

Dynamic changes of five neurotransmitters and their related enzymes in various rat tissues following β -asarone and levodopa co-administration

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Abstract. The aim of the present study was to investigate the dynamic changes of five neurotransmitters and their associated enzymes in the rat plasma and brain tissues following the co-administration of β -asarone and levodopa (L-dopa). The rats were divided into five groups, including the control group and four treatment groups that were intragastrically co-administered β -asarone and L-dopa and sacrificed at 1, 5, 18 and 48 h, respectively. Neurotransmitter levels in the brain tissues and plasma were detected using high performance liquid chromatograph and the related enzymes of dopamine (DA) were measured using an enzyme-linked immunosorbent assay. The results indicated that the striatal levels of L-dopa and 3,4-dihydroxyphenylacetic acid (DOPAC) peaked at 1 h and then returned to the normal levels, while the striatal levels of DA were stable within 48 h. In the cortex and hippocampus tissue, L-dopa, DA, DOPAC and homovanillic acid (HVA) levels peaked at 1 h and then returned to normal levels. In the plasma, L-dopa, DA, DOPAC and HVA levels peaked at 1 h. Compared with the control group, L-dopa, DA and HVA levels were higher between 18 and 48 h, whereas the DOPAC level was lower. By contrast, no statistically significant differences were observed in the serotonin (5-HT) levels among the plasma, hippocampus, cortex and striatum. Furthermore, the DA/L-dopa ratio in the brain tissues and plasma increased in the first 5 h, while (DOPAC + HVA)/DA ratios demonstrated a significant reduction. Striatal tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC) levels were higher compared with the control group; however,

catechol-O-methyltransferase (COMT) and monoamine oxidase B levels were reduced. In the rat plasma, TH and COMT peaked at 1 h, while AADC peaked at 5 h. In conclusion, the results of the present study indicate that the co-administration of L-dopa and β -asarone may be used to maintain a stable striatal DA level within 48 h. In addition, this treatment may promote DA generation by AADC and reduce the metabolism of DA by COMT.

Introduction

Parkinson's disease (PD) is predominantly caused by the death of dopaminergic neurons in the substantia nigra, and results in deficient striatal dopamine (DA) levels, which are responsible for the motor symptoms of PD, including bradykinesia, tremor and rigidity (1). Currently, the primary treatment for PD is supplemental DA therapy (2). Tyrosine hydroxylase (TH) is a rate-limiting enzyme of DA synthesis, which catalyzes the hydroxylation of tyrosine to levodopa (L-dopa) (3). L-dopa is subsequently converted into DA by aromatic amino acid decarboxylase (AADC) (4). In addition, DA is converted into 3,4-dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase B (MAO-B) (5), and into homovanillic acid (HVA) by catechol-O-methyltransferase (COMT) (6). Therefore, L-dopa, DA, DOPAC, HVA, AADC, MAO-B, COMT and TH may be implicated in the pathogenesis of PD.

It has been demonstrated that L-dopa is the most effective constituent of typical PD treatment (7). However, the long-term use of L-dopa is associated with severe side effects, including motor response fluctuations and the emergence of drug-induced involuntary movements (8). For instance, Madopar, which consists of L-dopa and benserazide, is the firstline treatment option for PD. Long-term administration of Madopar is complicated by the development of various types of motor response oscillation, as well as drug-induced dyskinesia, which is a complication characterized by erratic involuntary movements, hypotension and psychiatric symptoms (9). Hence, Madopar does not resolve the adverse effects of L-dopa.

β -asarone (cis-2,4,5-Trimethoxy-1-propenylbenzene) is a strong fat-soluble substance with a low molecular weight (208 g/mol), which is able to rapidly traverse the blood-brain

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barrier, with a peak traversal time of 12 min, and has a half-life of 54 min (10). Our previous experiments indicated that β -asarone has a wide range of pharmacological effects on the central nervous system (CNS) and may be widely distributed in the rat hippocampus and cortex (11). Notably, we demonstrated that β -asarone and L-dopa co-administration was able to significantly increase the striatal levels of DA in healthy rat tissue (11). In addition, the striatum, hippocampus and cortex were the three important parts of the CNS (12). However, to the best of our knowledge, there are no prior studies that describe the effects of β -asarone and L-dopa co-administration on the dynamic changes of neurotransmitters in the rat striatum, cortex, hippocampus and plasma.

