Anti-oxidative and Anti-inflammatory Protection from Carnosine in Striatum of MPTP-treated Mice

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ABSTRACT

Mice treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were used to examine the neuroprotective effects of carnosine. Carnosine at 0.5, 1 and 2 g/L was directly added to the drinking water for 4 wk. MPTP treatment significantly depleted striatal glutathione content, reduced the activity of glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase, increased malondialdehyde and reactive oxygen species levels, and elevated interleukin-6, nitrite and tumor necrosis factor-α production as well as enhanced inducible nitric oxide synthase (iNOS) activity in striatum ($P<0.05$). The pre-intake of carnosine significantly attenuated MPTP-induced glutathione loss, retained the activity of GPX and SOD, diminished oxidative stress, and lowered inflammatory cytokines and nitrite levels as well as suppressed iNOS activity ($P<0.05$). MPTP treatment significantly suppressed GPX mRNA expression and enhanced iNOS mRNA expression ($P<0.05$). Carnosine pre-intake significantly elevated GPX mRNA expression and declined iNOS mRNA expression ($P<0.05$). Pre-intake of carnosine also significantly improved MPTP-induced dopamine depletion and maintained 3,4-dihydroxyphenylacetic acid and homovanillic acid levels ($P<0.05$). These results suggest that carnosine could provide anti-oxidative and anti-inflammatory protection for the striatum against the development of Parkinson's disease.

KEYWORDS: carnosine; Parkinson's disease; oxidative stress; iNOS activity; mRNA expression

Abbreviations

DA, dopamine; DAT, dopamine transporter; DOPAC, 3,4-dihydroxyphenylacetic acid; GPX, glutathione peroxidase; GSH, glutathione; HVA, homovanillic acid; IL-1β, interleukin-1β; iNOS, inducible nitric oxide synthase; MDA, malonyldialdehyde; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; PD, Parkinson's disease; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-α;

INTRODUCTION

Parkinson's disease (PD) is one of the major neurodegenerative diseases in the world. It is characterized by massive degeneration of nigrostriatal dopamine (DA) neurons in the substantia nigra pars compacta and the resultant deficiency in the neurotransmitter DA at the nerve terminals in the striatum (*1, 2*). The biochemical and cellular changes that occur after administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in animals are remarkably similar to those seen in idiopathic PD; thus, MPTP-induced PD has been widely used as a model for investigating pathogenic mechanisms of PD (*3, 4*). Oxidative stress and neuroinflammatory processes have been implicated as important mechanisms responsible for neuronal death in PD because reactive oxygen species (ROS), oxidized DA metabolites, nitric oxide (NO) and inflammatory cytokines are toxic to nigral neurons (*5, 6*). Thus, there is an increasing interest to examine the use of appropriate agent(s) to prevent or attenuate oxidative and inflammatory damage in PD (*7, 8*).

Carnosine (beta-alanyl-l-histidne) is endogenously synthesized peptide present in brain, skeletal muscle and liver (*9*). It has been reported that carnosine concentration in rat tissues could be increased by dietary supplementation (*10*). Several studies have indicated that this compound could provide both anti-oxidative and anti-inflammatory protection against diabetic deterioration and ethanol-induced chronic liver injury in mice (*11, 12*). Shen et al. (*13*) and Fu et al. (*14*) reported that carnosine could attenuate N-methyl-D-aspartate- and Abeta42-induced neurotoxicity in differentiated rat PC12 cells through carnosine-histidine-histamine pathway and/or inhibiting glutamate release. Those previous studies support that carnosine is a potent neuroprotective agent against oxidative and inflammatory progression in neurodegenerative diseases; however, further animal study is necessary to demonstrate the *in vivo* neuroprotective effects of carnosine.

In this study, MPTP was used to induce neurotoxicity in mice. Both anti-oxidative and anti-inflammatory activities of carnosine were examined in the mouse striatum, in which the impact of this agent at various doses on striatal content of glutathione (GSH), ROS and nitrite, activity of glutathione peroxidase (GPX) and inducible nitric oxide synthase (iNOS), and level of tumor necrosis factor (TNF)-α and interleukin (IL)-6 was determined. Furthermore, the effect of this agent on striatal level of DA metabolites such as 3,4-dihydroxyphenylacetic acid (DOPAC) and mRNA expression of DA transporter (DAT) was also evaluated.

MATERIALS AND METHODS

Animals and Diets. Three- to four-week-old male C57BL/6 mice were obtained from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Mice were housed on a 12-h light-12-h dark schedule, and fed with water and mouse standard diet for one week acclimation. Use of the mice was reviewed and approved by both Chung Shan Medical University and China Medical University animal care committees.

Experimental Design. Carnosine (98%), purchased from Sigma Chemical Co. (St. Louis, MO, USA), at 0.5, 1 or 2 g/L, was directly added to the drinking water. After 4-wk supplementation, mice were treated by daily subcutaneous injection of vehicle saline or MPTP (24 mg/kg body weight) for 6 consecutive days. Mice were sacrificed by decapitation. Brain was quickly removed and the striatum was collected. The striatum at 0.15 g was homogenized on ice in 2 mL of phosphate buffer (pH 7.2) and the filtrate was collected. Protein concentration of striatal filtrate was determined by a commercial assay kit (Pierce Biotechnology Inc., Rockford, IL, USA) with bovine serum

albumin used as standard. In all experiments, the sample was diluted to a final concentration of 1 mg protein/mL.

Determination of MPP^+ Level. The MPP^+ level in striatum was determined according to HPLC method of Richardson et al. (*15*). Briefly, striata were sonicated in 5% trichloroacetic acid and centrifuged for 10 min at 14,000 *g*, and the supernatants were collected for analysis. HPLC was equipped with a reverse-phase C18 column (Alltech Associates Inc., Deerfield, IL, USA), and the ultraviolet detector was set at 290 nm. MPP^+ was identified by comparing retention time with a known standard and concentration was calculated from a standard curve.

