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Inhibitory Effects of Noscapine on Dopamine Biosynthesis in PC12 Cells

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The effects of noscapine, a phthalide isoquinoline alkaloid, on dopamine biosynthesis and tyrosine hydroxylase (TH) activity in PC12 cells were investigated. Noscapine showed 74.6% inhibition on dopamine content in PC12 cells at a concentration of 20 μM. IC_{50} of noscapine was 6.8 μM. TH activity was inhibited by the treatment of noscapine in PC12 cells (20.9% inhibition at 20 μM). Therefore, the inhibition of TH activity by noscapine might be involved in at least one component of the reduction of dopamine biosynthesis in PC12 cells.

Key words : Noscapine, Dopamine Biosynthesis, Tyrosine Hydroxylase, PC12 Cells.

INTRODUCTION

Noscapine, a phthalide isoquinoline alkaloid constituting 1~10% of the alkaloid content of opium, had been used clinically as an antitussive agent (Eddy *et al.*, 1969). Mechanisms of antitussive of noscapine are still unknown. Noscapine is one of the same order of potency as codeine in blocking cough (Empey *et al.*, 1979). But unlike codeine, noscapine lacks addictive, analgesia, respiratory depressant and sedative properties (Eddy *et al.*, 1969).

PC12 cells derived from rat adrenal pheochromocytoma exhibit many properties of the 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 tyrosine hydroxylase (TH), the rate-limiting enzyme of the catecholamine biosynthetic pathway (Greene and Rein, 1977).

Previously, protoberberine alkaloids such as berberine and palmatine have been found to inhibit catecholamine biosynthesis in PC12 cells, and to inhibit competitively bovine adrenal TH (Lee and Kim, 1996; Lee and Zhang, 1996; Lee *et al.*, 1996). These results suggest that some bioactive isoquinoline alkaloids might be able to inhibit the catecholamine biosynthesis. Therefore, we investigated the inhibitory effects of noscapine on dopamine content, TH activity and intracellular free Ca^{++} concentration ($[Ca^{++}]_i$) in PC 12 cells.

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MATERIALS AND METHODS

Materials

Noscapine hydrochloride (MW 449.9), L-tyrosine, isoproterenol, catalase, 3,4-dihydroxybenzylamine, DL-6-methyl-5,6,7,8-tetrahydropterine, alumina and fura-2 AM were purchased from the Sigma Chemical Company (St. Louis, MO, USA). All sera, antibiotics and RPMI 1640 for cell culture were obtained from GIBCO (Grand Island, NY, USA). All other chemicals were of reagent grade.

Cell culture

PC12 cells were maintained routinely in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum and 5% fetal calf serum plus 100 units /ml penicillin and 100 μg/ml streptomycin at 37°C (Tis- chler *et al.*, 1983). The PC12 cells (ca. 1×10^5 cells/cm^2) were treated with noscapine (4~20 μM) and then incubated for 6~12 hr. The cells (ca. $1.5\sim2\times10^5$ cells/cm^2) were harvested with phosphate buffered saline (PBS) and then centrifuged. Pellet was resuspended in PBS (500 μl) and the pellet extract was used for the measurement of dopamine content and TH activity.

Determination of dopamine content

Dopamine content was determined as described previously (Mitsui *et al.*, 1985; Lee and Kim, 1996). To the pellet extract, 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-M cartridge (Na^+, resin 1 ml, Toso, Tokyo, 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 1,2-diphenylethylenediamine. The final reaction mixture (100 μl) was injected into a HPLC system (Toso). The conditions of HPLC analysis were the same as described previously (Lee and Kim, 1996).

Assay for TH

TH activity was measured according to a slightly modified procedure of Nagatsu *et al.* (1979) as described previously (Lee and Zhang, 1996). 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 pellet extract (20 μl). The enzyme reaction took place 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 clean-up of the reaction mixture using the alumina cartridge (100 mg), the eluate (50 μl) was injected into the HPLC equipped with a CM8010 electrochemical detector (Toso) and a TSK-gel ODS-120T (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.

Measurement of $[Ca^{++}]_i$

PC12 cells were loaded with fura-2 AM to a final concentration of 5 M at 37°C for 40 min. After loading, the cells were washed twice with Locke's solution (pH, 7.4; NaCl, 154 mM; KCl, 5.6 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 2.2 mM; HEPES, 5.0 mM; glucose, 10 mM) to remove the extracellular dye. For the fluorimetric measurement of $[Ca^{++}]_i$, the cells (2×10^5 in 1 ml Locke's solution) were placed on a quartz cuvette. Fluorescence intensity was quantified with a fluorescence spectrophotometer (Perkin Elmer L225 0137, Beaconsfield, UK), with a single excitation at 340 nm and an emission at 500 nm. Calibration of the fluorescence signal in terms of $[Ca^{++}]_i$ was performed according to Grynkiewicz *et al.* (1985).

