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The Harman and Norharman Reduced Dopamine Content and Induced Cytotoxicity in PC12 Cells

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Abstract - The effects of harman and norharman on dopamine content and L-DOPA-induced cytotoxicity were investigated in PC12 cells. Harman and norharman decreased the intracellular dopamine content for 24 h. The IC_{50} values of harman and norharman were 20.4 μM and 95.8 μM, respectively. Tyrosine hydroxylase (TH) activity and TH mRNA levels were also decreased by 20 μM harman and 100 μM norharman. Under the same conditions, the intracellular cyclic AMP levels were decreased by harman and norharman. In addition, harman and norharman at concentrations higher than 80 μM and 150 μM caused cytotoxicity at 24 h in PC12 cells. Non-cytotoxic ranges of 10-30 μM harman and 50-150 μM norharman inhibited L-DOPA (20-50 μM)-induced increases of dopamine content at 24 h. Harman at 20-150 μM and norharman at 100-300 μM also enhanced L-DOPA (20-100 μM)-induced cytotoxicity at 24 h. These results suggest that harman and norharman decrease dopamine content by reducing TH activity and aggravate L-DOPA-induced cytotoxicity in PC12 cells.

Keywords: Harman, Norharman, Dopamine content, Tyrosine hydroxylase, PC12 cell

INTRODUCTION

β-Carbolines derivatives, harman and norharman (Fig. 1), may be formed by cyclization of indoleamines with aldehydes in the brain (Deitrich and Erwin, 1980) during conventional high-temperature cooking and tobacco smoking (Pfau and Skog, 2004; Breyer-Pfaff *et al.,* 1996). Harman and norharman are also found in groundwater, plants, grape juice and wine (Allen and Holmstedt, 1980; Pfau and Skog, 2004).

Harman, a 1-methylated derivative of norharman, has been reported to have a strong inhibitory action of monoamine oxidase type A (MAO-A, EC 1.4.3.4) (May *et al.,* 1991). Norharman inhibits preferentially MAO-B (May *et al.,* 1991) and is found in substantia nigra from humans (Matsubara *et al*., 1993). The plasma levels of harman and norharman are also increased in parkinsonian patients (Kuhn *et al.,* 1995).

MAO-A and -B in human brain tissue metabolize dopamine, which is the most affected neurotransmitter in Parkinson's disease (Ehringer and Hornykiewicz, 1960). It is, therefore, suggested that harman and norharman as MAO inhibitors may play neuroprotective roles in Parkinson's disease. In contrast, the dopamine metabolites of MAO could produce the cellular damages by the formation of reactive oxygen species (Cohen, 1983). In addition, β-carbolines have been proposed as neuronal toxins because of the structural similarity to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 1-methyl-4-phenylpyridinium (MPP^+) (Albores *et al*., 1990; Fields *et al*., 1992). However, these inconsistent functions of harman and norharman on dopamine biosynthesis and cytotoxicity could not be fully elucidated.

L-3,4-Dihydroxyphenylalanine (L-DOPA), the precursor of dopamine, is administered most frequently for controlling the symptoms of Parkinson's disease (Marsden, 1994). However, the long-term L-DOPA therapy produces neurotoxicity by generating reactive oxygen spe-

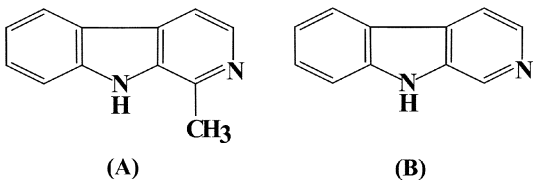

Fig. 1. Chemical structures of harman (A) and norharman (B).

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cies leading to apoptosis (Marsden, 1994; Sandstrom *et al*., 1994).

Rat adrenal pheochromocytoma PC12 cells have dopamine synthesizing, storing and releasing properties (Tischler *et al*., 1983). PC12 cells also express catecholamine biosynthetic enzymes such as tyrosine hydroxylase (TH, EC 1.14.16.2), aromatic L-amino acid decarboxylase (AADC, EC 4.1.1.28) (Tischler *et al*., 1983).