Measurement of DA, DOPAC and Homovanillic Acid (HVA). The levels of DA, DOPAC and HVA were determined by HPLC methods (*15*). Briefly, the striatum was homogenized in 0.1 mol/L of perchloric acid containing 0.1 mM ethylene-diaminetetraacetic acid. After centrifuging at 12,000 *g* for 60 min at 4°C, the supernatant was collected for analysis. HPLC equipped with a coulometric electrode array detector was used to quantify.

Determination of Lipid Oxidation and ROS. Malonyldialdehyde (MDA), an index of lipid peroxidation, was measured by using a commercial assay kit (OxisResearch, Portland, OR, USA). The method described in Gupta et al. (*16*) was used to measure ROS level. Briefly, 10 mg tissue was homogenized in 1 mL of ice cold 40 mM Tris–HCl buffer (pH 7.4), and further diluted to 0.25% with the same buffer. Then, samples were divided into two equal fractions. In one fraction, 40 μL 1.25 mM 2', 7'-dichlorofluorescin diacetate in methanol was added for ROS estimation. Another fraction, in which 40 μL methanol was added, served as a control for auto fluorescence. After incubating for 15 min at 37 °C, fluorescence was determined at 488 nm excitation and 525 nm emission

using a fluorescence plate reader.

Analyses for Carnosine, GSH and Total Antioxidant Capacity. Carnosine concentration was quantified according to the method described in Kamal et al. (*17*) by HPLC equipped with a 5-μm Waters Symmetry C18 column (250 x 4.6 mm). GSH concentration in striatal filtrate was determined by a commercial colorimetric GSH assay kit according to the manufacturer's instruction (OxisResearch, Portland, OR, USA). Reduced GSH was determined in this study. Total antioxidant capacity was measured via monitoring the change in absorbance at 593 nm by the method of Benzie and Strain (*18*), in which ferric tripyridyltriazine complex could be reduced by nonenzymatic antioxidants such as ascorbic acid presented in the sample.

Catalase, superoxide dismutase (SOD) and GPX Activity Assay. The activities of catalase, SOD, and GPX in the striatum were determined by catalase, SOD, and GPX assay kits (Calbiochem, EMD Biosciences, Inc., San Diego, CA, USA). The enzyme activity was expressed in U/mg protein.

Cytokine Measurements. Striatal levels of IL-1β, IL-6, TNF-α and monocyte chemoattractant protein (MCP)-1 were measured by ELISA methods using cytoscreener immunoassay kits (Bio-Source International, Camarillo, CA, USA). The sensitivities of assay with the detection limit were 5 pg/mL for IL-1β and IL-6, and 10 pg/mL for TNF-α and MCP-1.

Nitrite Assay and NOS Activity. The production of nitric oxide was determined by measuring the formation of nitrite. Briefly, 100 μL of supernatant was treated with nitrate reductase, NADPH and FAD, and incubated for 1 h at 37 ºC in the dark. After centrifuging at 6,000 g, the supernatant was mixed with Griess reagent for color

development. The absorbance at 540 nm was measured and compared with a sodium nitrite standard curve. The methods described in Sutherland et al. (*19*) were used to measure total NOS and iNOS activities. Total NOS activity was determined via incubating 30 μL of homogenate with 10 mM β-nicotinamide adenine dinucleotide phosphate, 10 mM L-valine, 3000 U/mL calmodulin, 5 mM tetrahydrobiopterin, 10 mM $CaCl_2$, and a mixture of 100 μM L-arginine containing L-[^{3}H]arginine. iNOS activity was measured excluding $CaCl_2$ and adding 10 mM ethylene glycol tetraacetic acid. Then, reaction was stopped by 1 mL of 20 mM HEPES buffer (pH 5.5). A dowex column was used to separate L-[^{3}H]arginine and L-[^{3}H]citrulline. The amount of L-[^{3}H]citrulline was assessed by liquid scintillation counter (Beckman Coultier, LS6500, Fullerton, CA, USA).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) for mRNA Expression. Part of the striatum was homogenized in guanidinethiocyanate, and total RNA was extracted. Two micrograms of total RNA was used to generate cDNA, which was amplified using Taq DNA polymerase. PCR was carried out in 50 mL of reaction mixture containing Taq DNA polymerase buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 200 mM dNTP, 2.5 mM $MgCl_2$, 0.5 mM of each primer) and 2.5 U Taq DNA polymerase. The specific oligonucleotide primers for DAT, GPX, iNOS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, the housekeeping gene) are as follows (*8, 20*), DAT: forward 5'-ATC AAC CCA CCG CAG ACA CCA GT-3', reverse, 5'-GGC ATC CCG GCA ATA ACC AT-3'; GPX: forward, 5'-CCT CAA GTA CGT CCG GCC TG-3', reverse, 5'-CAA CAT CGT TGC GAC ACA CC-3'; iNOS; forward, 5'-ATG ACC AGT ATA AGG CAA GC-3', reverse, 5'-GCT CTG GAT GAG CCT ATA TTG-3'; GAPDH: forward, 5'-TGA TGA CAT CAA GAA GGT GGT GAA G-3', reverse, 5'-CCT TGG AGG CCA TGT

AGG CCA T-3'. The cDNA was amplified under the following reaction conditions: 94 ºC for 1 min, 57 ºC for 1 min, and 72 ºC for 1 min. 28 cycles were performed for GAPDH and 32 cycles were performed for DAT, GPX and iNOS. A 10-μL aliquot of each PCR product was analyzed on a 2% agarose gel containing 0.5 μg/mL of ethidium bromide. Quantitative analysis for PCR products was performed by a BAS 2000 BIO-image analyzer (Fuji Photo Film Co., Tokyo, Japan), in which PCR products were illuminated by computerized densitometric scanning of the images. mRNA level was calculated as percentage of the control group.

Statistical Analysis. The effect of each treatment was analyzed from 10 mice (n = 10) in each group. All data were expressed as mean ± standard deviation (SD). Statistical analysis was done using one-way analysis of variance (ANOVA), and post-hoc comparisons were carried out using Dunnet's t-test. *P* values <0.05 were considered as significant.

RESULTS

As shown in Table 1, the intake of carnosine did not affect daily water intake and final body weight ($P>0.05$). The treatment of carnosine and/or MPTP did not significantly affect the weight of whole brain and striatum (data not shown). Compared with MPTP treatment alone, the pre-intake of carnosine did not significantly affect striatal MPP^+ level ($P>0.05$, data not shown).

