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Inhibition of L-DOPA-Induced Increase in Dopamine Content by (1R, 9S)-β-Hydrastine Hydrochloride in PC12 cells

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Abstract – The effects of BHSH on L-DOPA-induced increase in dopamine content in PC12 cells were investigated. L-DOPA treatment at 20 or 50 μM increased dopamine content after both 24 and 48 h of incubation in PC12 cells. However, the co-treatments of BHSH (10-50 μM) with L-DOPA (20 or 50 μM) significantly inhibited the increase of dopamine content induced by L-DOPA. BHSH treatment at 10-50 μM significantly inhibited basal aromatic L-amino acid decarboxylase (AADC) activity in a concentration-dependent manner at 15 min, and then AADC activity was rapidly recovered to the control level at about 2 h. These results indicate that the inhibition of AADC activity by BHSH was, in part, contributed to the early-stage decrease of dopamine content induced by L-DOPA in PC12 cells. Taken together, it is proposed that the short-term inhibition of dopamine biosynthesis by BHSH was mediated by the regulation of tyrosine hydroxylace (TH).
Keywords – (1R,9S)-β-Hydrastine hydrochloride; L-DOPA; Tyrosine hydroxylase; Aromatic L-amino acid decarboxylase; PC12 cells

Introduction

(1R,9S)-β-hydrastine is a phthalide isoquinoline alkaloid and have been isolated from the rhizomes and roots of goldenseal (*Hydrastis canadensis* L., Ranunculaceae), the herbaceous plant that has been traditionally used as a disinfectant by Cherokee Indians and was successfully employed in modern phytotherapy to cure infectious diseases of the gastrointestinal, urogenital and respiratory tracts (Fang *et al.*, 1981). Recently, it is proposed that (1R,9S)-β-hydrastine hydrochloride (BHSH) decreased the intracellular dopamine content and inhibited tyrosine hydroxylace (TH) activity in PC12 cells (Kim *et al.*, 2001). BHSH also inhibits bovine adrenal TH activity (Lee *et al.*, 1997).

L-3,4-Dihydroxyphenylalanine (L-DOPA) is an effective replacement therapy in parkinsonian patients due to its decarboxylation by the enzyme aromatic L-amino acid decarboxylase (EC 4.1.1.28; AADC) to dopamine (Baroni *et al.*, 1984; Hughes *et al.*, 1993; Kordower *et al.*, 1997). However, although many of parkinsonian patients experience good control of symptoms for several years after initiating L-DOPA therapy, the L-DOPA therapy eventually develops a troubling array of motor fluctuation, which appears to attribute to L-DOPA-induced down-regulation of AADC.

PC12 rat adrenal pheochromocytoma cells have dopaminergic properties similar to those neurons, which synthesize, store, and release catecholamines (Byrd *et al.*, 1986; Gebreyesus, 1993). In addition, L-DOPA treatments at low concentrations increase dopamine levels, but at high concentrations induce cytotoxicity by oxidative stress in PC12 cells. PC12 cells, therefore, have been used as a model system for investigating the effects on dopamine biosynthesis or cytotoxicity (Lee and Kim, 1996; Lee and Zhang, 1996; Lee *et al.*, 1996; Shin *et al.*, 1998a; 1998b; 1999; Kim *et al.*, 1998).

The aim of the present study was to determine whether BHSH similarly reduce L-DOPA-induced increase in dopamine content in PC12 cells and, if so, to determine whether it could be related to the biosynthetic pathway of inhibiting AADC activity.

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Abbreviation used: AADC: aromatic L-amino acid decarboxylase, BHSH: (1R,9S)-β-hydrastine hydrochloride, PD: Parkinsons disease , L-DOPA: L-3,4-dihydroxyphenylalanine

Materials and Methods

Materials – BHSH, EDTA, 3-(4,5-dimetythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and L-DOPA were purchased from the Sigma Chemical Company (St. Louis, Mo, USA). All sera, antibiotics and RPMI 1640 for cell cultures were obtained from Gibco (Grand Island, NY, USA). All other chemicals were of reagent grade.

Cell culture – PC12 cells were grown in RPMI medium 1640 supplemented with 10% heat-inactivated horse serum, 5% heat-inactivated fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO_2/humidified air (Tischler *et al.*, 1983).

Determination of dopamine content – Dopamine content was determined as described previously (Mitsui *et al.*, 1985; Shin *et al.*, 2000). Trichloroacetic acid (1 M, 100 μl) and isoproterenol (1 nmol/ml, 100 μl, internal standard) were added to the pellet extracts. The mixture was passed through a Toyopak SP-M cartridge (Na^+, resin 1 ml, Toso, Tokyo, Japan) and the cartridge eluate was derivatized with 1,2-diphenylethylenediamine. The final reaction mixture (100 μl) was injected into an HPLC system (Toso). The HPLC conditions were the same as described previously (Shin *et al.*, 2000).

