

Attenuation of Methamphetamine-induced Nigrostriatal Dopaminergic Neurotoxicity in Mice by Lipopolysaccharide Pretreatment

Yin Chiu Lin¹, Yu-Min Kuo², Pao-Chi Liao³, Chianfang G. Cherng⁴, Su-Wen Su¹ and Lung Yu¹

¹*Institute of Behavioral Medicine, ²Departments of Cell Biology and Anatomy*

³*Environmental and Occupational Health*

*National Cheng-Kung University College of Medicine
Tainan 701, Taiwan*

⁴*Aviation Safety Training and Research Center*

Chinese Air Force Academy

Kanshan 820, Taiwan, R.O.C.

Abstract

Immunological activation has been proposed to play a role in methamphetamine-induced dopaminergic terminal damage. In this study, we examined the roles of lipopolysaccharide, a pro-inflammatory and inflammatory factor, treatment in modulating the methamphetamine-induced nigrostriatal dopamine neurotoxicity. Lipopolysaccharide pretreatment did not affect the basal body temperature or methamphetamine-elicited hyperthermia three days later. Such systemic lipopolysaccharide treatment mitigated methamphetamine-induced striatal dopamine and 3,4-dihydroxyphenylacetic acid depletions in a dose-dependent manner. As the most potent dose (1 mg/kg) of lipopolysaccharide was administered two weeks, one day before or after the methamphetamine dosing regimen, methamphetamine-induced striatal dopamine and 3,4-dihydroxyphenylacetic acid depletions remained unaltered. Moreover, systemic lipopolysaccharide pretreatment (1 mg/kg) attenuated local methamphetamine infusion-produced dopamine and 3,4-dihydroxyphenylacetic acid depletions in the striatum, indicating that the protective effect of lipopolysaccharide is less likely due to interrupted peripheral distribution or metabolism of methamphetamine. We concluded a critical time window for systemic lipopolysaccharide pretreatment in exerting effective protection against methamphetamine-induced nigrostriatal dopamine neurotoxicity.

Key Words: neurotoxicity, methamphetamine, dopamine, striatum

Introduction

A long-term methamphetamine (MA) administration suppressed macrophages both in numbers and their antiviral activity (13). Acute MA treatment has been found to induce the expression of glial fibrillary acidic protein, oxidative stress, free radical formation, and the expression of genes known to regulate inflammatory responses (3, 6, 8, 28). Methamphetamine and certain virus-infected events

may synergistically produce an increase in inflammatory cytokine expression (7). Interestingly, a recent report documented that pretreatment of MK-801 and dextromethorphen were effective in reversing the MA-induced dopamine depletions in the striatum *via* blockade of microglia activation (25). Therefore, MA-associated immunological modulation may take part in its nigrostriatal dopamine neurotoxicity.

Prenatal exposure to Gram negative bacteriotoxin, lipopolysaccharide (LPS), may produce a long-lived

Corresponding author: Dr. Lung Yu, Behavioral Neuropharmacology Laboratory, Institute of Behavioral Medicine, National Cheng-Kung University College of Medicine, 1 University Rd., Tainan, Taiwan 70101, ROC. Tel: +886-6-2095616, Fax: +886-6-2353535 ext. 5114, E-mail: lungyu@mail.ncku.edu.tw

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dopaminergic cell loss and elevated pro-inflammatory cytokine, and tumor necrosis factor alpha (19). These conditions were suspected to potentiate neurotoxin-induced dopaminergic neuronal loss in adulthood (19). A neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, applied either simultaneously or in tandem with LPS, induced a progressive and selective degeneration of dopaminergic neurons in mesencephalic neuron-glia cultures (10). LPS alone selectively decreased tyrosine hydroxylase immunoreactive cells and increased levels of interleukin-1 beta, tumor necrosis factor-alpha as well as nitrite in culture media (11). These findings prompt investigators to form the hypothesis that the neurotoxic effects of LPS on nigrostriatal dopaminergic neurons could be mediated by microglial or astroglial activation (1, 12).