Therefore, the aim of the present study was to investigate the dynamic changes in the levels of L-dopa, DA, DOPAC, HVA and serotonin (5-HT) in the plasma, striatum, hippocampus and cortex of healthy rats following β -asarone and L-dopa co-administration, using high-performance liquid chromatography (HPLC) and fluorescence detection (FD). Furthermore, in order to observe the dynamic changes in the levels TH, COMT, AADC and MAO-B after co-administration within 48 h, the levels of these enzymes in the plasma and striatum were evaluated using an enzyme-linked immunosorbent assay (ELISA).

Materials and methods

Experimental design. A total of 40 Sprague Dawley rats (20 female and 20 male; weight, 220-250 g) were obtained from the Laboratory Animal Center of Guangzhou University of Chinese Medicine (ethical code no. TCMF1-2012028; Guangzhou, China). The animals were housed in a light- and temperature-controlled room with free access to standard food and water. The experimental protocols were approved by the Ethics Committee of Guangzhou University of Chinese Medicine and were consistent with the international guidelines.

Rats were divided into five groups (n=8 per group): Control group and four groups that received a single co-administration of β -asarone and L-dopa, at 15 and 60 mg/kg body weight, respectively. The rats in the four treatment group rats were subsequently sacrificed by cervical dislocation at 1, 5, 18 and 48 h, respectively. The control rats received an equal volume of normal saline vehicle, following the same procedure and were sacrificed at 48 h.

Preparation of β -asarone. The β -asarone used in this study was extracted from *Acorus tatarinowii* Schott according to a previously-reported procedure (13). The purity of β -asarone was ~99.55% (14), which was confirmed by gas chromatography-mass spectrometry, infrared spectrum and nuclear magnetic resonance detection, which was conducted at the China National Analytical Center (Guangzhou, China).

Sample collection. Following the co-administration, rats were anesthetized with 10% chloral hydrate (3.5 mg/kg, intraperitoneal injection) at the pre-specified times of 1, 5, 18 and 48 h after treatment. Next, the limbs of the anesthetized rats were fixed on an autopsy table and the rat hearts were exposed in the thoracic cavity by opening the abdominal cavity that is

below the xiphoids. Blood samples were collected from the aortic artery, and the plasma was separated by centrifugation at 3,000 x g for 10 min and stored at -80°C for subsequent HPLC analysis. Subsequently, in order to remove the blood from the rat brain, normal saline was perfused into the left ventricle and evacuated from the right atrial appendage. The perfusion was discontinued when the rat eyes and claws turned pale. The striatum, hippocampus and cortex areas were then dissected rapidly from the brains on ice and stored at -80°C for HPLC analysis. Sample collection from the control group rats was performed following the same procedure.

HPLC analysis of DA, L-dopa, DOPAC, HVA and 5-HT. Briefly, the striatum, hippocampus and cortex were weighed and homogenized in ice-cold 0.1 M perchloric acid (1:5 g/ml) by sonication at 40 kHz for 5 min. Homogenates were centrifuged at 12,000 x g for 15 min at 4°C, then the supernatants were collected and filtered through microporous membrane filters (0.22 μ m), and 20 μ l of each sample was injected into the HPLC column. In addition, 0.1 M HClO₄ was added to 500 μ l plasma at a ratio of 1:1 (v/v). The mixture was subjected to vortex mixing and centrifugation at 13,000 x g for 15 min at 4°C. Next, the supernatants were collected and filtered through microporous membrane filters (0.22 μ m), and 20 μ l of each sample was subjected to HPLC. The control substances of DA, 5-HT and L-dopa were obtained from National Institutes for Food and Drug Control (Beijing, China), while DOPAC and HVA were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The concentrations of DA and L-dopa were quantified by HPLC and FD, using a Waters 2695 separations module and a Waters 2475 multiwavelength fluorescence detector (Waters Corporation, Milford, MA, USA) at an excitation and absorption wavelength of 280 and 330 nm, respectively. Separation was performed using a 5- μ m Hypersil™ ODS2 column (150x4.6 mm; Dalian Elite Analytical Instruments Co., Ltd., Dalian, China) with column temperature of 30°C and a flow rate of 1 ml/min. The mobile phase was composed of 0.1 M KH₂PO₄ and methanol. The column was equilibrated with mobile phase for 30 min prior to analysis. This method was validated for the determination of neurotransmitter levels as reported in our previous study (15). Data were analyzed using Empower 2 chromatography data software (Waters Corporation) and the results were calculated and expressed as μ g/g and μ g/ml for tissue and plasma, respectively.