Determination of protein

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

RESULTS AND DISCUSSION

Some isoqunoline alkaloids have been reported to have the regulatory effects on catecholamine biosynthesis. Berberine, palmatine, hydrastine and bulbocapnine inhibit dopamine biosynthesis in PC12 cells (Lee and Kim, 1996; unpublished data). Berberine, palmatine and bulbocapnine also inhibit bovine adrenal TH. Berberine and palmatine show competitive inhibition (Lee and Zhang, 1996; Lee *et al.* 1996), and bulbocapnine does uncompetitive inhibition (Zhang *et al.* 1997). We, therefore, investigated the effects of noscapine, a phthalide isoquinoline alkaloid, on dopamine biosynthesis in PC12 cells.

Table I. Inhibitory effects of noscapine on dopamine content and TH activity in PC12 cells

Inhibitor	Dopamine content (% of control) (nmol/mg protein)	TH activity (% of control) (nmol/min/mg protein)
Control	3.54±0.23 (100)	3.68±0.21 (100)
Noscapine		
4 μM	2.02±0.14 (57.1)**	
12 μM	1.51±0.09 (42.6)**	
20 μM	0.90±0.11 (25.4)***	2.91±0.14 (79.1)*

PC12 cells were incubated for 24 hr and replaced by fresh media. The cells were treated with noscapine (4~20 μM) and then incubated for 12 hr. The cells were harvested with PBS, and the dopamine content and TH activities were measured by HPLC. Results represent the mean±SEM of 5 dishes. Significantly different from the control value: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ (Student's t test).

Treatment of noscapine significantly decreased the intracellular dopamine content dose-dependently in PC 12 cells. Noscapine exhibited 74.6% inhibition on dopamine content at a concentration of 20 μM (Table I). IC_{50} of noscapine was 6.8 μM. The cell viability was examined by lactate dehydrogenase activity and trypan blue exclusion test. Noscapine at concentrations up to 40 μM did not show cytotoxicity towards PC12 cells. The secretion of dopamine into the medium did not alter upon addition of noscapine (20 μM) (data not shown). Therefore, the dopamine content, stored in the cells and secreted into the medium, was reduced by noscapine. In this condition, TH activity was markedly inhibited by the treatment of noscapine (20.9 % inhibition at 20 μM; Table I).

Noscapine binds in brain specific and ion insensitive, indicating a specific receptor distinct from opiate receptors (Mourey *et al.*, 1992). Noscapine causes high levels of polyploidy and may be considered a potential carcinogen (Porter *et al.*, 1992). But the relation-

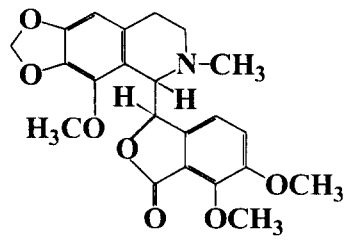

Fig. 1. Structure of noscapine.

Table II. Effects of noscapine on intracellular Ca^{++} concentration ($[Ca^{++}]_i$) in PC12 cells

Inhibitor	$[Ca^{++}]_i$ (nM) % of control
Control	19.5±0.35 (100)
Noscapine, 20 μM	15.1±0.25 (77.4) ***

PC12 cells were treated with noscapine (20 μM) and then incubated for 6 hr. The cells (2×10^5 cells/ml) were harvested, and the $[Ca^{++}]_i$ were measured by spectrofluorimetric method. Results represent the mean±SEM of 5 dishes. Further comments see materials and methods, and Table I.

ships between the polyploidy-carcinogenic action and the decrease of dopamine content in PC12 cells are unclear.

To study partially the mechanism of noscapine on TH activity, $[Ca^{++}]_i$ were investigated in PC12 cells. The $[Ca^{++}]_i$ showed 22.6% inhibition compared with the control by noscapine at 20 μM for 6 hr of the incubation time (Table II). Noscapine (1 mM) does not affect basal phosphoinositide turnover, but markedly inhibits carbachol-stimulated phosphoinositide turnover by monitoring the formation of CDP-diacylglycerol in guinea pig and rat brain slices (Mourey *et al.*, 1992). These results suggest that noscapine may have a possibility to decrease the $[Ca^{++}]_i$ level and to inhibit the activity of Ca^{++}-phospholipid kinase. TH activity in PC12 cells is responsible for various factors such as c-AMP, dexamethasone, protein kinase A and protein kinase C (Lewis *et al.*, 1987; Stachowiak *et al.*, 1988). So, we hypothesize that the decrease of $[Ca^{++}]_i$ level by noscapine may cause the reduction of TH activity in PC12 cells.

In this experiment, noscapine decreased the dopamine content and inhibited the TH activity in PC12 cells. Therefore, the inhibition of TH activity by noscapine might be involved in at least one component of the reduction of dopamine biosynthesis in PC12 cells. The intracellular mechanisms in PC12 cells on phthalide isoquinoline alkaloids such as noscapine and hydrastine need further investigation.

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