Assay for AADC activity – AADC activity was measured according to the method of Lee *et al.* (1986). Dopamine formed by enzyme reaction from a substrate L-DOPA was determined by an HPLC method using 1,2-diphenylethylenediamine as a fluorescent reagent.

Statistical analysis – Protein amounts were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. All data were expressed as means ± S.E.M. of at least four or five experiments. Statistical analysis was performed using ANOVA followed by Tukey's test.

Results and Discussion

L-DOPA at 20, 50, and 100 μM increased intracellular contents of dopamine to about 199%, 252%, and 285% of control values after 24 h of incubation, respectively (Fig. 1). Whereas, at 150 μM L-DOPA, the intracellular dopamine content was decreased and the values was about 197% of control value. Treatments with L-DOPA for 48 h, at concentrations of 20 and 50 μM L-DOPA also increased the content of dopamine to 124% and 138% of control values, respectively (Fig. 1).

L-DOPA at concentration of 20 μM did not decrease cell viability after 24 h or 48 h of incubation in PC12 cells (data not shown). A slight but not significant decrease in

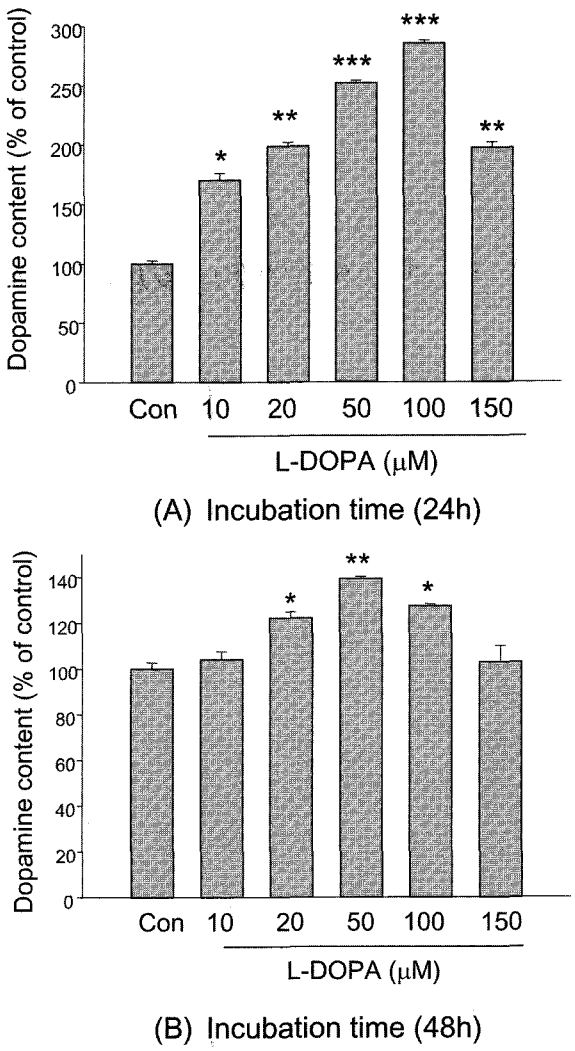

Fig. 1. Effects of L-DOPA on dopamine content in PC12 cells. PC12 cells were exposed for 24 h (A), or 48 h (B) to different concentrations of L-DOPA (10, 20, 50, and 100 μM). Dopamine content was measured using HPLC method. The control value of dopamine content was 3.64±0.21 and 3.67±0.28 nmol/mg protein, respectively. Results represent the means±S.E.M. of five experiments performed in triplicate. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ compared with the control (ANOVA followed by Tukey's test).

cell viability was observed when PC12 cells were exposed to 50 μM L-DOPA for 48 h. However, exposure to 100 and 150 μM L-DOPA resulted in a great decrease of cell viability at 24 and 48 h of incubation time. In this experiment, 20 and 50 μM concentrations of L-DOPA were used to investigate the effects of BHSH on L-DOPA-induced increase in dopamine content in PC12 cells.