TH, COMT, AADC and MAO-B analyses. The striatum was weighed and homogenized with ice-cold normal saline (1:3 μ l/mg), and then centrifuged at 3,000 x g for 10 min to obtain the supernatant. TH (T031FC), COMT (C033FC), AADC (A036FC) and MAO-B (M035FC) were determined separately using ELISA kits (Shanghai Saimo Biotechnology Co., Ltd., Shanghai, China) and an American Hyperion MR111 microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) according to the manufacturer's instructions. In addition, ELISA kits from the same supplier were used to separately determine the plasma levels of AADC (A036SC), COMT (C033SC) and TH (T031SC).

Statistical analysis. Data are expressed as the mean \pm standard deviation and statistical differences between groups

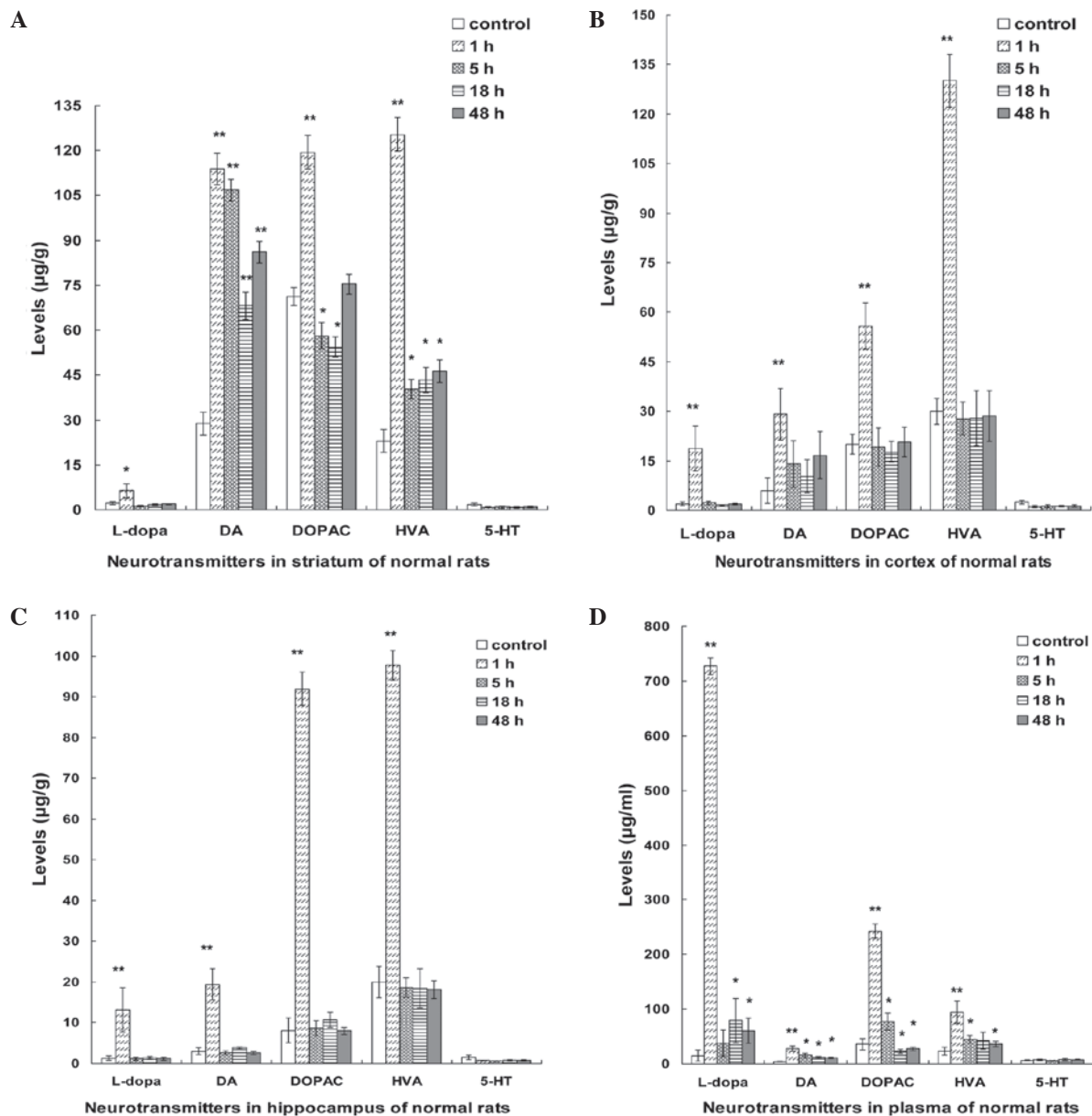


Figure 1. Dynamic changes in the levels of the neurotransmitters, L-dopa, DA, DOPAC, HVA and 5-HT, in the plasma and brain tissues of healthy rats at various time points after the co-administration of β -asarone and L-dopa. Differences in the levels of the five neurotransmitters in the (A) striatum, (B) cortex, (C) hippocampus and (D) plasma of the rats within 48 h ($\mu\text{g/g}$ tissue weight) are presented. Bars represent the mean \pm standard deviation of 8 rats. * $P < 0.05$ and ** $P < 0.01$ vs. control group (analysis of variance with Bonferroni test). L-dopa, levodopa; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, serotonin.