Although a synergistic effect of MA and LPS on modulating body temperature regulation was documented in a recent report (22), MA and LPS alone altered the core body temperature (16, 21). In this study, we determined to study the modulating effects of peripheral LPS delivery on MA-induced nigrostriatal dopamine neurotoxicity and MA-elicited hyperthermia in adult male mice. Besides, we also examined the modulating effects of peripheral LPS treatment on local MA infusion-produced dopamine neurotoxicity in the striatum.

Materials and Methods

Drugs and Chemicals

Methamphetamine hydrochloride was purchased from the National Bureau of Controlled Drugs (Taipei, Taiwan). HPLC-grade methanol was purchased from Fisher Scientific U.K. Ltd. (Loughborough, Leicestershire, UK), Na_2HPO_4 , citric acid and perchloric acid were obtained from Merck (Darmstadt, Germany), and ethylenediaminetetraacetic acid (EDTA) was purchased from Sigma (St. Louis, MO, USA). *Escherichia coli* LPS (Serotype 055:B5) was purchased from Sigma, too.

Animals

Male C57BL/6Ncrj mice, at 9-12 weeks of age, were used with free access to food (Purina Mouse Chow, Richmond, IN, USA) and tap water and housed in a facility located at National Cheng-Kung University (NCKU) Laboratory Animal Center (Tainan, Taiwan). The colony room was temperature- and humidity-controlled and maintained on a 12 h light/dark cycle (lights on at 0700). All experiments were conducted in a laboratory with temperature maintained at $23 \pm 1^\circ\text{C}$. This study was performed in accordance with the Guiding Principles in the Use of

Animals in Toxicology. All procedures were approved by the local Animal Care Committee at NCKU College of Medicine.

Drug Treatment Protocol

Methamphetamine dosing regimen consisted of three cumulative doses (10 mg/kg, s.c.) of MA at 2-hr intervals during the light cycle. One dose (0, 0.05, 0.1, 0.5, 1.0 mg/kg) of LPS was given intraperitoneally three days before the MA dosing regimen in an attempt to delineate the modulating effects of LPS on MA-induced dopamine neurotoxicity. The most potent protective dose (1 mg/kg) of LPS was then given 2 weeks, one day before or after the MA regimen for revealing the effective time window of LPS on attenuating MA-induced dopamine toxicity. Local MA infusion was conducted according to our previous protocol with a minor modification (29). A total of 50 μg MA in 2 μl saline was infused (at a rate of 1 $\mu\text{l}/\text{min}$) into the right striatum while an equivalent volume of saline was infused into the left striatum, serving as the controls, for each mouse.

Assay for Striatal Dopamine (DA) and 3,4-Dihydroxyphenylacetic Acid (DOPAC) Levels

A 14-day recovery period awaited following MA dosing regimen. The mice were killed by rapid decapitation, and a portion of striatum from two hemispheres was dissected. Striatal tissues were stored in liquid nitrogen until assayed by HPLC with an LC-4C amperometric detector (BAS, West Lafayette, IN, USA) for measuring DA and DOPAC levels. Striatal tissues were homogenized in 0.4 N perchloric acid and centrifuged at $16,000 \times g$ for 20 min at 4°C . The supernatant was filtered and delivered through a high-pressure valve fitted with a 20- μl loop onto a Phase-II ODS column (3 μm , 3.2 mm i.d. \times 10 cm), and oxidized with a +0.72-V potential between the glassy carbon electrode and the Ag/AgCl reference electrode. The mobile phase consisted of 0.1 M sodium phosphate dibasic, 0.1 M citric acid, 5 mg EDTA, and 7% methanol delivered at a 0.6-ml/min flow rate.