The effects of carnosine intake and/or MPTP treatment on striatal levels of carnosine, GSH, total antioxidant capacity, MDA and ROS are presented in Table 2. The intake of carnosine at 1 and 2 g/L significantly increased carnosine and GSH content in striatum

(P<0.05). MPTP treatment significantly decreased levels of carnosine, GSH and total antioxidant capacity, and increased the production of MDA and ROS (P<0.05). However, the pre-intake of carnosine dose-dependently diminished MPTP-induced GSH loss (P<0.05). Furthermore, carnosine pre-treatments at 1 and 2 g/L significantly retained carnosine and total antioxidant capacity levels, as well as decreased production of MDA and ROS (P<0.05). The effect of carnosine and/or MPTP treatment on the activity of GPX, SOD and catalase is presented in Table 3. Carnosine pre-treatments alone did not affect the activity of these enzymes (P>0.05). MPTP treatment significantly reduced the activity of three test enzymes (P<0.05); however, the pre-intake of carnosine dose-dependently attenuated MPTP-induced GPX activity loss; but only at 1 and 2 g/L significantly retained the activity of SOD and catalase (P<0.05).

The effect of carnosine and/or MPTP treatment on the level of IL-1β, IL-6, TNF-α and MCP-1 is presented in Table 4. Carnosine pre-treatments alone did not affect these cytokines (P>0.05); however, MPTP treatment significantly increased the release of four test cytokines (P<0.05). The pre-intake of carnosine dose-dependently decreased TNF-α production; but this agent significantly lowered IL-1β and IL-6 levels at 1 and 2 g/L (P<0.05). Carnosine pre-intake only at 2 g/L significantly reduced MPTP-caused MCP-1 release (P<0.05).

As shown in Figure 1, MPTP treatment significantly increased nitrite production and elevated total NOS and iNOS activities (P<0.05). The pre-intake of carnosine dose-dependently decreased nitrite production and iNOS activity (P<0.05); but this agent at 1 and 2 g/L significantly lowered total NOS activity (P<0.05). MPTP treatment significantly down-regulated DAT and GPX mRNA expression, and up-regulated iNOS

mRNA expression (Figure 2, $P<0.05$). The pre-intake of carnosine dose-dependently enhanced GPX expression and suppressed iNOS expression ($P<0.05$). Carnosine pre-treatments failed to affect DAT expression ($P>0.05$). As shown in Table 5, MPTP treatment significantly decreased the striatal content of DA, DOPAC and HVA ($P<0.05$). The pre-intake of carnosine dose-dependently attenuated MPTP-induced DA loss; but only at 1 and 2 g/L significantly retained DOPAC and HVA content ($P<0.05$).

DISCUSSION

In our present study, carnosine pre-intake markedly attenuated MPTP-caused oxidative and inflammatory stress by lowering ROS, NO and inflammatory cytokines production, as well as mediating activity and mRNA expression of GPX and iNOS in striatum. Because carnosine pre-intake did not affect the striatal MPP^+ level; thus, the observed antioxidant protective action from this agent was not due to its scavenging activity on MPP^+. Therefore, our results support that carnosine is an effective anti-oxidative and anti-inflammatory agent against the development of neurodegenerative diseases such as PD. Since carnosine could mediate the mRNA expression of GPX and iNOS, this agent might exert its functions at the level of transcription.

The increased carnosine content in brain via dietary intake as we observed implied that this compound was able to penetrate the blood-brain barrier. It is reported that carnosine could scavenge free radicals and chelate divalent metal ions (*21*). Thus, partial anti-oxidative protection for MPTP-treated mice from this compound should be ascribed to its free radical scavenging action. Postmortem study indicated that GSH content in substantia nigra of PD patients was decreased; and GSH depletion has been proposed as the first indicator of oxidative stress during PD process (*22*). Thus, maintaining GSH level in

brain may delay PD progression. In our present study, carnosine intake elevated GSH content in brain from mice without MPTP treatment. This finding suggested that carnosine might be able to spare GSH and favor GSH homeostasis, which definitely contributed to enhance anti-oxidative protection for brain. In addition, we notified that carnosine pre-intake effectively attenuated MPTP-caused decline in striatal GSH and total antioxidant capacity. These results implied that carnosine participated in the anti-oxidative defense to protect brain of MPTP-treated mice via sparing other antioxidant agents or elevating the overall reducing power of this tissue. On the other hand, we found that carnosine intake markedly alleviated subsequent MPTP-caused activity decrease in GPX, SOD and catalase, and dose-dependently up-regulated mRNA expression of GPX, which further diminished oxidative damage in this tissue. Therefore, the results of our present study support that carnosine could mitigate oxidative injury in brain of MPTP-treated mice via both non-enzymatic and enzymatic antioxidant protective actions.

Increased level of proinflammatory cytokines such as TNF-α and IL-6 in the nigrostriatal region of postmortem brains from patients with sporadic PD is reported (*23*). The inhibition of TNF-α response has been considered as a promising target for developing anti-parkinsonian drugs for inflammatory treatment in PD (*24*). Our present study found that carnosine dose-dependently decreased MPTP-induced TNF-α production, and also effectively lowered IL-1β and IL-6 release at 1 and 2 g/L. Thus, carnosine could alleviate inflammatory damage via diminishing inflammatory cytokines production. On the other hand, iNOS is one of three NOS forms in the central nerves system. Overexpressed iNOS and elevated NO production are the most important neurotoxic effectors contributed to the loss of dopaminergic neurons and inflammatory deterioration of PD (*25*). Furthermore,

marked up-regulation of iNOS in the nigrostriatal region of postmortem brains from PD patients has been observed (*26*). Thus, inflammatory response in PD could be also improved via suppressing iNOS activity and lowering NO level. In our present study, carnosine pre-intake dose-dependently suppressed iNOS mRNA expression and inhibited activity of total NOS and iNOS, which consequently lowered NO production. It is reported that TNF-α-mediated activation of NF-kappaB is responsible for iNOS upregulation (*27*). However, Eberhardt et al. (*28*) indicated that NO is required for the expression of proinflammatory cytokines in macrophages. Obviously, there is a closed link in inflammatory regulation between NO/iNOS and proinflammatory cytokines such as TNF-α. Thus, carnosine seems a more efficient anti-inflammatory agent because it could suppress both production and activity of NO, iNOS and proinflammatory cytokines.