were determined by one-way analysis of variance followed by Bonferroni post-hoc test for multiple comparisons at $P < 0.05$. $P < 0.05$ was considered to indicate a statistically significant difference. Correlations between the neurotransmitters were performed by Pearson correlation. All the statistical analyses were performed using SPSS statistical software, version 17.0 (SPSS, Inc., Chicago, IL, USA).

Results

Levels of the five neurotransmitters changed in the rat plasma, hippocampus, cortex and striatum following treatment. Subsequent to co-administration of β -asarone and L-dopa, the L-dopa levels increased in the striatum of the rats, peaking at 1 h, then decreasing markedly at 5 h and

remaining stable between 5 and 48 h. Compared with the control group, L-dopa levels increased significantly in the 1 h group ($P < 0.05$). DA levels also increased and peaked at 1 h, showing a slight reduction at 5 h, followed by a further reduction between 5 and 18 h and a subsequent increase. DA levels demonstrated a significant increase in the 1, 5, 18 and 48 h groups compared with the control group ($P < 0.05$). Similarly, DOPAC levels increased significantly in the 1 h group compared with the control group ($P < 0.01$), decreased linearly between 5 and 18 h ($P < 0.05$), and then exhibited a gradual increase in the 48 h group. In addition, HVA levels peaked at 1 h, then declined markedly at 5 h and increased slightly between 5 and 48 h. Compared to the control group, HVA levels demonstrated a significant increase in all the treatment groups (1, 5, 18 and 48 h; $P < 0.05$). By contrast,