Body Temperature Measurement

Core body temperature was recorded and monitored throughout the MA dosing regimen. Rectal temperature, as an index of core body temperature, was recorded with a 7000H microcomputer thermometer (Jenco Electronics Ltd., Taipei, Taiwan) coupled with a custom-made probe. The probe tip was 0.5 mm in diameter and lubricated with mineral oil before use. Mice were held by tails with their front paws grasping on a grid top and dorsiflexed while the probe was inserted to a

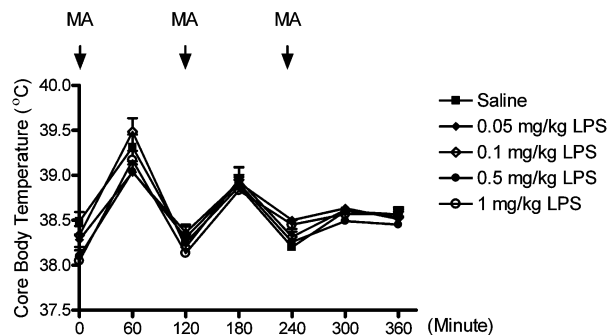


Fig. 1. Effects of lipopolysaccharide (LPS) pretreatment on methamphetamine (MA)-elicited hyperthermia. Regardless of LPS doses used, LPS treatment 3 days prior to the MA dosing protocol (3×10 mg/kg at 2-hour intervals) did not affect basal body temperature or MA-elicited hyperthermia. Arrows indicate the injection time points for MA.

depth of 12 mm to ensure a reliable measurement.

Statistical Analysis

Group differences in each experiment were examined by using one-way analysis of variance (ANOVA) and followed by Fisher PLSD post-hoc analyses if appropriate. A *P*-value of 0.05 was considered statistically significant.

Results

Attenuation of MA-induced Striatal DA and DOPAC Depletions by LPS Treatment Three Days Prior to the MA Regimen

Intraperitoneal injection of single LPS rendered a rapid decline in body temperature within the first 2 h. Then, their body temperatures elevated for the next ten hours, but, such LPS treatment did not alter basal body temperatures three days later or the MA-elicited hyperthermia throughout the MA dosing protocol (Fig. 1). Pretreatment with LPS at the highest dose used (1 mg/kg) did not alter DA or DOPAC levels in the striatum (Fig. 2A & 2B). LPS treatment three days before the MA dosing regimen effectively attenuated the MA-induced striatal DA depletions in a dose-dependent manner (Fig. 2A). Likewise, LPS pretreatment mitigated MA-induced DOPAC depletions in the striatum (Fig. 2B).

MA-induced DA or DOPAC Depletions in the Striatum Not Altered by LPS Treatment for 2 Weeks, 1 Day before or after the MA Protocol