DAT is involved in DA homeostasis and sensitivity to dopaminergic neurotoxicants (*29*). As reported by others, MPTP depleted the striatal DA level (*1*) and suppressed gene expression of DAT (*30*). The results of our present study agreed those previous studies. However, we found the pre-intake of carnosine substantially locked MPTP-induced DA depletion in the striatum without alleviating MPTP-induced DAT depletion. Apparently, the increased DA level from this compound was not associated with DAT expression. It is highly possible that carnosine by its anti-oxidative and anti-inflammatory actions directly protected nigrostriatal dopaminergic neurons and ameliorated DA degeneration in the substantia nigra pars compacta. Since DA depletion was improved, the increased levels of DOPAC and HVA, metabolites of DA, could be explained. Carnosine is a naturally occurring dipeptide. Thus, the supplement of this compound might be safe. The major food source of carnosine is muscle foods such as chicken and beef (*31*). These muscle

foods also contain considerable fat. Based on healthy consideration, it may not be practical to increase muscle foods consumption in order to obtain carnosine.

In conclusion, the pre-intake of carnosine effectively alleviated MPTP-induced oxidative stress, inflammatory damage and DA loss. This agent exhibited anti-oxidative and anti-inflammatory activities by increasing GSH and carnosine content, elevating the activity of GPX and SOD, decreasing IL-6 and TNF-α levels, suppressing NO production and iNOS activity, as well as regulating mRNA expression of GPX and iNOS in striatum, which consequently retained levels of neurotransmitters such as DA, DOPAC and HVA. These results suggest that carnosine is a potent neuroprotective agent against the development of PD.

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Table 1. Water intake (WI) and body weight of mice consumed 0.5, 1 or 2 g/L carnosine (Car) at 1 and/or 4 week. Data are mean ± SD (n=10).

	WI, mL/mouse/d		Body weight, g
Time, wk	1	4	4
Control	2.0 ± 0.5[a]	3.1 ± 0.6[a]	25.0 ± 0.9[a]
Car			
0.5	2.3 ± 0.6[a]	3.4 ± 0.4[a]	24.3 ± 0.7[a]
1	2.1 ± 0.4[a]	3.2 ± 0.6[a]	24.7 ± 0.6[a]
2	2.4 ± 0.5[a]	3.3 ± 0.3[a]	25.1 ± 0.8[a]

[a]Means in a column without a common letter differ, $P<0.05$.

Table 2. Effect of carnosine (Car) alone or plus MPTP treatment on content of Car, GSH, total antioxidant capacity (TAC), MDA and ROS in the striatum. Values are mean ± SD, n=10.

	Car	GSH	TAC	MDA	ROS
	mg/100 g	ng/mg protein	nmol/mg protein	μmol/mg protein	nmol/mg protein
control	6.04±0.91^{c}	94±6^{e}	129±14^{d}	0.25±0.04^{a}	0.23±0.06^{a}
Car, 0.5	6.13±0.97^{c}	98±8^{e}	130±10^{d}	0.19±0.06^{a}	0.20±0.04^{a}
Car, 1	6.81±1.08^{d}	118±10^{f}	137±12^{d}	0.22±0.03^{a}	0.18±0.05^{a}
Car, 2	7.08±1.19^{d}	123±12^{f}	136±15^{d}	0.26±0.04^{a}	0.21±0.06^{a}
MPTP	4.07±0.70^{a}	34±4^{a}	66±6^{a}	1.40±0.11^{d}	1.23±0.13^{d}
Car, 0.5 + MPTP	3.98±0.51^{a}	45±5^{b}	73±5^{a}	1.29±0.10^{d}	1.19±0.12^{d}
Car, 1 + MPTP	4.85±0.55^{b}	60±3^{c}	90±7^{b}	0.87±0.07^{c}	0.93±0.10^{c}
Car, 2 + MPTP	5.11±0.47^{b}	72±5^{d}	108±10^{c}	0.57±0.08^{b}	0.75±0.09^{b}

Means in a column without a common superscript letter differ, $P < 0.05$

Table 3. Effect of carnosine (Car) alone or plus MPTP treatment on activity of GPX, SOD and catalase in the striatum. Values are mean ± SD, n=10.

	GPX	SOD	Catalase
	U/mg protein	U/mg protein	U/mg protein
Control	22.0±1.6^{e}	7.3±1.0^{c}	2.8±0.5^{c}
Car, 0.5	21.6±1.7^{e}	7.5±0.9^{c}	2.6±0.4^{c}
Car, 1	22.3±1.9^{e}	7.0±0.5^{c}	3.0±0.8^{c}
Car, 2	21.8±1.5^{e}	7.2±0.7^{c}	2.7±0.7^{c}
MPTP	9.1±1.0^{a}	1.9±0.4^{a}	0.9±0.3^{a}
Car, 0.5 + MPTP	11.3±1.2^{b}	2.5±0.5^{a}	1.2±0.4^{a}
Car, 1 + MPTP	14.2±1.3^{c}	4.0±0.6^{b}	1.9±0.6^{b}
Car, 2 + MPTP	17.4±1.5^{d}	4.7±0.9^{b}	2.0±0.5^{b}