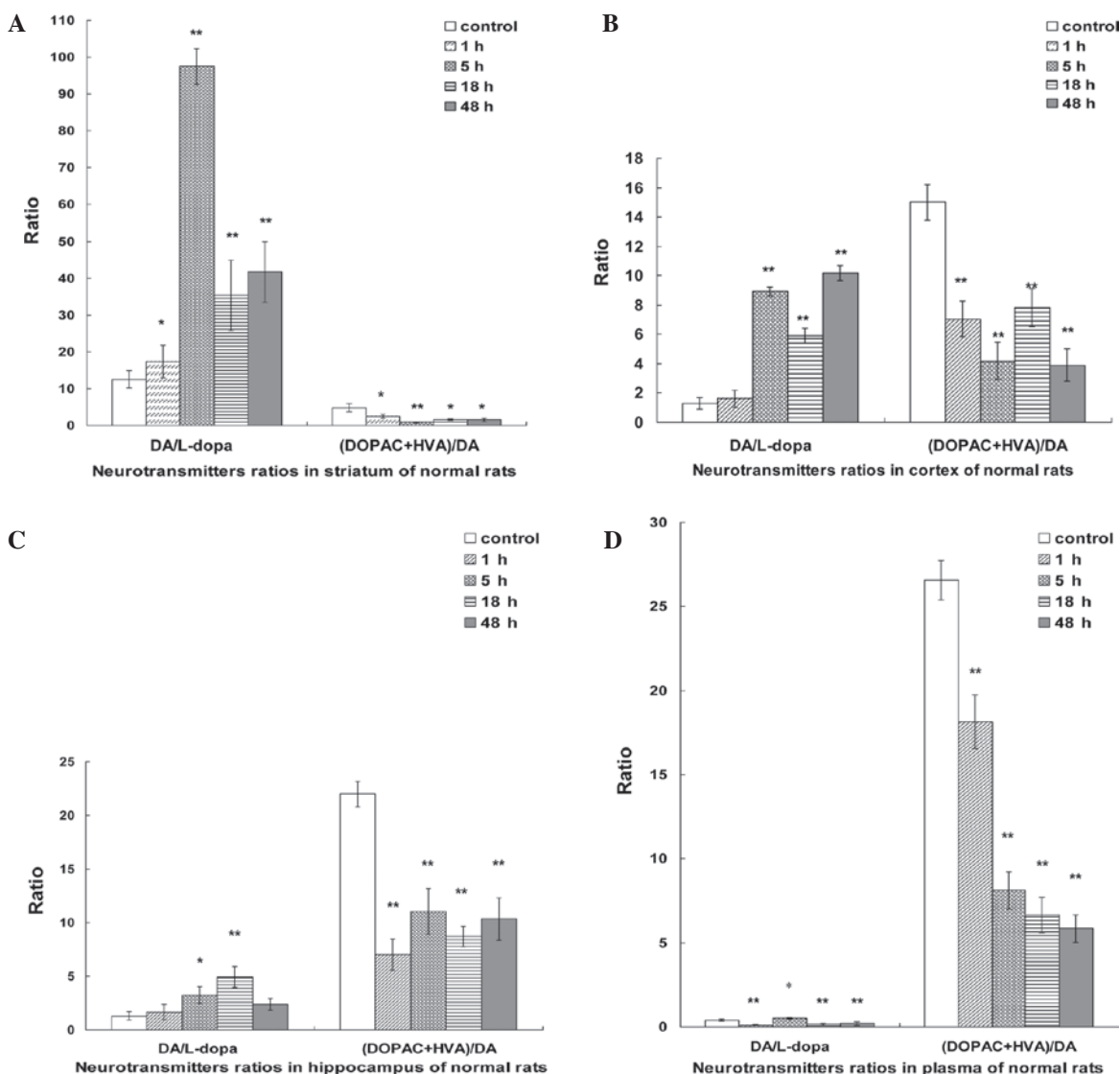


Figure 2. Dynamic changes in the ratios of DA/L-dopa and (DOPAC + HVA)/DA in the plasma and brain tissues of healthy rats at various time points after the co-administration of β -asarone and L-dopa. The DA/L-dopa and (DOPAC + HVA)/DA ratios in the (A) striatum, (B) cortex, (C) hippocampus and (D) plasma of normal rats are presented. Error bars represent the mean \pm standard deviation of 8 rats. * $P < 0.05$ and ** $P < 0.01$ vs. control group (analysis of variance with Bonferroni test). L-dopa, levodopa; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid.

5-HT levels remained stable between 1 to 48 h and were not significantly different compared with the control group (Fig. 1A).

In the cortex and hippocampus, the levels of L-dopa, DA, DOPAC and HVA peaked at 1 h, followed by a sharp decline at 5 h and a stable level between 5 and 48 h. Compared with the control group, the levels of L-dopa, DA, DOPAC and HVA were significantly increased in the 1 h group ($P < 0.01$); however, the 5-HT levels remained stable between 1 and 48 h and showed no significant difference compared with that of the control group (Fig. 1B and C).

In the rat plasma, L-dopa levels increased and peaked at 1 h, then decreased markedly at 5 h, increased slightly at 18 h before finally declining slightly at 48 h. In addition, DA levels increased and peaked at 1 h, and subsequently decreased slightly between 5 and 48 h. In addition, DOPAC levels increased and peaked at 1 h, then showed a sharp decline at 5 h, followed by a further reduction between 18 and 48 h. HVA levels peaked at 1 h, decreased sharply at 5 h, then declined slowly between

18 and 48 h. 5-HT levels remained a stable between 1 and 48 h and showed no significant difference compared with the control group. Furthermore, the levels of L-dopa, DA, DOPAC and HVA demonstrated a marked increase in the 1 h group compared with the control group ($P < 0.01$; Fig. 1D).

Comparisons of DA/L-dopa and (DOPAC + HVA)/DA ratios in the plasma, striatum, hippocampus and cortex. In the striatum, the DA/L-dopa ratio showed a significant increase in the initial 5 h, followed by a sharp reduction at 18 h, and then increased slightly at 48 h. However, the (DOPAC + HVA)/DA ratio exhibited a marked reduction at 1 h, reaching a minimum at 5 h and then increasing slightly between 18 and 48 h (Fig. 2A).

In the cortex, the DA/L-dopa ratio demonstrated a sharp increase in the first 5 h, followed by a rapid decline at 18 h, and then increased significantly at 48 h. By contrast, the (DOPAC + HVA)/DA ratio showed a sharp decline in the first 5 h, followed by a marked increase at 18 h, and subsequently decreased at 48 h (Fig. 2B).