To further study the effective time window for

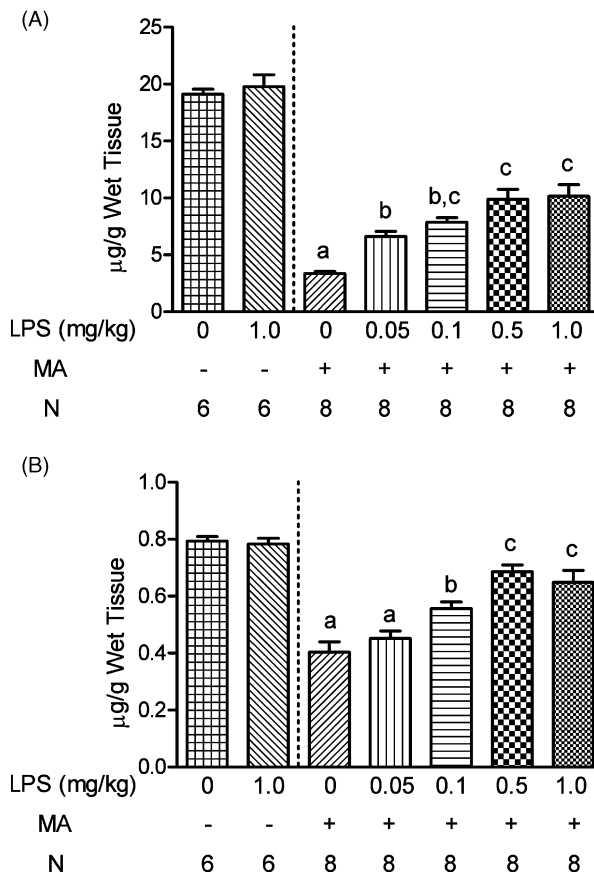


Fig. 2. Modulating effects of lipopolysaccharide (LPS) pretreatment on methamphetamine (MA)-induced dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) depletions in mouse striatum. Lipopolysaccharide treatment 3 days prior to the MA dosing protocol (3×10 mg/kg at 2-hour intervals) attenuated the MA-induced striatal A) DA and B) DOPAC depletions in a dose-dependent manner. Significant differences found between groups with different lower case alphabets.

the protective effects of LPS against the MA-induced striatal DA and DOPAC depletions, the most potent dose (1 mg/kg) of LPS was used two weeks, 1 day before, or 1 day after the MA dosing regimen. We found that treatment with LPS two weeks or one day before the MA regimen did not alter basal body temperature or MA-elicited hyperthermia (Fig. 3). Besides, LPS treatment 2 weeks, one day before, and one day after the MA dosing regimen did not affect the MA-induced DA or DOPAC depletions in the striatum (Fig. 4A & 4B).

MA-produced DA and DOPAC Depletions Effectively Reversed by Peripheral LPS Treatment Three Days Prior to Intra-striatal MA Infusion

To exclude the possibility that systemic LPS

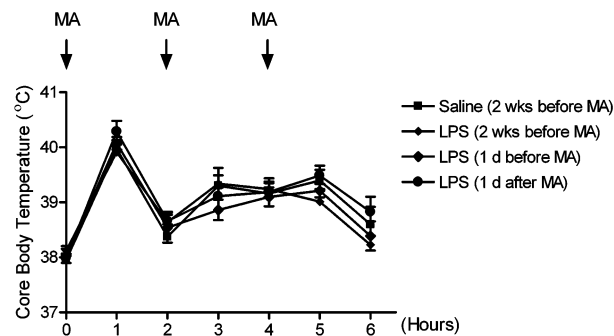


Fig. 3. Effects of lipopolysaccharide (LPS) treatment at various timings relative to the methamphetamine (MA) dosing regimen (3×10 mg/kg at 2-hour intervals) on core body temperature. Pretreatment with LPS at 1 mg/kg two weeks or one day before the MA dosing regimen did not alter the MA-elicited hyperthermia.