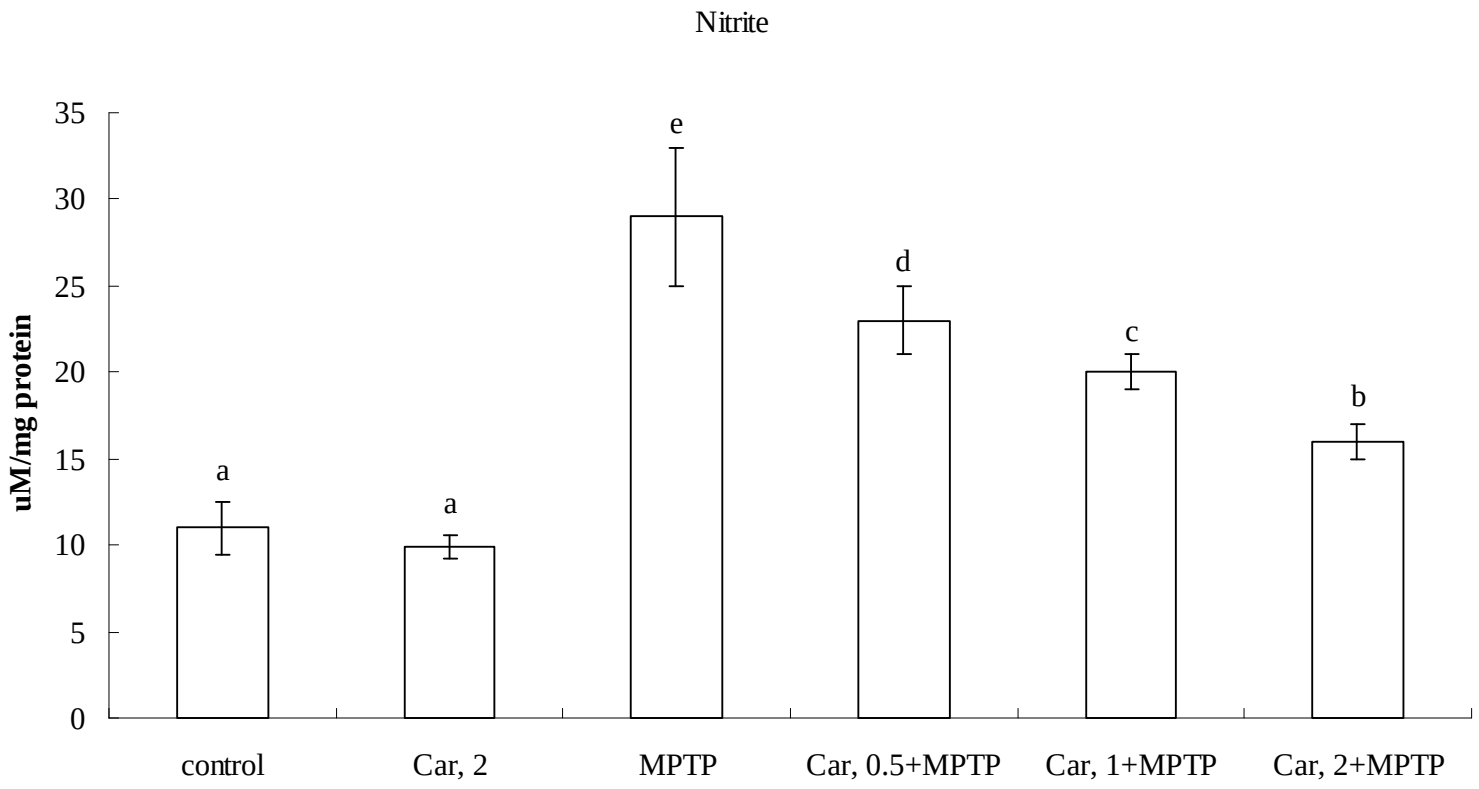
Means in a column without a common superscript letter differ, $P< 0.05$.

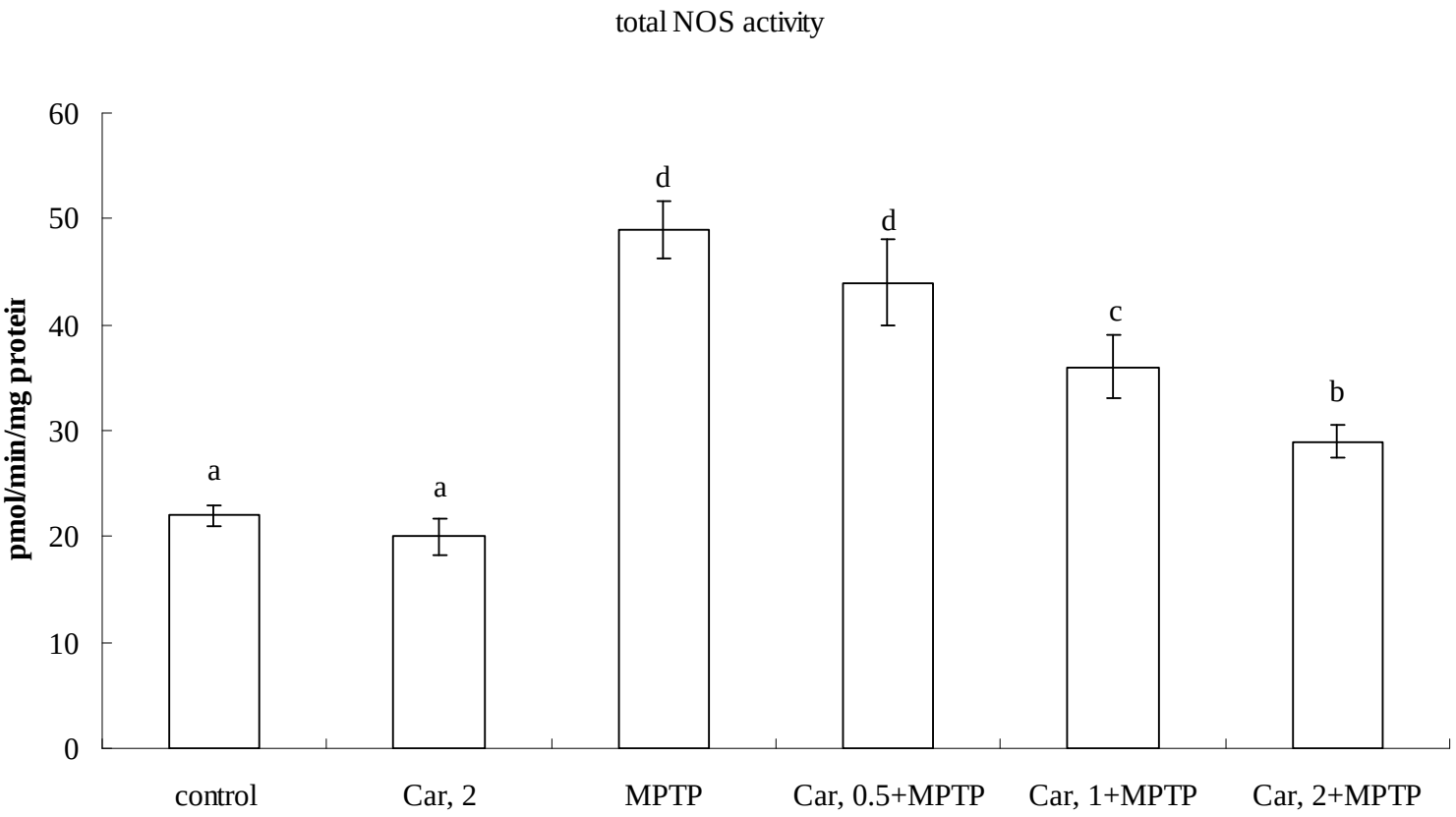
Table 4. Effect of carnosine (Car) alone or plus MPTP treatment on level (pg/mL) of IL-1β, IL-6, TNF-α and MCP-1 in the striatum. Values are mean ± SD, n=10.