In this study, therefore, the effects of harman and norharman on dopamine content and cytotoxicity were investigated using PC12 cells as a model system.

MATERIALS AND METHODS

Materials

Harman, norharman, L-DOPA and 3-(4,5-dimethyl-2-thiazolyl)-2,5-di-phenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cyclic AMP enzyme immunoassay kit was purchased from Amersham Parmacia Biotech (St. Freiburg, Germany). All sera, antibiotics and RPMI 1640 for cell culture were obtained from Gibco (Rockville, MD, USA). All other chemicals were of reagent grade.

Cell culture

PC12 cells were maintained routinely (Tischler *et al.,* 1983). PC12 cells (ca. 1×10^5 cells/cm^2) were treated with harman and norharman in the absence or presence of L-DOPA with indicated times.

Determination of dopamine content and TH activity

Dopamine content was determined by using an HPLC system (Toso, Tokyo, Japan) as described previously (Shin *et al.,* 2000). TH activity was also measured according to Nagatsu *et al.* (1979) as described previously (Shin *et al*., 2000) with a slight modification. The HPLC analysis for the determination of TH activity was performed as described previously (Shin *et al*., 2000).

RNA extraction and Northern blotting

Total RNA was extracted and RNA samples (10 μg/lane) were fractionated by electrophoresis on 1% agarose containing 0.66 M formaldehyde gel and transferred to a nylon membrane (ICN, East Hills, NY, USA). The Northern blot analysis for TH mRNA was performed according to the method of Kim *et al.* (1993). The blots were hybridized to the coding regions of the 0.7 kb rat TH cDNA probe labeled with [α-^{32}P] dCTP using a Random Primer labeling system (DuPont NEN, Boston, MA, USA).

Measurement of cyclic AMP levels

The cells were incubated with harman and norharman for 30 min and agitated after the addition of the lysis reagent. Cyclic AMP levels were measured by using an enzyme immunoassay system kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK).

Assessment of cell viability

Cell viability was determined by the conventional MTT assay. Cells were treated with various concentrations of harman, norharman and L-DOPA, alone or associated, for 24 h and then treated with the MTT solution (final concentration, 1 mg/ml) for 4 h in an incubator. The reaction was stopped by adding 0.8 M HCl in isopropanol. The absorbance was measured at 570 nm by using a Bauty Diagnostic Microplate Reader (Molecular Devices, CA, USA).

Statistical analysis

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

RESULTS

Inhibition of dopamine content

Treatments of PC12 cells with harman at 5-25 μM and norharman at 40-120 μM significantly decreased the intracellular dopamine content in a concentration-dependent manner for 24 h (Fig. 2). The IC_{50} values of harman and norharman were 20.4 μM and 95.8 μM, respectively. Harman and norharman caused cytotoxicity at 24 h with

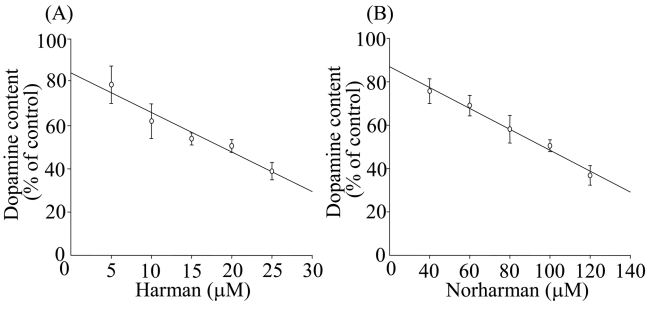

Fig. 2. Inhibitory effects of harman (A) and norharman (B) on dopamine content in PC12 cells. PC12 cells were treated with harman and norharman for 24 h and dopamine content was measured by an HPLC method. Dopamine content of control levels were 3.46 ± 0.23 nmol/mg protein. Results represent means ± S.E.M. of four experiments.

the concentrations up to 80 μM and 150 μM, respectively (Fig. 3).

