I reverse transcriptase and inhibits the enzyme activity (Gudima *et al.*, 1994).

In the present study, palmatine competitively inhibited the bovine adrenal TH activity. Despite of palmatine's interaction with DNA, we suggest that the inhibition of TH activity by palmatine may be par-

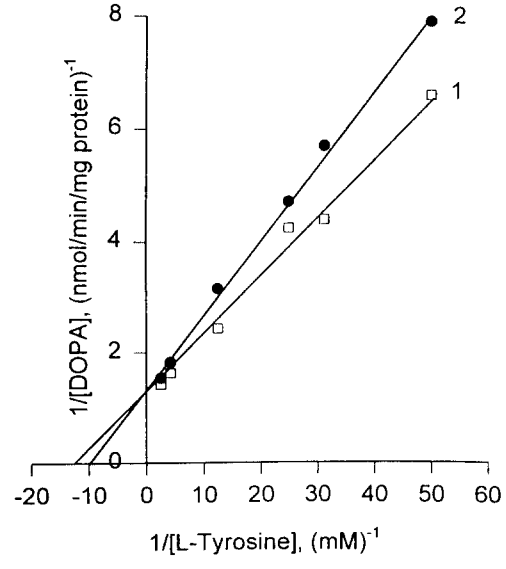

Fig. 1. Inhibition of adrenal TH by palmatine added in the enzyme reaction mixture. The data were plotted by linear regression analysis. Palmatine concentration: 1, nil; 2, 200 μM

tially involved in the reduction of dopamine biosynthesis in PC12 cells. The intracellular mechanisms of palmatine need further investigation.

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REFERENCES CITED

Bembeneck, M. E., Abell, C. W., Chrisey, L. A., Rozwadowska, M. D., Gessner, W. and Brossi, A., Inhibition of monoamine oxidases Aand B by simple isoquinoline alkaloids: Racemic and optically active 1,2,3,4-tetrahydro-, 3,4-dihydro-, and fully aromatic isoquinolines. *J. Med. Chem.*, 33, 147-152 (1990).

Chen, S. H. and Sha, N. H., Dose dependent relationship of analgesic effect of Corydalis app. (Papaveraceae) in mice. *Chin. J. Pharm. Anal.*, 4, 162-163 (1984).

Gudima, S. O., Memelova, L. V., Borodulin, V. B., Pokholok, D. K., Mednikov, B. M., Tolkachev, O. N. and Kochetkov, S. N., Kinetic analysis of interaction of human immunodeficiency virus reverse transcriptase with alkaloids. *Molekul. Biolog. (Russia)*, 28, 1308-1314 (1994).

Hsieh, M.-T., Su, S.-H., Tsai, H.-Y., Peng, W.-T., Hsieh, C.-C. and Chen, C.-F., Effects of palmatine on motor activity and the concentration of central monoamines and its metabolites in rats. *Japan J. Pharmacol.*, 61, 1-5 (1993).

Joh, T. H. and Ross, M. E., Preparation of catecholamine-synthesizing enzymes as immunogens for immunohistochemistry, In Cuello, A. C. (Ed.), *Immunohistochemistry*. 1983, Oxford IBRO Handbook, Wiley, New York, pp. 121-138.

Kuo, C. L., Chou, C. C. and Yung, B. Y. M., Berberine complexes with DNA in the berberine-induced apoptosis in human leukemic HL-60 cells. *Cancer Lett.*, 93, 193-200 (1995).

Lee, M. K., Park, W. K. and Kim, H. S., Inhibitory effects of the root of *Coptis japonica* on catecholamine biosynthesis in PC12 cells. *Arch. Pharm. Res.*, 17, 269-272 (1994).

Lee, M. K. and Kim, H. S., Inhibitory effects of the protoberberine alkaloids from the root of *Coptis japonica* on catecholamine biosynthesis in PC12 cells. *Planta Med.*, 62, 31-34 (1996).

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265-275 (1951).

Ma, S. S., Chen, K. G., Ma, Y. L. and Bau, S. F., Screening test of antiarrythmia effect of *Corydalis turtschaninovii* (Papaveraceae) in rats. *Bull. Chin. Materia Med.*, 10, 41-42 (1985).

Minami, M., Takahashi, T., Maruyama, A., Takahashi, A., Dostert, P., Nagatsu, T. and Naoi, M., Inhibition of tyrosine hydroxylase by R and S enantimers salsolinol, 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline. *J. Neurochem.*, 58, 2097-2101 (1992).

Nagatsu, T., Levitt, M. and Udenfriend, S., Tyrosine hydroxylase: The initial step in norepinephrine biosynthesis. *J. Biol. Chem.*, 239, 2910-2917 (1964).

Nagatau, T., Oka, K. and Kato, K., Highly sensitive assay for tyrosine hydroxylase activity by high-performance liquid chromatography. *J. Chromatogr.*, 163, 247-252 (1979).

Naoi, M. and Nagatsu, T., Inhibition of type A monoamine oxidase by methylquinolines and structurally related compounds. *J. Neurochem.*, 50, 1105-1110 (1988).

Naoi, M., Takahashi, T., Darrez, H., Kabeya, R. and Taguchi, E., N-Methylisoquinolinim ion as an inhibitor of tyrosine hydroxylase, aromatic L-amino acid decarboxylase and monoamine oxidase. *Neurochem. Int.*, 15, 315-320 (1989).

Vauquelin, G., De Keyser, J., Kanyinda, B. and Vanhaelen, M., (±)Tetrahydroanisocycline and (±) tetrahydropalmatine binding to D_1 and D_2 dopaminergic receptors in human putamen. *Neurochem. Int.*, 15, 321-324 (1989).

Vennerstrom, J. L. and Klayman, D. L., Protoberberine alkaloids as antimalarials. *J. Med. Chem.*, 31, 1084-1087 (1988).