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Inhibitory Effect of the Root of *Coptis japonica* on Catecholamine Biosynthesis in PC12 Cells

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The effect of the root of *Coptis japonica* (COPT), both the dichloromethane soluble (CH_2Cl_2) and insoluble (H_2O) fractions, on catecholamine contents and tyrosine hydroxylase (TH) activity in PC12 cells was investigated. CH_2Cl_2 and H_2O fractions showed 21 and 53% inhibitions on dopamine content, respectively, at a concentration of 40 μg/ml in medium: the H_2O fraction provided a greater inhibitory effect. The TH activity was reduced by the treatment of COPT (H_2O fraction). These results suggest that COPT has an inhibitory effect on the catecholamine biosynthesis by the reduction of TH activity in PC12 cells.

Key words: *Coptis japonica*, Catecholamines, Tyrosine Hydroxylase, PC12 Cells

INTRODUCTION

The root of *Coptis japonica* (COPT; Coptidis Rhizoma, Ranunculaceae, Hwanglyun in Korean) has been used in traditional medicine as an anxiolytic. COPT has proved to have antibacterial (Franzblau and Cross, 1986), antihypertensive (Chen and Hsieh, 1986), antiulcer (Takase *et al.*, 1987), anticancer and cytotoxic (Kim *et al.*, 1994), CNS depressant (Yamahara, 1976), anticonvulsant (Hong *et al.*, 1988), alkaline phosphatase stimulatory (Kumazawa *et al.*, 1991), tyrosinase inhibitory (Fukushima and Kimura, 1989), and histamine release inhibitory (Hirai *et al.*, 1983) activities. The well-known components of COPT are alkaloids such as berberine, coptisine, worenine, magnoflorine, and palmatine.

PC12 cells, origined from rat adrenal pheochromocytoma, exhibit many properties of adrenal medullary chromaffin cells, including the synthesis, storage, and secretion of catecholamines (Greene and Rein, 1977; Tischler *et al.*, 1983). The PC12 cells also express the catecholamine biosynthetic enzymes such as tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase, and dopamine β-hydroxylase (Greene and Rein, 1977; Riberio *et al.*, 1991). The cells are therefore useful for studies of environmental factors that modulate catecholaminergic functions.

Previously, we have examined 29 herbal medicines traditionally used in Korea on the bovine adrenal TH activity, and found that several of them including COPT contain TH inhibitors (unpublished data; Hwang *et al.*, 1994). Furthermore, it has not reported whether COPT has an inhibitory effect on the catecholamine biosynthesis. In this study, we have investigated the effect of COPT on the catecholamine contents and TH activity in PC12 cells. Both the dichloromethane soluble and insoluble fractions have been used for the experiments.

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MATERIALS AND METHODS

Preparation of Extracts

The dried roots of *Coptis japonica* (50 g) were extracted three times with MeOH at 70℃ for 6 hr, and the extract was concentrated, frozen and dried to give a powder (MeOH extract, 7.1 g), which was further dissolved in water and partitioned two times with dichloromethane (CH_2Cl_2). Removal of the solvent under reduced pressure gave a solid residue (CH_2Cl_2 fraction, 2.5 g). The aqueous layer was frozen and dried to give a powder (H_2O fraction, 3.2 g). These extracts were dissolved in water and/or DMSO as indicated and tested for the experiments. The concentration of DMSO used did not show cytotoxicity in PC12 cells.

Cell Culture

PC12 cells were grown routinely on 60-mm tissue

culture dishes (Falcon Labware, Oxnard, CA) in RPMI 1640 medium (Sigma, St. Louis, MO) with 10% heat-inactivated horse serum and 5% fetal calf serum (GIBCO, Grand Island, NY) plus 100 units/ml penicillin and 100 μg/ml streptomycin (Sigma) (Tischler *et al.*, 1983). Cultures were maintained at 37℃ in a 5% CO_2 humidified atmosphere.

The PC12 cells (ca. 1×10^5 cells/cm^2) were incubated for 24 hr and replaced with fresh medium. The cells were treated with each extract (40 μg/ml medium) and then incubated for 48 hr. The cells (ca. $1.5-2\times10^5$ cells/cm^2) were harvested with phosphate buffered saline (PBS) and then centrifuged. The pellet was resuspended with PBS (400 μl) and the pellet extract was used for the measurement of catecholamine contents and TH activity.

Determination of Catecholamine Contents

Catecholamine contents were determined as described (Mitsui *et al.*, 1985; Lee *et al.*, 1986). To the extract (200 μl), 3 M trichloroacetic acid (100 μl) and isoproterenol (1 nmol/ml, internal standard, 100 μl) was added. The mixture was passed through a Toyopak SP cation cartridge (Toso, Japan) for clean-up. The absorbed amines were eluted with 2 ml of 0.6 M KCl-acetonitrile (1 : 1, v/v) and the eluate was derivatized with diphenylethylenediamine. The final reaction mixture (100 μl) was injected into a high-performance liquid chromatograph system (Toso). Chromatographic conditions were as follows: column, TSK-gel ODS 120T (5 μm, 15×0.45 cm, Toso); mobile phase, acetonitrile-methanol-0.1 M sodium acetate buffer (pH 5.0) (55 : 5 : 45, v/v); flow rate, 1 ml/min; detector, F1000 fluorescence spectrophotometer (Hitachi, Japan) (Ex. 350 nm, Em. 470 nm).