Dopamine content was decreased by 20 μM harman and 100 μM norharman at 6 h, and reached minimal levels at 24-48 h, respectively (Fig. 4). Dopamine content in the medium, which was secreted from the intracellular dopamine, was not altered by 20 μM harman and 100 μM norharman for 24-48 h (data not shown).

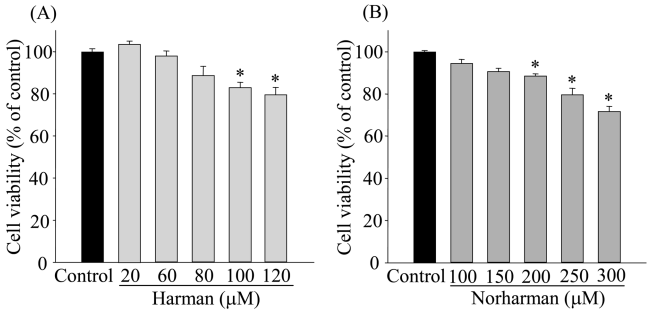

Fig. 3. Effects of harman (A) and norharman (B) on PC12 cell viability. PC12 cells were exposured to harman and norharman for 24 h. The cell viability was assessed by the MTT methods, in which viable cells convert the soluble dye MTT to in soluble blue formazan crystals. Results represent means ± S.E.M. of four experiments. *P<0.05; **P<0.01 compared to control levels (ANOVA followed by Tukey's test).

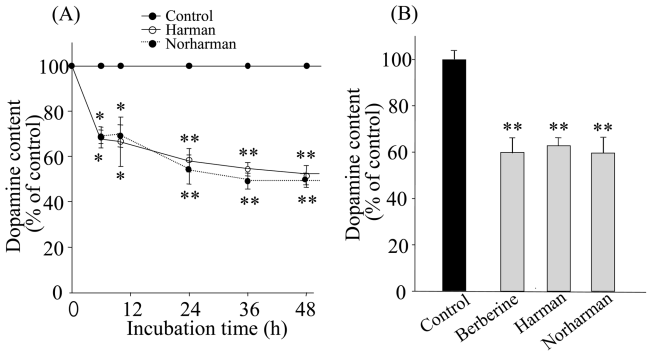

Fig. 4. Time courses of intracellular dopamine content by 20 μM harman and 100 μM norharman in PC12 cells (A). Berberine at 10 μM was used as a positive control at 24 h (Shin *et al*., 2000) (B). Dopamine content of control levels was 3.54 ± 0.34 nmol/mg protein. Results represent means ± S.E.M. of four experiments. Significantly different from the control values: *P<0.05; **P<0.01 (ANOVA followed by Tukey's test).

Inhibition of TH activity and TH gene expression

TH activity was inhibited by 20 μM harman and 100 μM norharman to 48.6-51.2% and 53.2-57.5% compared to control levels at 12-48 h (Table I). However, AADC