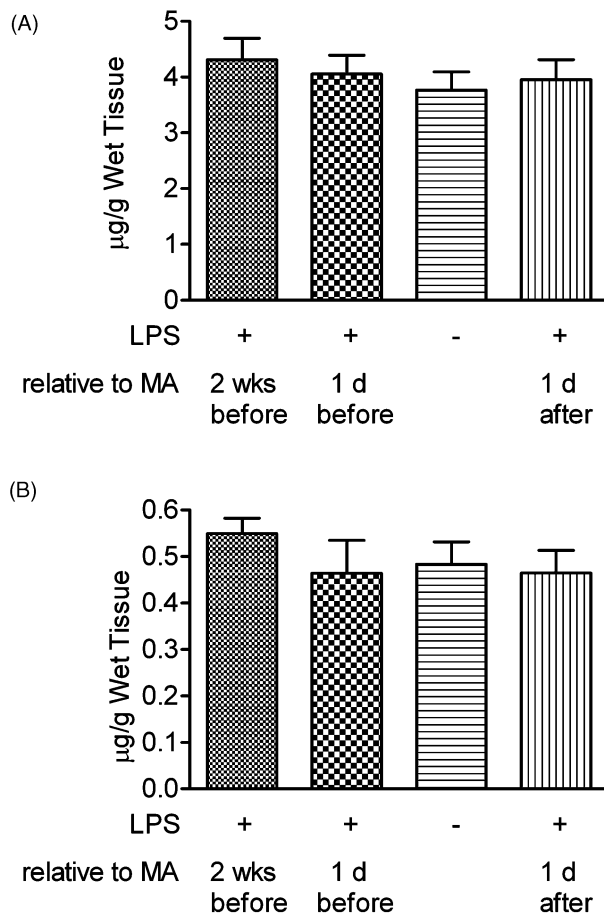


Fig. 4. Effects of lipopolysaccharide (LPS) treatment at different timings on methamphetamine (MA)-induced striatal A) DA and B) DOPAC depletions. Pretreatment with LPS (1 mg/kg) two weeks, one day before, or one day after the MA dosing regimen (3×10 mg/kg at 2-hour intervals) did not alter the MA-induced DA depletions in mouse striatum.

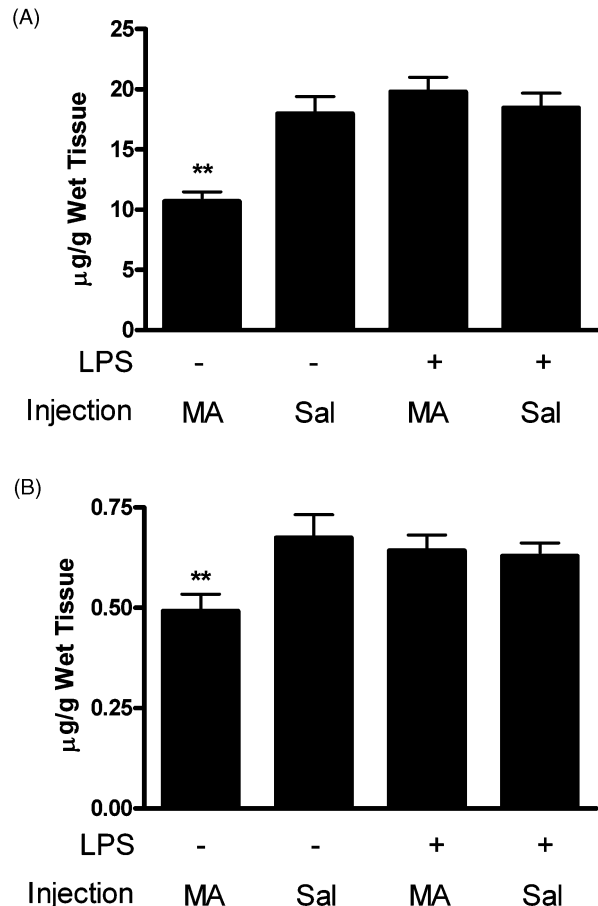


Fig. 5. Modulating effects of lipopolysaccharide (LPS) pre-treatment on intra-striatal methamphetamine (MA, 50 µg/2 µl/side) injection-induced A) dopamine (DA) and B) 3,4-dihydroxyphenylacetic acid (DOPAC) depletions in mouse striatum. Systemic LPS treatment (1 mg/kg) 3 days prior to the intra-striatal MA infusion protocol abolished the MA-induced DA and DOPAC depletions in the infusion side of the striatum. **Significantly lower than matched saline-infused side.

treatment mitigated MA-induced striatal dopamine toxicity *via* its disruption on MA distribution in brain, we infused MA directly into the right striatum for each animal. An equivalent volume of saline was infused into the left striatum, serving as the controls for baseline DA and DOPAC levels. Local MA infusion (totally 50 µg in 2 µl) produced significant depletions in both DA (Fig. 5A) and DOPAC in striatum (Fig. 5B). However, systemic injection of LPS (1 mg/kg) three days prior to the intra-striatal MA infusion protocol reversed the MA-induced DA (Fig. 5A) and DOPAC (Fig. 5B) depletions in the striatum.