	IL-1β	IL-6	TNF-α	MCP-1
Control	19.7±1.3[a]	17.5±2.1[a]	20.8±1.5[a]	16.2±1.0[a]
Car, 0.5	18.6±1.4[a]	18.7±1.6[a]	21.2±2.4[a]	17.0±1.4[a]
Car, 1	20.3±1.6[a]	19.0±1.5[a]	19.7±1.8[a]	16.6±0.9[a]
Car, 2	19.2±1.0[a]	17.2±1.3[a]	20.5±1.7[a]	15.9±1.1[a]
MPTP	120.6±10.2[d]	105.8±7.4[d]	133.9±9.2[e]	90.5±5.6[c]
Car, 0.5 + MPTP	111.8±8.1[d]	97.2±5.9[d]	109.1±7.0[d]	85.4±3.8[c]
Car, 1 + MPTP	84.4±6.7[c]	75.4±4.0[c]	76.4±5.2[c]	81.1±4.0[c]
Car, 2 + MPTP	63.6±5.1[b]	49.3±3.8[b]	55.3±4.5[b]	62.0±2.5[b]

Means in a column without a common superscript letter differ, $P < 0.05$.

Figure 1. Effect of carnosine (Car) alone or plus MPTP treatment on nitrite level, total NOS and iNOS activity in the striatum. Data are mean ± SD (n=10). [a-e]Means among bars without a common letter differ, $P<0.05$.


Nitrite
uM/mg protein
35
30
25
20
15
10
5
0
a
a
e
d
c
b
control
Car, 2
MPTP
Car, 0.5+MPTP
Car, 1+MPTP
Car, 2+MPTP


total NOS activity
pmol/min/mg protein
60
50
40
30
20
10
0
a
a
d
d
c
b
control
Car, 2
MPTP
Car, 0.5+MPTP
Car, 1+MPTP
Car, 2+MPTP