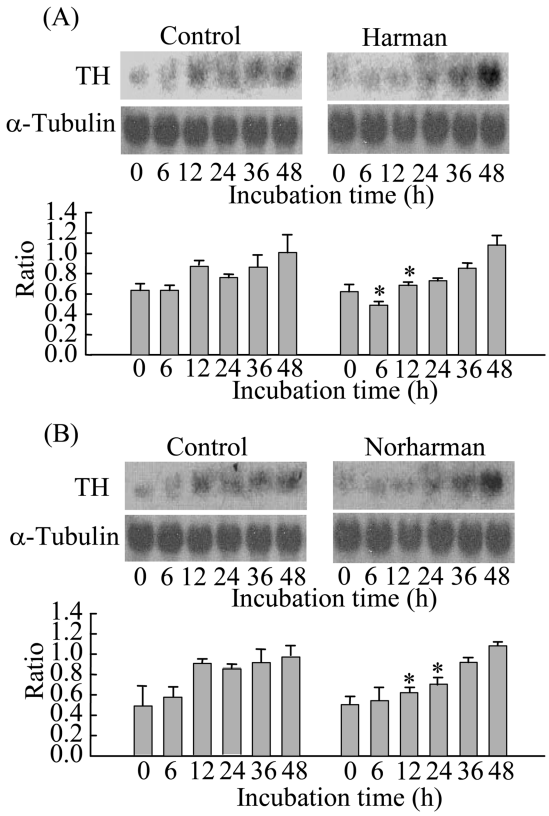

Fig. 5. Time courses of TH mRNA levels by 20 μM harman (A) and 100 μM norharman (B) in PC12 cells. Total RNA was extracted and 10 μg aliquots were subjected to electrophoresis on formaldehyde gels, blotted onto nylon and probed with ^{32}P-labeled cDNA probes for rat TH and α-tubulin. Relative density ratio in control was expressed as 1 arbitrary unit. Results represent means ± S.E.M. of four experiments. *P<0.05 compared to control levels (ANOVA followed by Tukey's test).

Table I. Effects of tyrosine hydroxylase (TH) activity on harman and norharman in PC12 cells

Compounds	TH activity (nmol/min/mg protein) (% of control)		
	Incubation time (12 h)	Incubation time (24 h)	Incubation time (48 h)
Control	3.72 ± 0.26 (100)	3.91 ± 0.32 (100)	4.02 ± 0.38 (100)
Harman (20 μM)	1.88 ± 0.12 (50.5)*	1.90 ± 0.14 (48.6)*	2.05 ± 0.16 (51.2)*
Norharman (100 μM)	1.98 ± 0.15 (53.2)*	2.19 ± 0.21 (56.0)*	2.31 ± 0.18 (57.5)*

PC12 cells were treated with harman and norharman for 12-48 h and TH activity was assayed by an HPLC method. Results represent means ± S.E.M. of four experiments. Significantly different from the control values: *P<0.01 (ANOVA followed by Tukey's test).

activity was not inhibited by 20 μM harman and 100 μM norharman (data not shown). TH mRNA levels were also decreased by harman at 6-12 h and by norharman at 12-24 h (Fig. 5A and 5B).

Reduction of cyclic AMP levels

Harman at 20 μM and norharman at 100 μM significantly reduced the intracellular cyclic AMP levels to 50.5% and 70.1% of control levels at 30 min, respectively (Table II).

L-DOPA-induced dopamine content

Treatments with L-DOPA at 20 μM and 50 μM for 24 h increased dopamine content to 121% and 131% compared to control levels, respectively (Fig. 6). Harman (10, 20 and 30 μM) reduced L-DOPA (20 and 50 μM)-induced increases of dopamine content at 24 h (Fig. 6). Norharman (50, 100 and 150 μM) also reduced L-DOPA (20 and 50 μM)-induced increases of dopamine content at 24 h (Fig. 6).

Table II. Effects of harman and norharman on cyclic AMP levels in PC12 cells

Compounds	Cyclic AMP levels (nmol/min/mg protein) (% of control)
Control	3.84 ± 0.32 (100)
Harman (20 μM)	1.94 ± 0.31 (50.5)**
Norharman (100 μM)	2.69 ± 0.38 (70.1)*

PC12 cells were treated with harman and norharman and incubated at 37°C for 30 min. The intracellular cyclic AMP levels were measured by an enzyme immunoassay. Results represent means±S.E.M. of four experiments. Significantly different from the control values: *P<0.05; **P<0.01 (ANOVA followed by Tukey's test).

