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Effects of (−)-Sesamin on Dopamine Biosynthesis in PC12 Cells

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Abstract – The present study investigated the effects of (−)-sesamin on dopamine biosynthesis in PC12 cells. Treatment with (−)-sesamin (25 and 50 μM) increased intracellular dopamine levels and enhanced L-DOPA-induced increase in dopamine levels in PC12 cells. (−)-Sesamin (25 and 50 μM) also induced the phosphorylation of cyclic AMP-dependent kinase A (PKA), cyclic AMP-response element binding protein (CREB) and tyrosine hydroxylase (TH) in PC12 cells. These results suggest that (−)-sesamin induces dopamine biosynthesis via the PKA-CREB-TH pathways in PC12 cells. (−)-Sesamin needs to be studied further to serve as an adjuvant phytonutrient in neurodegenerative disease.
Keywords – (−)-Sesamin, Dopamine biosynthesis, PKA, CREB, Tyrosine hydroxylase, PC12 cells

Introduction

Parkinson's disease (PD) is mainly due to the loss of dopaminergic neurons in the substantia nigra pars compacta. Thereby, the dopamine levels and dopamine biosynthesis are reduced in the regions of substantia nigra and striatum.[1] L-3,4-Dihydroxyphenylalanine (L-DOPA), the natural precursor of dopamine, is the most frequently prescribed drug for controlling the symptoms of PD patients.[2] However, long-term administration of L-DOPA can accompany by disabling motor adverse effects[3,4] and also accelerate the progression of PD.[5] In the dopamine biosynthetic pathway, tyrosine hydroxylase (TH; EC 1.14.16.2) is a rate-limiting step, and TH activity and its gene expression are regulated by cyclic AMP, cyclic AMP-dependent kinase A (PKA) and cyclic AMP-response element binding protein (CREB) in dopaminergic neuronal and PC12 cells.[6,7] It is, therefore, suggested that the modulating agents of dopamine biosynthesis can be applied to the clinical candidates of PD.[8]

(−)-Sesamin is a major lignan constituent of Asiasari Radix (*Asiasarum heterotropoides* F. Maekawa var. *mandshuricum* F. Maekawa, Aristolochiaceae). (−)-Sesamin has an anti-cancer function in human lung cancer cells,[9] and has an inhibitory effect on nitric oxide production in BV-2 microglial cells.[10] (+)-Sesamin is a primary lignan compound in *Sesamum indicum DC* (Sesame seeds) and shows cholesterol-lowering, lipid-lowering, anti-inflammatory and anti-cancer effects.[11-13] (+)-Sesamin also has a protective effect on hypoxia-induced cell death in PC12 and BV-2 microglia cells through the suppression of reactive oxygen species (ROS) generation.[14,15] Recently, it has been reported that (+)-sesamin enhances dopamine biosynthesis and reduces L-DOPA-induced cytotoxicity in rat adrenal pheochromocytoma (PC12) cells.[16] (−)-Sesamin and (+)-sesamin are epimeric isomer lignans.[17] However, the effects of (−)-sesamin on dopamine biosynthesis were not examined. PC12 cells have been widely used as a model to investigate dopamine biosynthesis, oxidative stress-induced cytotoxicity, proliferation and differentiation.[18-21]

In this study, we investigated the effects of (−)-sesamin on dopamine biosynthesis in PC12 cells to evaluate the pharmacological functions. (−)-Sesamin enhanced dopamine biosynthesis via PKA-CREB-TH system in PC12 cells.

Experimental

Materials – (−)-Sesamin was isolated from *A. heteropoides* and identified previously described (Fig. 1).[22] A voucher specimen was deposited in the herbarium of College of Pharmacy, Youngnam University. L-DOPA,

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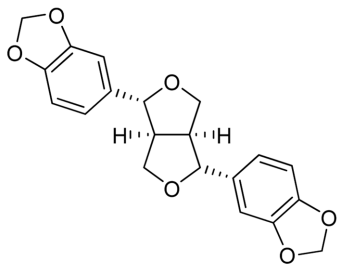

Fig. 1. Structure of (−)-sesamin.

dopamine, L-tyrosine, isoproterenol and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). RPMI 1640, donor horse serum, fetal bovine serum and antibiotics were purchased from Gibco BRL (Grand Island, NY, USA). Primary antibodies for Western blot analysis were purchased from Cell Signaling Tech (Beverly, MA, USA). All other chemicals were of analytical grade.