To determine whether BHSH similarly inhibit the formation of dopamine from exogenous L-DOPA in PC12 cells, the cells were exposed to L-DOPA and BHSH, alone or in combination, for 24 or 48 h. As shown in Fig. 2, the

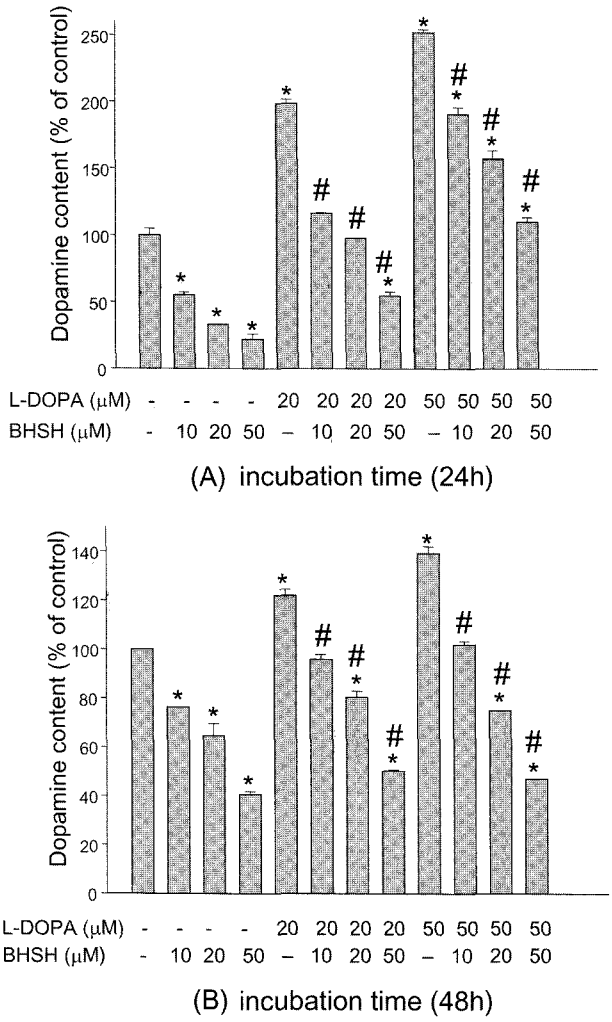

Fig. 2. Effects of BHSH on dopamine content induced by L-DOPA in PC12 cells. PC12 cells were treated with BHSH (10, 20, 50 μM) or L-DOPA (20, 50 μM), alone or in combination, for 24 h (A) or 48 h (B). The control value of dopamine content was 3.81±0.26 and 3.85±0.29 nmol/mg protein, respectively. Results represent the means ± S.E.M. of five experiments performed in triplicate. *, P<0.05 compared with the control; #, P<0.05 compared with the corresponding L-DOPA concentrations (ANOVA followed by Tukey's test).

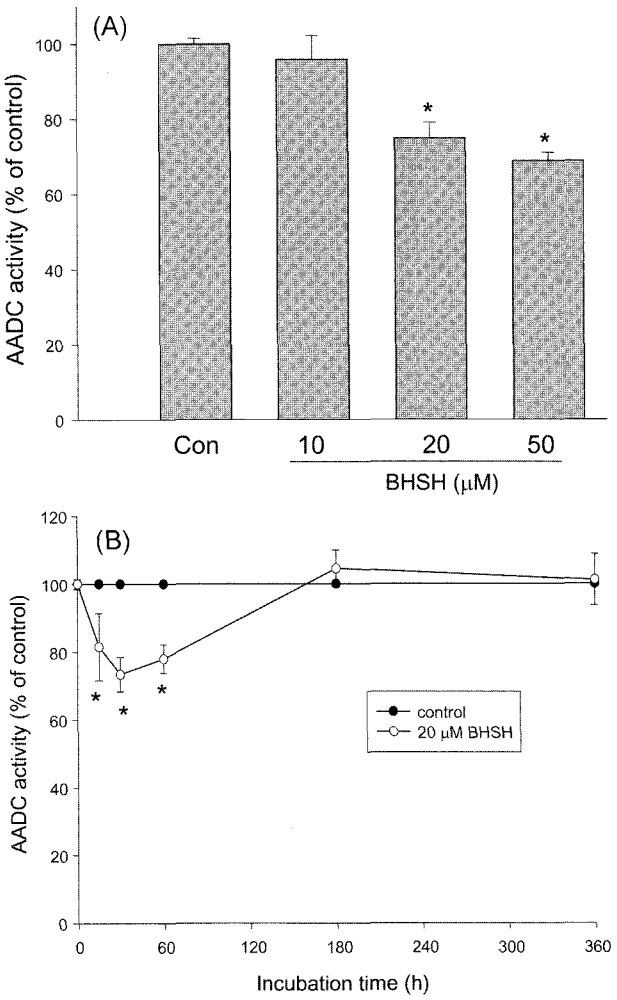

Fig. 3. Effects of BHSH on the intracellular AADC activity in PC12 cells (A). PC12 cells were exposed for 15 min to different concentrations of BHS (10, 20, and 50 μM). AADC activity was measured using HPLC method. The control value of AADC activity was 37.2±0.15 nmol/min/mg protein. Results represent the means±S.E.M. of five experiments performed in triplicate. *, P<0.05 compared with the control (ANOVA followed by Tukey's test). Time courses of AADC activity by BHSH (20 μM) in PC12 cells (B). The control value of AADC activity was 36.2±0.21 nmol/min/mg protein. Results represent the means±S.E.M. of five experiments performed in triplicate. *, P<0.05 compared with the control (ANOVA followed by Tukey's test).