L-DOPA-induced cytotoxicity

Harman and norharman exhibited cytotoxicity at con-

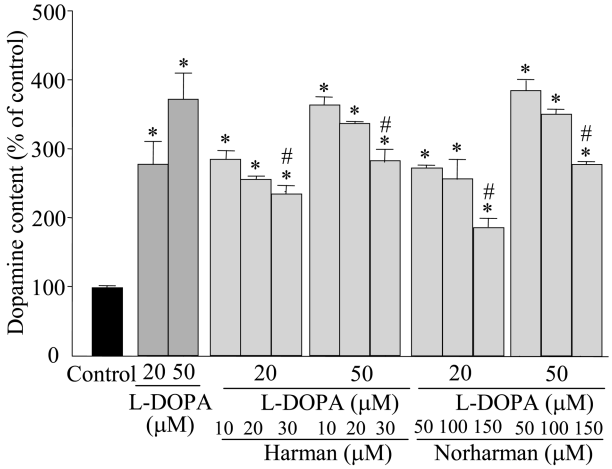

Fig. 6. Effects of harman and norharman on L-DOPA-induced dopamine content in PC12 cells. PC12 cells were exposed to harman (10, 20 and 30 μM) and norharman (50, 100 and 150 μM) in the absence or presence of L-DOPA (20 and 50 μM) for 24 h. Dopamine content of control levels was 3.43 ± 0.28 nmol/min/mg protein. Results represent means ± S.E.M. of four experiments. *P<0.05 compared to control levels; #P<0.05 compared to the corresponding L-DOPA concentrations. (ANOVA followed by Tukey's test).

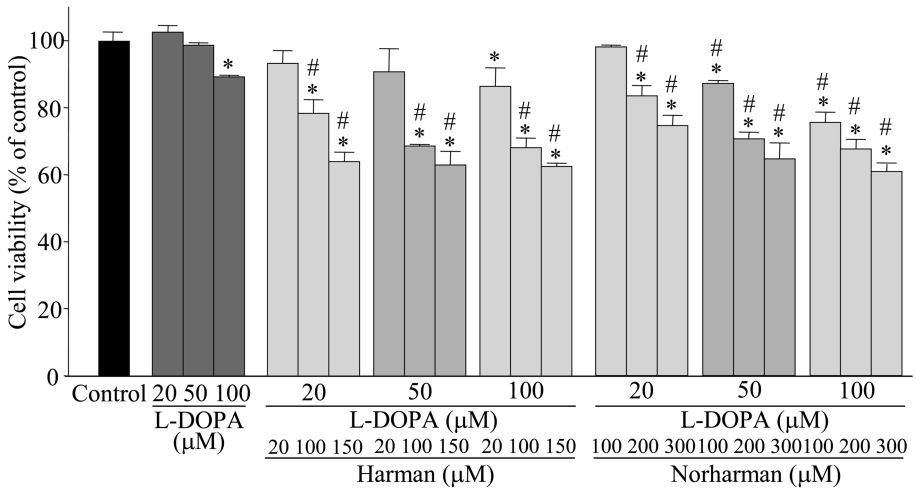

Fig. 7. Effects of harman and norharman on L-DOPA-induced cytotoxicity in PC12 cells. PC12 cells were incubated in the absence or presence of harman (20, 100 and 150 μM) and norharman (100, 200 and 300 μM) associated with L-DOPA (20, 50 and 100 μM) for 24 h. The cell viability was assessed by the MTT method. Results represent means ± S.E.M. of four experiments. *P<0.05 compared to control levels; #P<0.05 compared to the corresponding L-DOPA concentrations.

centrations higher than 100 μM and 200 μM at 24 h, respectively (Fig. 3). Treatments with L-DOPA at 100 μM, but not 20 and 50 μM, for 24 h reduced the cell viability to 90.5% of control levels, respectively (Fig. 7). Harman (20, 100 and 150 μM) associated with L-DOPA (20, 50 and 100 μM) showed the enhancing effects on the cell death at 24 h compared to L-DOPA alone (Fig. 7). Norharman (100, 200 and 300 μM) associated with L-DOPA (20, 50 and 100 μM) also exhibited the same trends on the cell viability (Fig. 7).

DISCUSSION

L-DOPA has been commonly used as the prior drug of the Parkinson's disease. However, L-DOPA accelerated the deterioration of the patient's condition in the long-term therapy (Marsden, 1994). TH is the rate-limiting enzyme in the dopamine biosynthetic pathways and involved for dopamine formation through the conversion of L-tyrosine to L-DOPA followed decarboxylation.