In the hippocampus, the DA/L-dopa ratio exhibited an increasing tendency in the first 18 h, and then returned to the normal levels. In addition, the (DOPAC + HVA)/DA ratio showed a sharp decline in the first 1 h, followed by a slight increase between 5 and 48 h (Fig. 2C).

In the plasma, when compared with the control group, the DA/L-dopa ratio showed a significant decline in the first 1 h, and then peaked at 5 h followed by a further reduction between 18 and 48 h. Furthermore, the (DOPAC + HVA)/DA ratio presented a sharp reduction after 48 h, when compared with the control group (Fig. 2D).

Comparison of DA, L-dopa, DOPAC, HVA and 5-HT alterations in the plasma, striatum, hippocampus and cortex following the co-administration. Following co-administration of β -asarone and L-dopa, the L-dopa levels increased and peaked at 1 h, followed by a sharp decline at 5 h, and remained stable between 5 and 48 h in the plasma, cortex, hippocampus and striatum. Among these, the L-dopa levels in the plasma were the highest. Furthermore, DA levels increased and peaked at 1 h in the plasma, hippocampus, cortex and striatum. Among these, the striatal levels of DA were the highest within 48 h, showing a slight reduction at 5 h, followed by a further reduction at 18 h and subsequent increase. However, DA levels in the cortex, hippocampus and plasma demonstrated a sharp decline at 5 h, followed by stable levels between 5 and 48 h. In addition, the striatal levels of DOPAC peaked at 1 h, followed by a sharp decline at 5 h, and remained stable until 18 h, prior to increasing at 48 h. DOPAC levels in the cortex, hippocampus and plasma exhibited a rapid decrease at 5 h, followed by a steady level between 5 and 48 h. Furthermore, striatal levels of HVA showed a marked reduction between 1 and 5 h, followed by a gradual increase between 5 and 48 h. Similarly, HVA levels in the cortex, hippocampus and plasma displayed a sharp reduction between 1 and 5 h, but remained stable between 5 and 48 h. By contrast, the 5-HT levels remained stable and showed no statistically significant differences from the control group levels within 48 h among the plasma, striatum, cortex and hippocampus.

Neurotransmitter ratio alterations in the plasma and brain following the co-administration. The DA/L-dopa ratio in the striatum and cortex showed a marked increase in the first 5 h after treatment, followed by a sharp reduction at 18 h, and subsequently returned to normal levels. In addition, the DA/L-dopa ratio in the hippocampus exhibited a rapid increase in the first 18 h, followed by a sharp reduction at 48 h. Furthermore, the DA/L-dopa ratio in the plasma increased and peaked at 5 h, followed by a rapid reduction at 18 h, and remained stable between 18 and 48 h.

The (DOPAC + HVA)/DA ratio in the plasma and striatum showed a rapid reduction within 48 h. In addition, the (DOPAC + HVA)/DA ratio in the cortex demonstrated a reduction in the first 5 h, followed by a rapid increase at 18 h. Similarly, the (DOPAC + HVA)/DA ratio in the hippocampus showed a sharp decline at 1 h, followed by a reduction at 18 h, and subsequently returned to normal levels.

Alterations in TH, COMT, AADC and MAO-B levels at various time points. In the striatum, TH levels increased and peaked