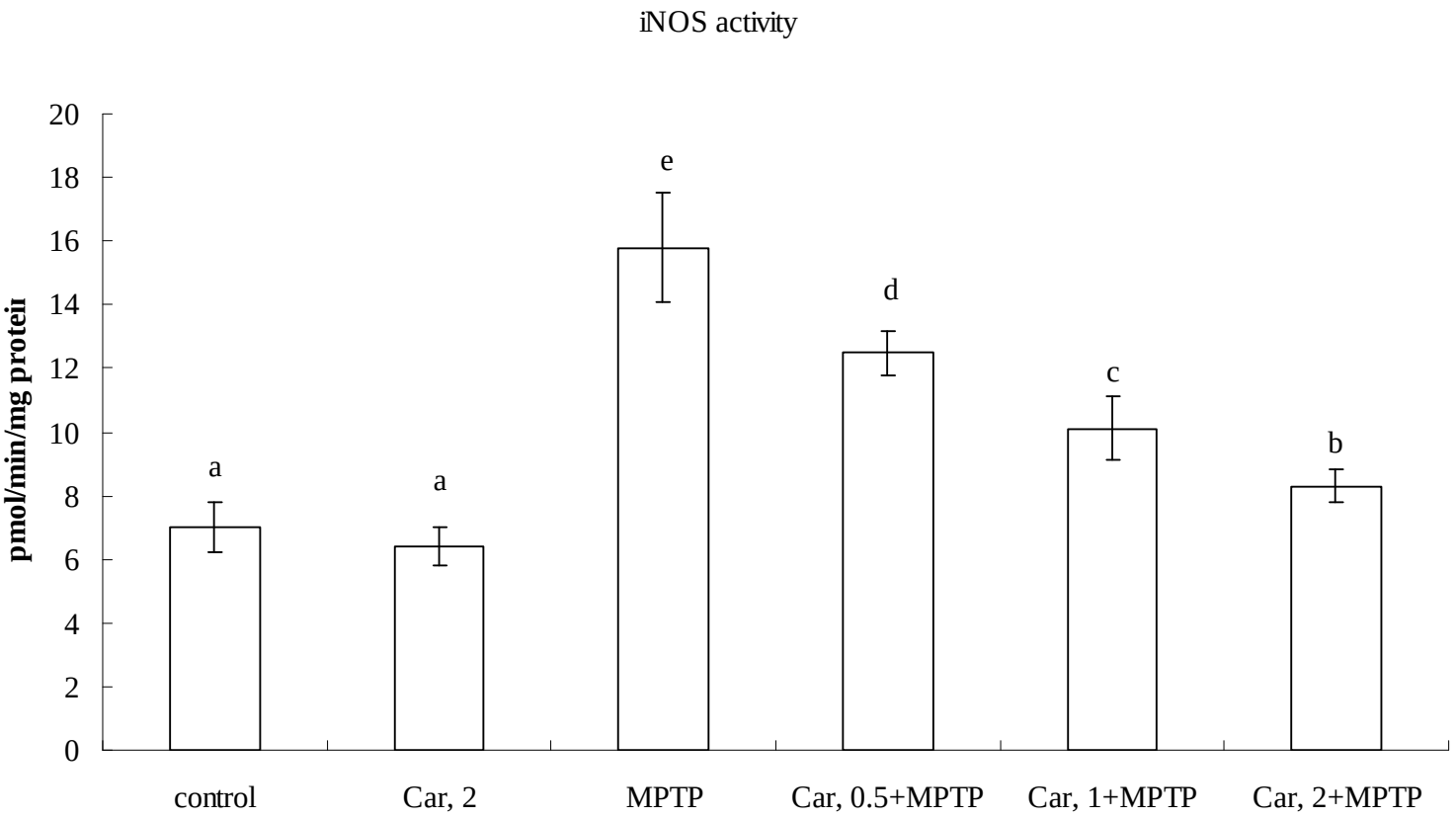
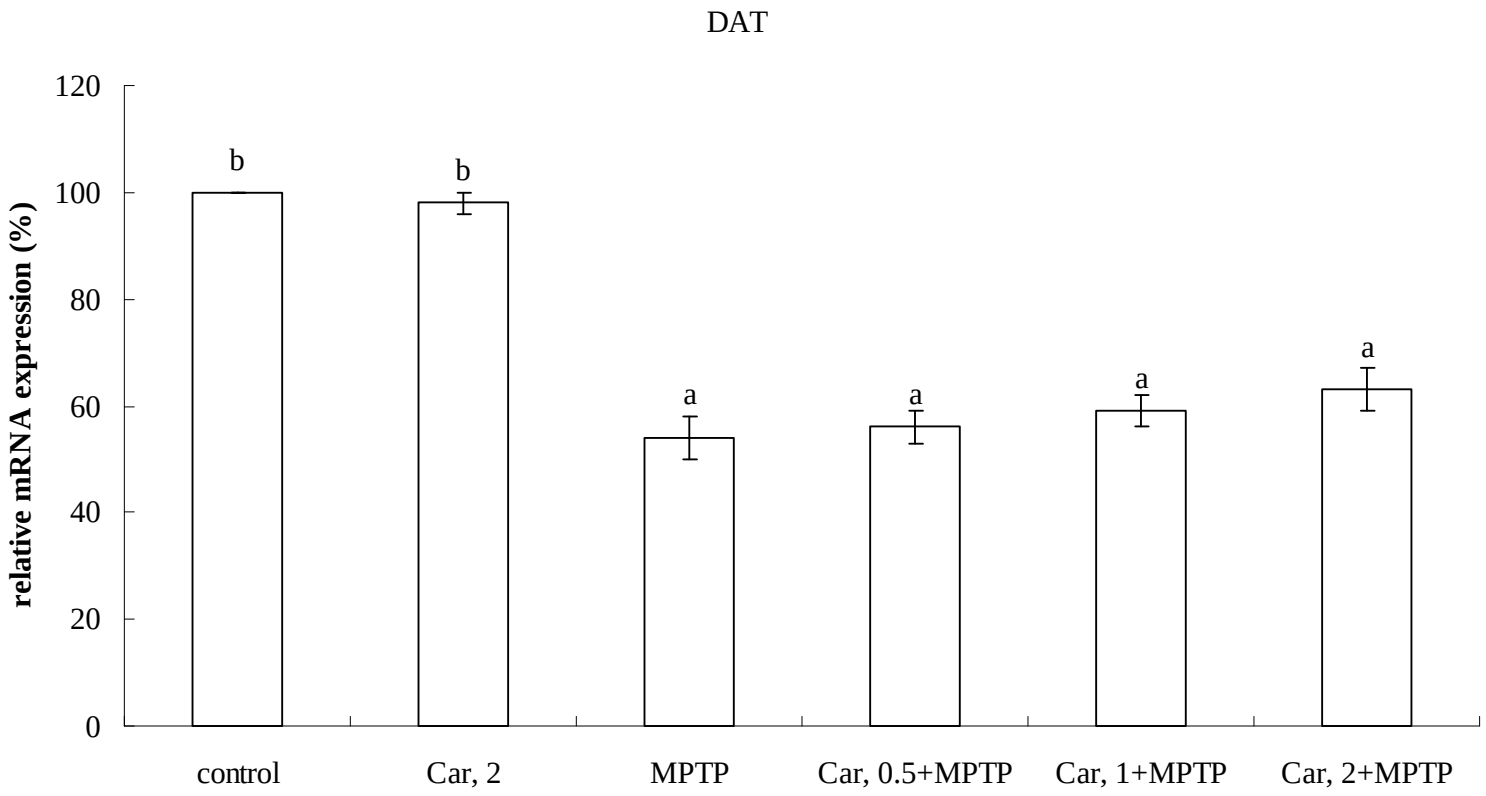
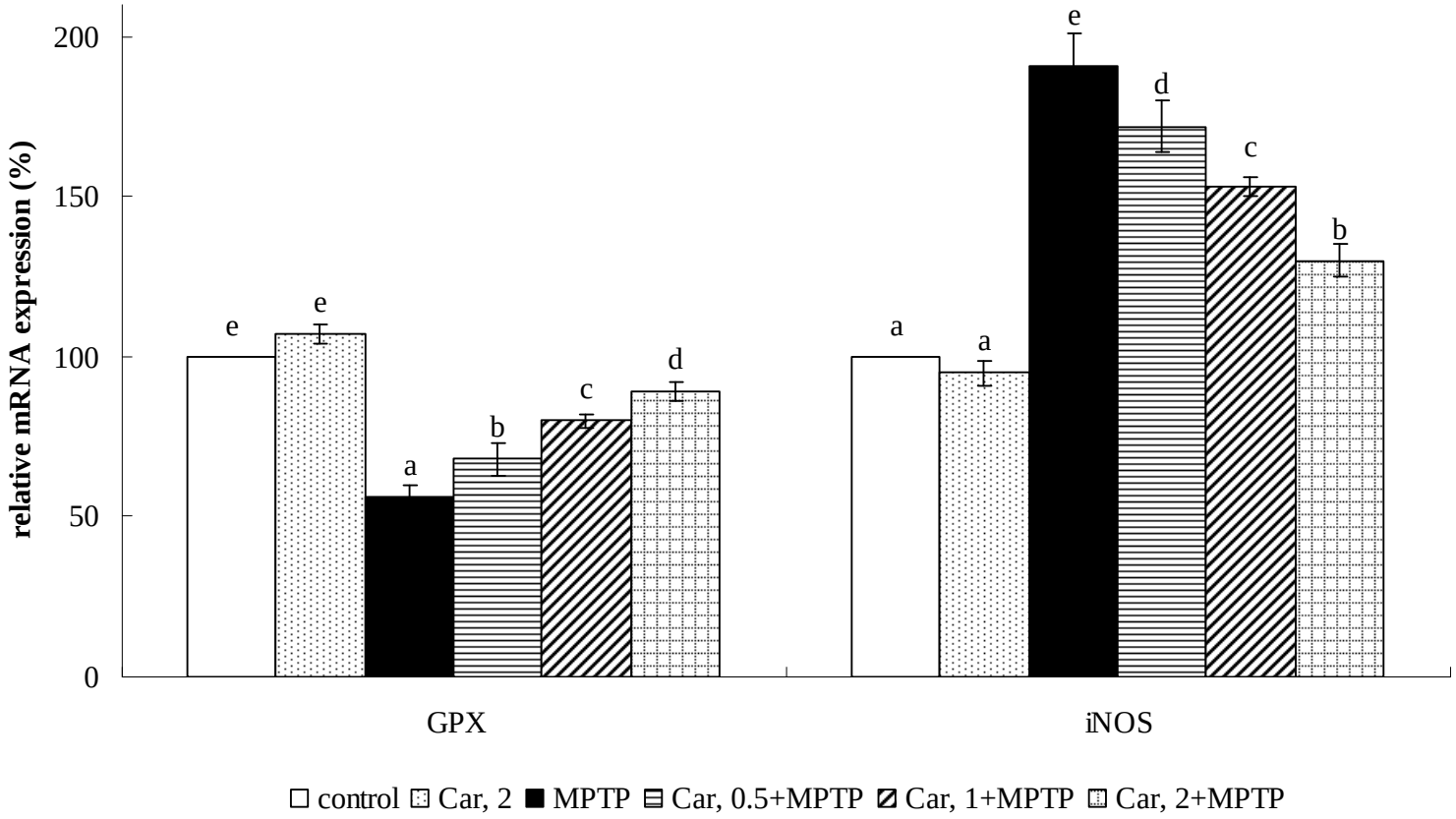
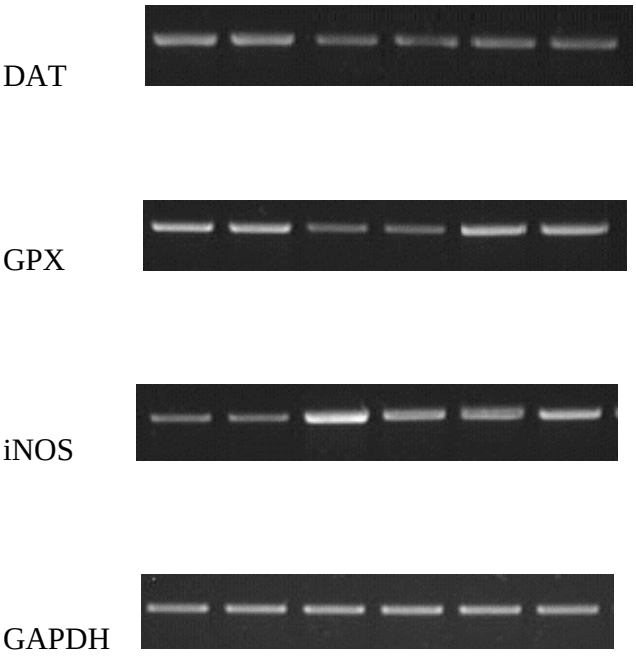