β-Carboline derivatives, harman and norharman, inhibit MAO activity, which is suggested that they have a possibly protective effect on Parkinson's diseases. In contrast, β-carbolines are structurally similar to MPTP, which causes a Parkinson-like syndrome by forming MPP^+ (Fields *et al.,* 1992). Therefore, the effects of harman and norharman on dopamine biosynthesis and L-DOPA-induced cytotoxicity were investigated in PC12 cells.

The intracellular dopamine content was significantly decreased by harman and norharman in a concentration-dependent manner without the secretion of dopamine. Under the same conditions, the intracellular TH activity and TH mRNA levels were reduced by harman and norharman. The intracellular cyclic AMP levels were also significantly reduced by harman and norharman. TH activity and TH gene expression could be activated by various factors including cyclic AMP, PKA, Ca^{2+}, PKC and Ca^{2+}/calmodulin kinase (Goldstein, 1995; Kumer and Vrana, 1996). These results suggest that harman and norharman inhibit dopamine biosynthesis by reducing TH activity and TH mRNA expression, which are mediated by the intracellular cyclic AMP levels.

L-DOPA at 20-100 μM increases dopamine content after 24 or 48 h of incubation in PC12 cells (Migheli, 1999; Lee *et al*., 2003). L-DOPA at concentrations higher than 100 μM also produces the intracellular cytotoxicity for 24 h, which is mediated by oxidative stress (Migheli, 1999; Lee *et al*., 2003). In this study, harman (10-30 μM) and norharman (50-150 μM) reduced L-DOPA (20-50 μM)-induced increases of dopamine content for 24 h in PC12 cells. High concentrations of harman (100 μM) and norharman (300 μM) caused cytotoxicity. Harman (20-150 μM) and norharman (100-300 μM) associated with L-DOPA (20-100 μM) enhanced L-DOPA-induced cytotoxicity. In addition, harman and norharman associated with L-DOPA exhibited a greater cytotoxicity than harman, norharman or L-DOPA alone. Harman also showed a stronger ability for the inhibition of dopamine biosynthesis and the aggravation of L-DOPA-induced cytotoxicity than norharman.

Many isoquinoline derivatives such as berberine, palmatine, bulbocapnine, higenamine, tetrahydropapaveroline, ethaverine and hydrastine inhibit the intracellular dopamine content in PC12 cells (Lee and Kim, 1996; Shin *et al*., 1998; Shin *et al*., 1999; Kim *et al*., 2005; Shin *et al*., 2001; Yin *et al*., 2004a). Tetrahydropapaveroline and hydrastine aggravate L-DOPA-induced cytotoxicity in PC12 cells (Lee *et al.,* 2003; Yin *et al*., 2004b). Isoquinoline derivatives are reported to have a similar structure with MPTP (McNaught *et al*., 1998) and their intracellular cytotoxic effects are also found to be mediated by oxidative stress (Nagatsu *et al.,* 1997).

Carbolines are converted to N-methylated β-carbolinium cations, which can be toxic to dopaminergic neurons, by β-carboline 9N-methyltransferase (Matsubara *et al.,* 1993). N-Methylated β-carbolinium ions such as 2-methyl-norharman induce large lesions after injection in the substantia nigra of rats (Neafsey *et al*., 1989) and are also increased in the frontal cortex of parkinsonians (Gearhart *et al.,* 2000). 2-Methylated β-carbolines are comparable to MPP^+ as inhibitors of mitochondrial respiration (Albores *et al.,* 1990). In Parkinson's diseases, the biosynthesis of N-methylated β-carboline derivatives is enhanced probably due to the failure of further catabolisation and detoxification of those N-methylated compounds (Green *et al.,* 1991). These results suggest that N-methylated derivatives are more toxic than the parent compounds. In addition, the precursors of those N-methylated β-carbolines such as harman and norharman were significantly higher in the substantia nigra than in the cortex of human brain without degeneration of substantia nigra (Matsubara *et al*., 1993). It is, therefore, suggested that the 1-methyl group of harman may play similar important roles regardless of N-position of methyl group. Further studies need to be determined the mechanisms responsible for the different effects on the N- or 1-position of methyl group.