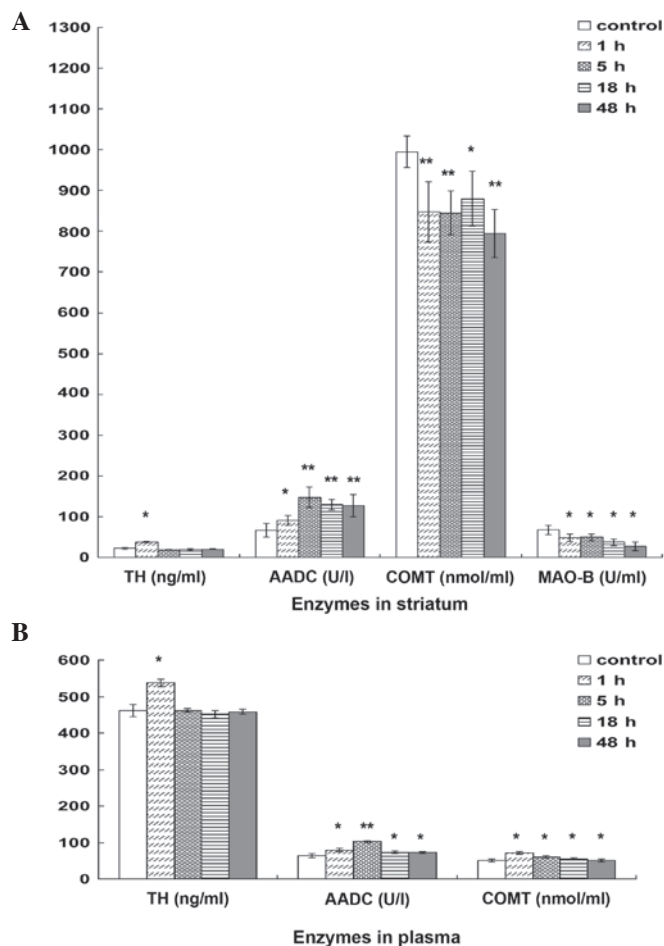


Figure 3. Dynamic changes in the levels of enzymes associated with DA synthesis at various time points after the co-administration of β -asarone and L-dopa. (A) Changes in the TH, AADC, COMT and MAO-B levels in the striatum of rats. (B) Changes in the TH, AADC and COMT levels in the plasma of the rats. Error bars represent the mean \pm standard deviation of 8 rats. * $P < 0.05$, ** $P < 0.01$ vs. control group (analysis of variance with Bonferroni test). TH, tyrosine hydroxylase; AADC, aromatic amino acid decarboxylase; COMT, catechol-*O*-methyltransferase; MAO-B, monoamine oxidase B.

at 1 h, followed by a marked reduction at 5 h, remaining stable between 5 and 48 h. The AADC levels demonstrated a rapid increase in the first 5 h followed by a further reduction at 18 h. By contrast, the COMT levels showed a sharp reduction at 1 h, followed by a further reduction at 48 h. In addition, MAO-B levels showed a slight reduction between 1 and 48 h (Fig. 3A).

In the plasma, TH levels increased at 1 h, followed by a rapid reduction at 5 h, and remained at a stable level between 5 and 48 h. AADC levels exhibited a gradual increase in the first 5 h and were subsequently reduced at 18 h, followed by a stable level between 18 and 48 h. Furthermore, AADC levels within 48 h were elevated compared with the control group, while COMT levels showed a significant reduction between 1 and 48 h (Fig. 3B).

Correlation between DA and 5-HT levels in the plasma, hippocampus, cortex and striatum following the co-administration. In order to investigate the association between the neurotransmitters following the co-administration of β -asarone and L-dopa, the correlations between DA and 5-HT in the cortex, striatum, hippocampus and plasma were analyzed using

Table I. Correlation between DA and 5-HT in rat plasma and brain tissues within 48 h.

Group	r-value			
	Striatum	Cortex	Hippocampus	Plasma
1 h	0.289	0.871	0.501	0.214
5 h	0.961 ^a	0.178	0.831	0.182
18 h	0.296	0.700	0.976 ^a	0.989 ^a
48 h	0.579	0.692	0.965 ^a	0.944

Pearson correlation analysis demonstrated that there were significant positive correlations between DA and 5-HT in the plasma, striatum, hippocampus and cortex. Data represent the mean \pm standard deviation of 8 rats. ^aP<0.05, vs. control group. DA, dopamine; 5-HT, serotonin.

Pearson correlation analysis (Table I). The correlation between DA and 5-HT was positive and statistically significant in the striatum at 5 h ($r=0.961$, $P<0.05$). In addition, the correlations between DA and 5-HT in the hippocampus at 18 and 48 h were 0.976 and 0.965, respectively ($P<0.05$). Furthermore, the correlation between DA and 5-HT in the plasma at 18 h was 0.989 ($P<0.05$). The results indicated that there were significant positive correlations between DA and 5-HT in the striatum at 5 h, in the hippocampus at 18 and 48 h and in the plasma at 18 h.

Discussion

β -asarone is a major component of *Acorus tatarinowii* Schott, and has a significant pharmacological effect in attenuating neuronal apoptosis, thus protecting against neurotoxicity (16). Furthermore, L-dopa remains the most effective medicine for the treatment of PD (7). To date, researchers have combined L-dopa with other medicines, such as carbidopa, for the treatment of PD, in order to mitigate the side-effects associated with L-dopa (17,18); however, such combinations have not resolved the adverse reactions that result from chronic use of L-dopa. Notably, we demonstrated in a previous study that β -asarone and L-dopa co-administration is able to significantly increase the striatal dopamine (DA) levels in healthy rats (11). However, the dynamic changes in the levels of DA, L-dopa, DOPAC, HVA and 5-HT in the plasma and brain of healthy rats within 48 h of the co-administration treatment remain unknown. Therefore, a quantitative HPLC method was employed to analyze the levels of these five neurotransmitters in the striatum, hippocampus, cortex and plasma of rats at 1, 5, 18 and 48 h following treatment. In addition, we determined the dynamic change in a number of enzymes associated with these neurotransmitters in the plasma and striatum within 48 h.