Assay for TH

Enzyme activity was measured by a modification of the method of Nagatsu *et al.* (1979). The reaction mixture contained 1.5 M NaAc (pH 5.8) 20 μl, 10 mM tyrosine 10 μl, 10 mM 6-methyltetrahydropterine 10 μl, 2 mg/ml catalase 10 μl and enzyme extract 50 μl. The enzyme reaction was performed at 37℃ 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 5 ml of EDTA (2%), 1.5 ml of KH_2PO_4 (0.35 M) and an aliquot of N-NaOH were added to adust the pH to 8.4-8.6, the reaction mixture was passed through the alumina cartridge (100 mg). DOPA and dihydroxybenzylamine absorbed were eluted with 500 μl of 0.5 M HCl. A 100 μl of the eluate was injected into the high-performance liquid chromatograph with a CM8010 electrochemical detector (Toso) and a TSK-gel ODS 120T column (5 μm, 15×0.45 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.

Determination of Protein

Protein amounts were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

RESULTS AND DISCUSSION

PC12 cells express the catecholamines and TH, the first rate-limiting enzyme of the catecholamine biosynthetic pathway (Greene and Rein, 1977; Riberio *et al.*, 1991). We have applied the PC12 cells for screening of bioactive substances from the herbal medicine, COPT.

The MeOH extract of COPT (40 μg/ml medium) showed an inhibitory effect on dopamine and norepinephrine production in intracellular PC12 cells (Table I). A 39% decrease in the dopamine biosynthesis was observed. The inhibitory action of COPT (MeOH extract) on dopamine biosynthesis was shown at concentrations of 20 to 100 μg/ml medium (a concentration of 50% inhibition, IC_{50}; 52 μg/ml medium). We, therefore, examined the activity of TH in PC12 cells. The TH activity was decreased by the treatment of COPT (MeOH extract) (Table I).

The MeOH extract was further fractionated with dichloromethane. Both the dichloromethane soluble (CH_2Cl_2) and insoluble (H_2O) fractions showed considerable inhibitions of dopamine and norepinephrine biosyntheses (Table II). The CH_2Cl_2 and H_2O fractions showed 21 and 53% inhibitions on dopamine content, respectively, at a concentration of 40 μg/ml in medium: the H_2O fraction provided a greater inhibitory effect. And, the TH activity was significantly decreased by the treatment of COPT (H_2O extract) (Table II). The secretion of catecholamines (dopamine and norepinephrine) into the medium was slightly increased, but not significant, by addition of the MeOH extract of COPT (data not shown). The total catecholamine contents, both stored inside the cells and released into the medium, was significantly reduced by COPT in PC12 cells. These results suggest that COPT has an inhibitory effect on the catecholamine biosynthesis by the reduction of TH activity in PC12 cells.

Treatment with berberine chloride, a main alkaloid from COPT, decreased the dopamine content in PC12 cells (IC_{50}; 9.5 μg/ml medium) (Table II). It seemed that berberine might partially possess the inhibitory effect on dopamine biosynthesis in PC12 cells. Berberine and the other alkaloids from COPT were mainly partitioned into the H_2O fraction, which was confirmed

Table I. Effect of the root of *Coptis japonica* (COPT) on the catecholamine contents and TH activity in PC12 cells

Herbal medicine	Catecholamine contents (% of control)		TH activity (% of control) (nmol/min/mg protein)
	Norepinephrine (pmol/mg protein)	Dopamine (nmol/mg protein)	
Control	98.0± 4.3 (100)	4.66± 0.29 (100)	106± 11.8 (100)
COPT, 20 (μg/ml)	68.6± 2.3 (70.1)**	3.79± 0.28 (81.3)	
40 (μg/ml)	62.6± 6.2 (63.8)**	2.86± 0.06 (61.4)**	82.4± 5.9 (77.7)
100 (μg/ml)	34.9± 5.1 (35.6)***	1.71± 0.21 (36.8)***	

Cells were incubated for 24 hr and replaced with fresh medium. The cells were treated with COPT (MeOH extract, 20-100 μg/ml medium) and then incubated for 48 hr. The cells were harvested with PBS and the intracellular catecholamine contents and TH activity were measured according to the text. The results represent the mean± SE of five dishes. Significantly different from the control value: **, $P<0.01$; ***, $P<0.001$ (Student's t test).

Table II. Effect of COPT on the catecholamine contents and TH activity in PC12 cells

Herbal medicine	Catecholamine contents (% of control)		TH activity (% of control) (nmol/min/mg protein)
	Norepinephrine (pmol/mg protein)	Dopamine (nmol/mg protein)	
Control	87.1± 5.1 (100)	4.42± 0.51 (100)	96.4± 6.4 (100)
H_2O fraction (40 μg/ml)	34.8± 3.8 (40.0)***	2.10± 0.24 (47.5)**	68.4± 8.7 (70.9)*
CH_2Cl_2 fraction (40 μg/ml)	56.7± 8.1 (65.1)*	3.49± 0.19 (79.0)	
Berberine (10 μg/ml)	48.8± 10.2 (56.0)*	2.12± 0.68 (47.9)*	75.8± 10.4 (78.4)

Cells were incubated for 24 hr and replaced with fresh medium. The cells were treated with herbal medicine and then incubated for 48 hr (n=5). The cells were harvested with PBS and the intracellular catecholamine contents and TH activity were measured according to the text. Significantly different from the control value: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (Student's t test).

by TLC analysis.

TH from PC12 cells is responsive to elevation of c-AMP by forskolin and activation of protein kinase C by phorbol ester, TPA (Lewis *et al.*, 1987; Stachowiak *et al.*, 1988). The important influence of cellular regulatory mechanisms on the response to herbal medicine(s) may well contribute to the diversity of the *in vivo* actions.

Our observation indicated that the catecholamine contents were remarkedly decreased and the TH activity was reduced by COPT in cultured PC12 cells. The separation of bioactive component(s) from COPT and its mechanism in intracellular PC12 cells need further investigation.

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