co-treatments with L-DOPA (20 and 50 μM) plus BHSH (10-50 μM) markedly reduced the elevation in intracellular contents of dopamine induced by L-DOPA after 24 and 48 h of incubation compared with treatment of L-DOPA alone. Treatments with 20 or 50 μM L-DOPA plus 20 μM BHSH for 48 (or 24) h showed about 34% or 46% (or about 51% or 48%) reduced dopamine content compared with corresponding L-DOPA concentrations, respectively.

In addition, in order to determine whether the reduction of L-DOPA-induced increase in dopamine content by BHSH is related to cytotoxicity, the effects of BHSH combined with L-DOPA on cell viability in PC12 cells were investigated. BHSH at concentration ranges of 10-150 μM did not affect 20-50 μM L-DOPA-induced cytotoxicity after 24 or 48 h of incubation (data not shown).

The previous study indicated that BHSH treatment caused a decrease in basal intracellular dopamine content by the inhibition of TH activity without increasing the extracellular dopamine content and inducing cell death in PC12 cells (Kim *et al.*, 2001). In this study, the effects of BHSH on AADC activity in PC12 cells were also investigated. As shown in Fig. 3A, BHSH at 10-50 μM

significantly inhibited AADC activity in a concentration-dependent manner at 15 min compared with the control values. After the treatment with 20 μM BHSH, the AADC activity was decreased at 15 min, reached minimal level about 73% of control value at 30 min, and then returned rapidly to the control level at 3 h (Fig. 3B). These data suggest that the inhibition of AADC activity by BHSH appears to be contributed to the reduction of the increased dopamine content induced by L-DOPA in PC12 cells at early stage period.

Isoquinoline derivatives have been proposed to have a function as neuromodulators, and may be involved in the regulation of monoamine function through the inhibition of monoamine biosynthetic and metabolic enzymes such as TH, AADC, catechol-O-methyltransferase, and MAO-A and B (Deitrich and Erwin, 1980; Rommelspacher *et al.*, 1991; Myers, 1989; Thull *et al.*, 1995; Lee and Kim, 1996; Lee and Zhang, 1996; Lee *et al.*, 1996; Shin *et al.*, 1998a; 1998b; 1999; Kim *et al.*, 1998). BHSH also inhibits dopamine biosynthesis and TH activity with a long-term regulation (submitted).

A number of observations in humans suggest that the amount of AADC in the normal human brain is exceedingly small and the neurodegeneration of nigrostriatal dopamine neurons in Parkinsons disease lowers the AADC activity further (Gjedde *et al.*, 1993; Lloyd and Hornykiewicz, 1970; Zhu and Juorio, 1995). In addition, the prolonged administration of L-DOPA to nigral-lesioned rats precipitated deterioration in the responsiveness of striatal cells to L-DOPA, which they attributed to a diminished capacity of the dopamine neurons to synthesize transmitter (Chang and Webster, 1997). The suppression of decarboxylation by AADC with an acute injection of L-DOPA is already compromised in parkinsonism. Theses evidences suggest that AADC may become rate-limiting in the parkinsonian brain and the changes of AADC is important for parkinsonian patients treated with exogenous L-DOPA.

Therefore, it is conceivable that chronic exposure to BHSH including various isoquinoline derivatives such as berberine, palmatine, tetrahydropapaveroline, and tetrahydoisoquinolines might accelerate the deterioration of parkinsonian patients, especially for the advanced patients. However, it is proved that BHSH showed a very weak cytotoxicity compared with other isoquinoline derivatives in PC12 cells. BHSH at concentrations higher than 500 μM showed a cytotoxic towards PC12 cells and the IC_{50} value of BHSH for dopamine biosynthesis was 9.3 μM (Kim *et al.*, 2001).

Taken together, it is proposed that the inhibition of dopamine biosynthesis by BHSH was mediated in part by the short-term regulation of TH and AADC activities. The pharmacological actions of BHSH need to be studied further.

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