In conclusion, harman at 10-30 μM and norharman at 50-150 μM reduced dopamine content and aggravated L-DOPA (20-50 μM)-induced cytotoxicity in PC12 dells. It is,

therefore, suggested that the patient in the long-term L-DOPA therapy should be carefully monitored for the drug interaction with the various neurotoxicants such as β-carbolines and isoquinoline derivatives.

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REFERENCES

Albores, R., Neafsey, E.J., Drucker, G., Fields, J.Z. and Collins, M.A. (1990). Mitochondrial respiratory inhibition by N-methylated β-carboline derivatives structurally resembling N-methyl-4-phenylpyridine. *Proc. Natl. Acad. Sci. USA*. **87,** 9368-9372.

Allen, J.R.F. and Holmstedt, B.R. (1980). The simple β-carboline alkaloids. *Phytochem*. **19,** 1573-1582.

Breyer-Pfaff, U., Wiatr, G., Stevens, I., Gaertner, H.J., Mundle, G. and Mann, K. (1996). Elevated norharman plasma levels in alcoholic patients and controls resulting from tobacco smoking. *Life Sci.* **58,** 1425-1432.

Cohen, G. (1983). The pathobiology of Parkinson's disease: Biochemical aspects of dopamine neuron senescence. *J. Neural Transm.* **19**(Suppl.)**,** 89-103.

Deitrich, R. and Erwin, V. (1980). Biogenic amine-aldehyde condensation products: Tetrahydroisoquinolines and trytolines (β-carbolines). *Annu. Rev. Pharmacol. Toxicol*. **20,** 55-80.

Ehringer, H. and Hornykiewicz, O. (1960). Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. *Klin. Wochenschr*. **38,** 1236-1239.

Fields, J.Z., Albores, R., Neafsey, E.J. and Collins, M.A. (1992). Similar inhibition of mitochondrial respiration by 1-methyl-4-phenylpyridinium (MPP^+) and a unique N-methylated beta-carboline analogue, 2,9-dimethyl-norharman (2,9-Me_2NH). *Ann. N.Y. Acad. Sci*. **648,** 272-274.

Gearhart, D.A., Collins, M.A., Lee, J.M. and Neafsey, E.J. (2000). Increased brain β-carboline-9N-methyltransferase activity in the frontal cortex in Parkinson's disease. *Neurobiol. Dis.* **7,** 201-211.

Goldstein, M. (1995). Long- and short-term regulation of tyrosine hydroxylase. In: Bloom F.E., Kupfer D.J. (Eds.), *Psychopharmacology*: the Fourth Generation of Progress. Raven Press, New York, 189-195.

Green, S., Buttrum, S., Molloy, H., Steventon, G., Sturman, S., Waring, R., Pall, H. and Williams, A. (1991). N-methylation of pyridines in Parkinson's disease. *Lancet* **338,** 120-121.

Kim, K.S., Park, D.H. and Joh, T.H. (1993). Parallel up-regulation of catecholamine biosynthesis enzymes by dexamethasone in PC12 cells. *J. Neurochem*. **60,** 946-951.

Kim, Y.M., Kim, M.N., Lee, J.J. and Lee, M.K. (2005). Inhibition of dopamine biosynthesis by tetrahydropapaveroline. *Neurosci. Lett*. **386,** 1-4.

Kuhn, W., Muller, T., Grobe, H., Dierks, T. and Rommelapacher, H. (1995). Plasma levels of the β-carbolines harman and norharman in Parkinson's disease. *Acta Neurol. Scand*. **92,** 451-454.

Kumer, S.C. and Vrana, K.E. (1996). Intricate regulation of tyrosine hydroxylase activity and gene expression. *J. Neurochem*. **67,** 443-462.

Lee, J.J., Kim, Y.M., Yin, S.Y., Park, H.D., Kang, M.H., Hong, J.T. and Lee, M.K. (2003). Aggravation of L-DOPA-induced neurotoxicity by tetrahydropapaveroline in PC12 cells. *Biochem. Pharmacol*. **66,** 1787-1795

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

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

Marsden, C.D. (1994). Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* **57,** 672-681.