Discussion

Cumulative doses of MA may produce

nigrostriatal dopaminergic neurotoxicity (9, 14, 18, 26). Although MA has been known to modulate the immune system, it was not revealed until lately that immunological activation may play a role in this MA-induced dopaminergic toxicity (24, 25). Early exposure to LPS caused nigrostriatal dopaminergic cell loss by immunological activation (19). Likewise, intracranial LPS administration produced nigrostriatal dopaminergic toxicity by immunological activation in adult animals (1). In this study, we examined the modulating effects of systemic LPS on MA-induced nigrostriatal dopamine neurotoxicity in adult mice. Since LPS may exert temperature-regulating effects following its administration, we also studied the effects of LPS treatment on MA-elicited hyperthermia. Systemic LPS treatment three days prior to the MA dosing regimen did not affect the MA-elicited hyperthermia. Surprisingly, such LPS treatment mitigated MA-induced striatal DA and DOPAC depletions in a dose-dependent manner. However, LPS treatment did not alter the MA-induced striatal dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) depletions or MA-elicited hyperthermia as LPS was administered two weeks, one day before or one day after the MA dosing protocol. Besides, systemic pretreatment with LPS (1 mg/kg) 3 days prior to the MA dosing protocol completely reversed local MA infusion-produced DA and DOPAC depletions in the striatum.

While activity levels and ambient temperature affected the MA-elicited hyperthermia, this drug-elicited hyperthermia has been thought to exacerbate the MA-induced dopamine neurotoxicity (15). Lately, accrued evidence indicated that several pharmacological ligands protected the nigrostriatal dopaminergic terminals from the MA-induced toxicity but did not affect the MA-elicited hyperthermia (2, 5, 17, 18, 27). We found that acute LPS-induced hyperthermia did not last for 24 hours, nor did it affect MA-elicited hyperthermia. We hereby provided another line of evidence that LPS-induced peripheral immunological activation attenuated MA-induced nigrostriatal dopaminergic neurotoxicity *via* a temperature-independent mechanism. These results, taken together, all support the notion that certain neurotoxic mechanisms specific to MA treatment, other than hyperthermia itself, are responsible for the MA-induced dopaminergic toxicity.

Although prenatal exposure to LPS may potentiate neurotoxin-induced dopaminergic neuronal loss in adulthood (19), preconditioning with LPS has been shown to confer the neuroprotective effects against the following ischemic injury (23). Moreover, LPS preconditioning dose-dependently decreased the number of circulating leukocytes and those recruited to the brain parenchyma (4). Thus, systemic LPS

administration exerted neuroprotective effects on the injured central nervous system by reducing the inflammatory infiltrate from periphery to the brain and spinal cord (4). Consistent with these findings, we demonstrated that peripheral LPS treatment within a limited time window may exert protective effects against the MA-induced central dopaminergic neurotoxicity. We also showed that systemic LPS pretreatment effectively mitigated local MA-induced dopaminergic neurotoxicity in the striatum. Considering the fact that LPS is not allowed to penetrate the blood brain barrier and delay of over 24 hr is required for substantiating the protective effects of systemic LPS treatment, we suspected the involvement of gene transcription and translation, time-consuming transport and relocation for active metabolites of immunological activation-associated factors following the LPS administration. Specifically, LPS-associated dopaminergic protection probably stems from the activation of inflammation-associated signaling pathways that activate anti-inflammatory mechanisms. For example, induction of tumor necrosis factor- α (TNF- α) and TNF- α -dependent signaling pathways could be responsible for the neuroprotective effects of LPS treatment in ischemic injury (20, 23). In addition, peripheral immunological activation-associated synthesis and metabolism of cytokines, interleukins and the other messengers, their transport *via* blood circulation to the local area, protein synthesis and secretion of central glial and neuronal cells are all suspected to participate in this protective effect of LPS against the MA-induced dopaminergic neurotoxicity.

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