Cell cultures – PC12 cells were grown in an RPMI 1640 medium supplemented with 5% heat-inactivated fetal bovine serum, 10% heat-inactivated horse serum, 100 μg/ml streptomycin and 100 units/ml penicillin in a humidified atmosphere with 5% CO_2 and 95% air at 37°C.[18]

Determination of dopamine levels – Dopamine levels were determined according to a slightly modified procedure.[21] Pellet extracts with trichloroacetic acid (100 μmol) and isoproterenol (200 pmol, internal standard) were passed through a Toyopak SP-M cartridge (Na^+, resin 1 ml, Toso, Tokyo, Japan). The cartridge eluate was then derivatized with 1,2-diphenylethylenediamine. The final reaction mixture was injected into an HPLC system (Toso) with a fluorescence detector (F1000, Hitachi, Tokyo) (Ex/Em, 350/460 nm). The analytical conditions of HPLC were the same as described previously.[21]

Western blot analysis – Activation of the phosphorylation of PKA at Thr197 [phospho-PKA (Thr197)], CREB at Ser133 [phospho-CREB (Ser133)], TH at Ser40 [phospho-TH (Ser 40)] and β-actin were determined by a Western blot analysis.[21,23] Proteins in samples (30 μg in each lane) were electrophoresed in 12 - 15% sodium dodecyl sulfate-polyacrylamide gels and transferred to a polyvinylidene difluoride membrane at 300 mA for 1 - 3 h. The blots were blocked for 1 h at room temperature in fresh blocking buffer [TBS-T containing 5% bovine serum albumin (BSA)], and then incubated overnight at 4 °C using primary antibodies diluted 1 : 1,000 in TBS-T with 5% BSA, and for 1 h at room temperature using secondary antibodies (dilutions, 1 : 5,000 in TBS-T with 5% BSA). The blots were then washed, and the transferred proteins were incubated with ECL substrate solution (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA) for 5 min, according to the manufacturer's instructions, and visualized with radiographic film.

Statistical analysis – Protein content was determined using bovine serum albumin as a standard.[24] All data were expressed as the means ± S.E.M. of dopamine levels (n = 7 – 10) and Western blot analysis (n = 4). Statistical analysis was performed using one-way ANOVA followed by Dunnett's test, and a p value < 0.05 was considered statistically significant.

Results and Discussion

Treatment with (−)-sesamin at 25 - 100 μM showed an increase in the intracellular levels of dopamine at 24 h in PC12 cells. (−)-Sesamin at 25 and 50 μM increased dopamine levels by 165 - 177% ($p < 0.05$), compared with control group (Fig. 2A). However, (−)-sesamin at 100 μM did not further increase dopamine levels in PC12 cells, which might be resulted in sesamin-induced cytotoxicity. (−)-Sesamin at concentrations higher than 150 μM showed cytotoxicity in PC12 cells, which was determined by the MTT method (data not shown). The intracellular levels of dopamine were increased by treatment with L-DOPA at 25 - 100 μM at 24 h in PC12 cells.[21] Treatment with L-DOPA at 25 and 50 μM significantly increased the intracellular levels of dopamine by 126 - 145% ($p < 0.05$) at 24 h in PC12 cells (Fig. 2B). In addition, the levels of dopamine were further increased by 222 - 255% ($p < 0.05$) and 232 - 265% ($p < 0.05$) by co-treatments with (−)-sesamin (25 and 50 μM) and L-DOPA (25 and 50 μM) (Fig. 2B). Next, (−)-sesamin at 25 μM was selected for the following experiments.

In dopamine biosynthetic pathways, TH is a rate-limiting enzyme and its activity is regulated by PKA-CREB system.[7] L-DOPA at 20 - 100 μM also elevates the intracellular levels of dopamine by the stimulation of TH activity via the cyclic AMP-PKA-CREB signaling pathways in PC12 cells.[21] Therefore, the effects of (−)-sesamin on the activation of TH, PKA and CREB in PC12 cells were examined.

(−)-Sesamin (25 μM) induced TH phosphorlation to 1.6-fold the control groups ($p < 0.05$) for 1 h in PC12 cells (Fig. 3). The phosphorylation of TH, which was induced by L-DOPA (50 μM), was further increased by co-treatments with (−)-sesamin (25 μM) and L-DOPA (50 μM) (2.4-fold, $p < 0.05$), compared with L-DOPA-treated