In the present study, it was observed that the striatal levels of L-dopa, DA, DOPAC and HVA increased after the co-administration and peaked at 1 h, followed by reduction or return to the normal levels between 5 and 48 h. Notably, DA metabolism occurs primarily in the striatum (19). The present results suggested that striatal DA levels were increased compared with those in the hippocampus, cortex and plasma

within 48 h, indicating that the co-administration may aid in maintaining DA levels in the striatum.

Compared with the control group, the DA/L-dopa ratio in the striatum exhibited a rapid increase and peaked within the initial 5 h after treatment, followed by a sharp decline at 18 h, and subsequent increase. In addition, the DA/L-dopa ratio in the striatum was higher compared with that in the hippocampus and cortex between 1 and 48 h. These results indicated that the transformation of L-dopa to DA occurred primarily in the striatum after co-administration. In addition, the (DOPAC + HVA)/DA ratios in the striatum, hippocampus and cortex exhibited a significant reduction compared with the control group. These results indicated that the co-administration of β -asarone and L-dopa may be able to reduce the metabolism of DA.

In order to identify the mechanism through which DA levels are elevated predominantly in the striatum, the functions of TH, AADC, COMT and MAO-B were investigated. TH is the primary regulator and rate-limiting enzyme of L-dopa synthesis (3). The results of the present study showed that striatal TH levels increased and peaked at 1 h, and subsequently returned to the normal levels at 5 h. This may explain why L-dopa level at in the striatum were higher at 1 h compared with at 5-48 h. In addition, L-dopa is known to be converted into DA by AADC (4). The current results indicated that striatal AADC levels in the treatment groups increased within 48 h compared with the control group, which was consistent with the observation that striatal DA levels within 48 h were elevated compared with levels in the hippocampus and cortex. Furthermore, DA may be degraded into DOPAC by MAO-B and into HVA by COMT (5,6). In the present study, striatal levels of MAO-B and COMT in the treatment groups were reduced compared with the control group, which may explain why the (DOPAC + HVA)/DA ratio in the striatum was reduced compared with the control group during 48 h.

In the plasma, the levels of L-dopa, DA, DOPAC and HVA increased and peaked at 1 h after the co-administration, and then showed a sharp decrease between 5 and 48 h. Based on the ratios of DA/L-dopa and (DOPAC + HVA)/DA, the turnover rate of L-dopa was found to peak at 5 h after co-administration, followed by a rapid decline between 5 and 48 h. However, the turnover rate of DA exhibited a sharp decrease between 1 and 48 h compared with control group. In addition, TH levels increased and peaked at 1 h prior to returning to the normal levels. AADC levels peaked at 5 h, and were higher compared with the control group. In addition, COMT levels exhibited a decreasing trend within 48 h compared with the control group. Thus, collectively the present results suggest that the co-administration may promote the generation of DA by enhancing the activity of AADC, in addition to reducing the metabolism of DA by inhibiting the activity of COMT.

In the present study, the 5-HT levels in the plasma, striatum, hippocampus and cortex of the rats exhibited no statistically significant differences when compared with the control group rats. However, the correlation between DA and 5-HT was positive and significant in the striatum, cortex, hippocampus and plasma at 5, 1, 18-48 and 18 h, respectively. The results indicated that the statistically significant correlation identified between DA and 5-HT was associated with the brain and plasma areas in healthy rats following the co-administration.

In conclusion, the present study reported the dynamic changes in the levels of L-dopa, DA, DOPAC, HVA and 5-HT in the striatum, cortex, hippocampus and plasma of rats within 48 h of the co-administration of β -asarone and L-dopa. Notably, the co-administration exerted the effect of maintaining a steady DA level in the striatum during the 48-h period. Furthermore, the co-administration appeared to promote the generation of DA by enhancing the activity of AADC, while reducing the metabolism of DA by inhibiting the activity of COMT. Therefore, the co-administration of β -asarone and L-dopa may provide a beneficial intervention for the treatment of PD.

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