Matsubara, K., Collins, M.A., Akane, A., Ikebuchi, J., Neafsey, E.J., Kagawa, M. and Shiono, H. (1993). Potential bioactivated neurotoxicants, N-methylated β-carbolinium ions, are present in human brain. *Brain Res.* **610,** 90-96.

May, T., Rommelspacher, H. and Pawlik, M. (1991). [^{2}H]harman binding experiments: I. A reversible and selective radioligand for monoamine oxidase subtype A in the CNS of the rat. *J. Neurochem*. **56,** 490-499.

McNaught, K.St.P., Carrupt, P.A., Altmore, C., Cellamare, S., Carotti, A., Testa, B., Jenner, P. and Marsden, C.D. (1998). Isoquinoline derivatives as endogenous neurotoxins in the aetiology of Parkinson's disease. *Biochem. Pharmacol*. **56,** 921-933.

Migheli, R., Godani, C., Bciola, L., Delodu, M.R., Serra, P.A., Zangani, D., Natale, G.D., Miele, E. and Desole, M.S. (1999). Enhancing effect of managanese on L-DOPA-induced apoptosis in PC12 cells: role of oxidative stress. *J. Neurochem*. **73,** 1155-1163.

Nagatsu, T. (1997). Isoquinoline neurotoxins in the brain and Parkinson's disease. *Neurosci. Res.* **29,** 99-111.

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

Neafsey, E.J., Drucker, G., Raikoff, K. and Collins, M.A. (1989). Striatal dopaminergic toxicity following intranigral injection in rats of 2-methyl-norharman, a beta-carbolinium analog of N-methy-4-phenylpyridinium ion (MPP^+). *Neurosci. Lett*. **105,** 344-349.

Pfau, W. and Skog, K. (2004). Exposure to β-carbolines norharman and harman. *J. Chromatogr. B.* **802,** 115-126.

Sandstrom, P.A., Mannie, M.D. and Buttke, T.H. (1994). Inhibition of activation-induced death in T cell hybridomas by thiol antioxidant: oxidative stress as a mediator of apoptosis. *J. Leukocyte Biol*. **55,** 221-226.

Shin, J.S., Kim, E.I., Kai, M. and Lee, M.K. (2000). Inhibition of dopamine biosynthesis by protoberberine alkaloids in PC12 cells. *Neurochem. Res*. **25,** 363-368.

Shin, J.S., Kim, K.T. and Lee, M.K. (1998). Inhibitory effects of bulbocapnine on dopamine biosynthesis in PC12 cells. *Neurosci. Lett.* **244,** 161-164.

Shin, J.S., Lee, J.J., Kim, Y., Lee, C.K., Yun, Y.P. and Lee, M.K. (2001). Inhibitory effects of ethaverine, a homologue of papaverine, on dopamine content in PC12 cells. *Biol. Pharm. Bull*. **24,** 103-105.

Shin, J.S., Yun-Choi, H.S., Kim, E.I. and Lee, M.K. (1999). Inhibitory effects of higenamine on dopamine content in PC12 cells. *Planta Med*. **65,** 452-455.

Tischler, A.S., Perlman, R.L., Morse, G.M. and Sheard, B.E. (1983). Glucocorticoids increase catecholamine synthesis and storage in PC12 cells pheochromocytoma cell culture. *J. Neurochem.* **40**, 364-370.

Yin, S.Y., Kim, Y.M., Lee, J.J., Jin, C.M., Yang, Y.J., Ma, J.J., Kang, M.H., Kai, M. and Lee, M.K. (2004a). Enantio-selective inhibition of (1R,9S)- and (1S,9R)-β-hydrastines on dopamine biosynthesis in PC12 cells. *Neuropharmacol.* **47,** 1045-1052.

Yin, S.Y., Lee, J.J., Kim, Y.M., Jin, C.M., Yang, Y.J., Kang, M.H., Kai, M. and Lee, M.K. (2004b). Effects of (1R,9S)-β-hydrastine on L-DOPA-induced cytotoxicity in PC12 cells. *Eur. J. Pharmacol*. **488,** 71-77.