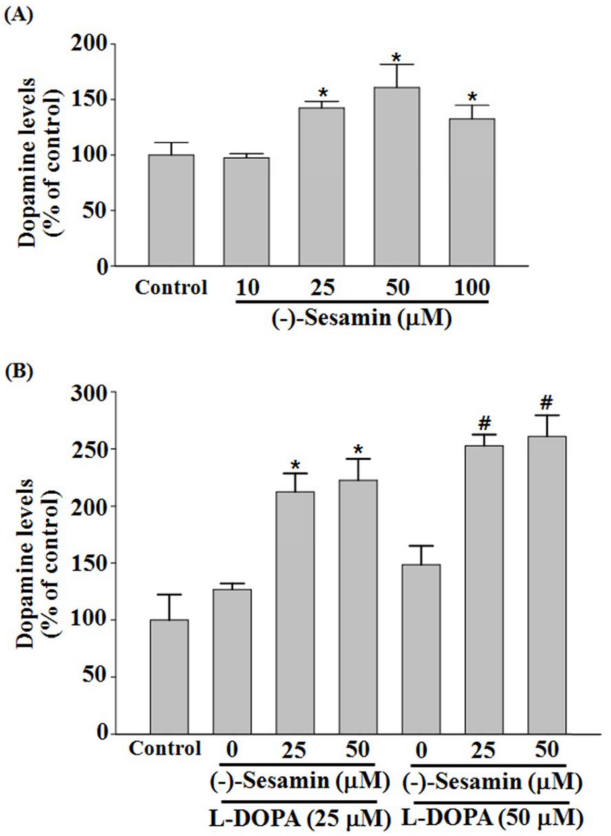

Fig. 2. Effects of (−)-sesamin on the intracellular levels of dopamine (A) and L-DOPA-induced dopamine biosynthesis (B) in PC12 cells. PC12 cells were treated with (−)-sesamin in the presence and absence of L-DOPA (20 and 50 μM) for 24 h, and then dopamine levels were determined by an HPLC method. The mean control levels of intracellular dopamine were 6.43 ± 0.21 nmol/mg protein. The results are expressed as the means ± S.E.M. ($n = 710$). $^{*}p < 0.05$ compared with control group; $^{\#}p < 0.05$ compared with L-DOPA-treated group (ANOVA followed by Dunnett's test).

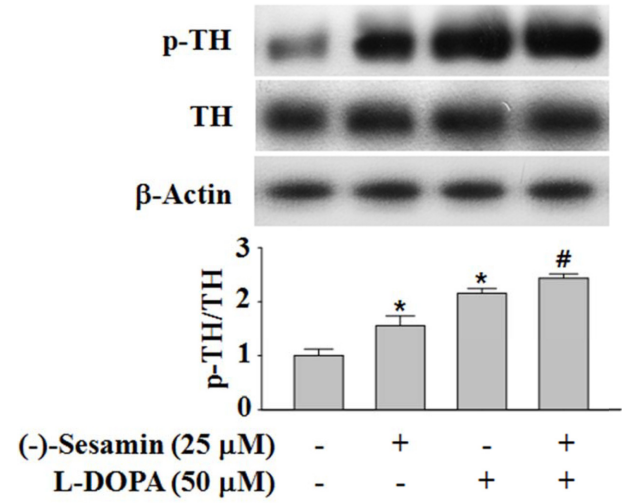

Fig. 3. Effects of (−)-sesamin on the phosphorylation of tyrosine hydroxylase (TH) in PC12 cells. PC12 cells were treated with (−)-sesamin (25 μM) in the presence and absence of L-DOPA (50 μM) for 13 h. Immunoblots of lysates of cell pellets were probed with phospho-TH antibodies, and then the phosphorylation of TH (p-TH) at Ser40 and the total TH were analyzed with Western blotting. The values of the relative density ratios of p-TH/TH are expressed in arbitrary units. The results are expressed as the means ± S.E.M. ($n = 4$). $^{*}p < 0.05$ compared with (−)-sesamin-untreated group; $^{\#}p < 0.05$ compared with L-DOPA-treated group (ANOVA followed by Dunnett's test).

group in PC12 cells (Fig. 3). In addition, (−)-Sesamin (25 μM) induced the phosphorylation of PKA and CREB to 1.6- and 1.4-fold the control group ((−)-sasamin-untreated group) ($p < 0.05$) for 1 h and 3 h, respectively in PC12 cells (Fig. 4). The phosphorylation of PKA and CREB, which was induced by L-DOPA (50 μM), was further increased to 2.5- and 1.8-fold ($p < 0.05$) by co-treatment with (−)-sesamin (25 μM) and L-DOPA (50 μM), compared with L-DOPA-treated group in PC12 cells (Fig. 4A and B). These results suggest that (−)-sesamin enhances dopamine biosynthesis via PKA-CREB-TH system in PC12 cells, and they showed the similar results obtained by (+)-sesamin.[16]

L-DOPA therapy is the current treatment of choice for PD patients. However, chronic treatment of L-DOPA induces oxidative stress-induced neurotoxicity by ROS formation against striatal dopaminergic neurons and PC12 cells.[19,25] (+)-Sesamin has the preventive effects on the loss of dopaminergic neuronal cells in the striatal regions of a rotenone- and MPTP-induced parkinsonian rat model.[26,27] Recently, (+)-sesamin increases dopamine levels by regulating TH activity via cyclic AMP-PKA-CREB system and inhibits L-DOPA-induced cytotoxicity through the suppression of ROS activity in PC12 cells.[16]

(−)-Sesamin and (+)-sesamin are epimeric lignans which have a furofuran backbone.[17] In general, the furofuran derivatives, including (−)-sesamin and (+)-sesamin, show an anti-oxidant function.[28] (−)-Sesamin inhibits nitric oxide production in BV-2 microglia cells.[10] (+)-Sesamin also shows free radical or superoxide anion scavenging activity in PC12 and BV-2 microglia cells.[14,15,29,30] In this study, (−)-sesamin showed very similar effects on dopamine biosynthesis in PC12 cells, compared with (+)-sesamin.[16] These results suggest that the effects of (−)-sesamin on dopamine biosynthesis are related with the ROS scavenging functions.