iNOS activity
pmol/min/mg protein
20
18
16
14
12
10
8
6
4
2
0
a
a
e
d
c
b
control
Car, 2
MPTP
Car, 0.5+MPTP
Car, 1+MPTP
Car, 2+MPTP

Figure 2. mRNA expression of DAT, GPX and iNOS in striatum from mice treated with carnosine (Car) alone or plus MPTP. Data are mean ± SD (n=10). [a-e]Means among bars without a common letter differ, $P<0.05$.


DAT
relative mRNA expression (%)
120
100
80
60
40
20
0
b
b
a
a
a
a
control
Car, 2
MPTP
Car, 0.5+MPTP
Car, 1+MPTP
Car, 2+MPTP


relative mRNA expression (%)
200
150
100
50
0
e
e
a
b
c
d
a
a
e
d
c
b
GPX
iNOS
control
Car, 2
MPTP
Car, 0.5+MPTP
Car, 1+MPTP
Car, 2+MPTP



Number 1 to 6 (from left to right) represents control, Car2, MPTP, Car0.5+MPTP, Car1+MPTP, Car2+MPTP, respectively.

Table 5. Effect of carnosine (Car) alone or plus MPTP treatment on content (ng/mg) of DA, DOPAC and HVA in the striatum. Values are mean ± SD, n=10.

	DA	DOPAC	HVA
control	9.43±1.05^{e}	0.75±0.10^{c}	0.94±0.09^{c}
Car, 0.5	9.51±0.92^{e}	0.78±0.08^{c}	0.92±0.10^{c}
Car, 1	9.37±0.88^{e}	0.74±0.12^{c}	0.95±0.11^{c}
Car, 2	9.40±0.98^{e}	0.73±0.07^{c}	0.91±0.06^{c}
MPTP	1.42±0.08^{a}	0.41±0.05^{a}	0.46±0.07^{a}
Car, 0.5 + MPTP	2.67±0.10^{b}	0.45±0.07^{a}	0.52±0.06^{a}
Car, 1 + MPTP	4.71±0.25^{c}	0.57±0.04^{b}	0.65±0.05^{b}
Car, 2 + MPTP	6.93±0.31^{d}	0.61±0.06^{b}	0.73±0.08^{b}

Means in a column without a common superscript letter differ, $P < 0.05$.