L-DOPA treatment increases the production of hydroxyl radicals in the substantia nigra *in vivo*.[31] In addition, an approach for improving L-DOPA therapy by co-administration of ROS scavenging agents/anti-oxidants in the substantia nigra has been suggested.[8] Anti-oxidant

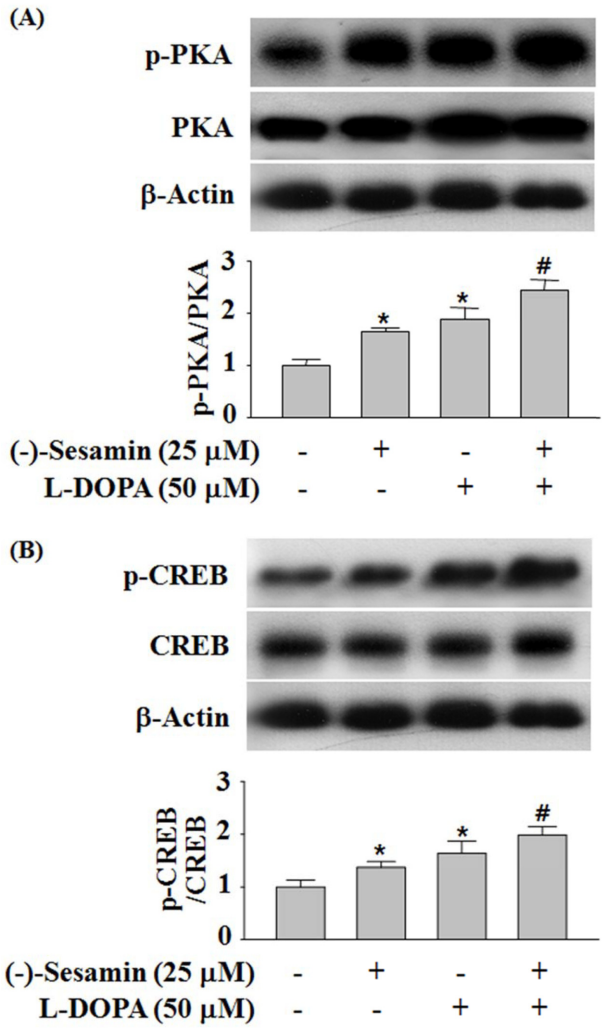

Fig. 4. Effects of (−)-sesamin on the phosphorylation of PKA (A) and CREB (B) in PC12 cells. PC12 cells were treated with (−)-sesamin (25 μM) in the presence and absence of L-DOPA (50 μM) for 1 h (A) and 3 h (B). Immunoblots of lysates of cell pellets were probed with phospho-PKA and phospho-CREB antibodies, and then the phosphorylation of PKA (p-PKA) at Thr197 and total PKA (A) and the phosphorylation of CREB (p-CREB) at Ser133 and the total CREB (B) were analyzed with Western blotting. The values of the relative density ratios of p-PKA/PKA and p-CREB/CREB are expressed in arbitrary units. The results are expressed as the means ± S.E.M. ($n = 4$). $^{*}p < 0.05$ compared with (−)-sesamin-untreated group; $^{\#}p < 0.05$ compared with L-DOPA-treated group (ANOVA followed by Dunnett's test).

therapies, such as selegiline, rasagiline, coenzyme Q10 and vitamin E, have been reported in *in vivo* applications.[8] It has been also found that (−)-sesamin protected 6-hydroxydopamine-induced cytotoxicity in PC12 cells (data not shown). With respect to this study, it is proposed that (−)-sesamin can be an adjuvant therapeutic agent for PD patients. Furthermore, the methanol extract of Asiasari Radix has not only been found to have inhibitory effects on AMPA-induced cytotoxicity through the activation of ERK in PC12 cells, but also to enhance memory through the inhibition of cholinesterase activity in rats.[32] These data can partially support our results.

In conclusion, (−)-sesamin increased dopamine biosynthesis by enhancing TH activity via the PKA-CREB signaling pathways in PC12 cells. (−)-Sesamin needs to be studied further to apply as an adjuvant phytonutrient in PD.

Acknowledgements

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