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Original research article

Neuronal nitric oxide synthase has a role in the detrimental effects of lipopolysaccharide on spatial memory and synaptic plasticity in rats

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ABSTRACT

Background: The role of neuronal nitric oxide synthase (nNOS) in lipopolysaccharide (LPS)-induced memory and synaptic plasticity impairment was investigated.
Methods: The rats were divided and treated as follows: (1) control (saline), (2) LPS, (3) 7NI (7-nitroindazole as a nNOS inhibitor)-LPS and (4) 7NI.
Results: In a Morris water maze, the LPS group took a longer amount of time and traveled a greater distance to reach the platform, this was prevented by 7NI. Malondialdehyde (MDA) and nitric oxide (NO) metabolites in the hippocampus of the LPS group were higher while the total thiol, superoxide dismutase and catalase were lower than that of the controlled specimen. Pre-treatment using 7NI prevented the changes in the biochemical criteria. The slope and amplitude of the field excitatory post-synaptic potential (fEPSP) in the LPS group decreased, whereas in 7NI–LPS group they increased.
Conclusion: It is suggested that inhibition of nNOS by 7NI improves the deleterious effects of LPS by reducing NO metabolites and the brain tissues oxidative damage.

Introduction

Both inflammation [1] and oxidative stress [2] are considered to have an important role in memory loss. Systemic inflammation and overproduction of multiple inflammatory cytokines in the brain, such as tumor necrosis factor-α (TNFα), interleukin1-β (IL-1β) and interleukin-6 (IL-6), have been shown to disrupt working memory and long-term potentiation (LTP) induction in the hippocampus. These contribute to memory loss in neurodegenerative diseases including Alzheimer's disease (AD) [3–7]. In addition, oxidative stress, which is accompanied with increased levels of reactive oxygen species (ROS) superoxide, has been proposed to have a crucial role in neuronal death and memory impairment [8].

Lipopolysaccharide (LPS), a particle extracted from the cell wall of Gram-negative bacteria, has been shown to trigger excessive production of free radicals and inflammatory cytokines that are accompanied with neuroinflammation, neuronal death and memory deficits [9]. Even a single systemic injection of LPS impairs spatial memory and long-term potentiation (LTP) and declines neurogenesis in the hippocampus [10]. It is suggested that the activation of cytokines is followed by overproduction of nitric oxide (NO) through the activation of nitric oxide synthase (NOS) isoforms which lead to superoxide anion formation cell death [11,12]. It is suggested that expression of iNOS increases after injection of LPS [13], however, the role of nNOS still needs to be evaluated. The aim of this study was to further investigate the effects of nNOS on LPS-induced learning, memory and LTP impairments in rats.

Materials and methods

Animals and drugs

Fifty-six Wistar rats were divided into four groups: (1) control, (2) LPS, (3) 7NI–LPS and (4) 7NI ($n = 8$ in each group). LPS was injected (1 mg/kg; *ip*) 2 h prior to the behavioral and electrophysiological experiments [14]. 7NI (30 mg/kg; *ip*) was injected 30 min

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before LPS or saline in the 7NI–LPS and 7NI groups, respectively. In the LPS and control groups, the animals were treated with saline, supplemented with dimethyl sulfoxide (3%, DMSO) (2 ml/kg) instead of 7NI. Thirty-two of the animals were treated by drugs or vehicles for 6 constitutive days and used in a behavioral test. The remaining animals (24) were used for electrophysiological experiments after receiving a single dose of drugs or vehicles. LPS and 7NI were purchased from Sigma (Sigma–Aldrich Chemical Co., St. Louis, MO, USA). Other chemicals such as DMSO, and those which were used for biochemical assessments, were purchased from Merck Company (Darmstadt, Germany).

Morris water maze (MWM) test

Spatial learning and memory were investigated using the MWM test as described in our previous studies [15]. Briefly, the rats were placed into the MWM tank for 4 trials each day for 6 days. In the first 5 days, an escape platform was hidden 2 cm beneath the surface of the water in the center of the quadrant of the apparatus and the rats were given 60 s to find it in each trial. The time spent and distance traveled in order to reach the platform was recorded. On the last day, the platform was removed and a probe test was performed. The time spent and the traveled path in the target quadrant (Q1) was compared between groups.

Electrophysiological study

The results of MWM showed that there was a significant difference between 7NI–LPS and LPS groups but no significant difference was observed between 7NI and control groups. For electrophysiological experiments, 24 of the animals were divided into three groups: (1) control, (2) LPS and (3) 7NI–LPS (n = 8 in each group).

The animals were deeply anesthetized with urethane (1.6 g/kg) and two small holes were drilled into the skull using stereotaxic apparatus in order to place stimulating and recording electrodes. Field potential was recorded from the CA1 area of the hippocampus. For this purpose, a bipolar stimulating electrode (stainless steel, 0.125 mm diameter, A-M System, Sequim, WA, USA) was infixed into the ipsilateral Schafer collateral pathway (AP = 3 m, ML = 3.5 mm, DV = 2.8–3 mm) and a unipolar recording electrode was lowered into the stratum radiatum of the right CA1 area of the hippocampus (AP = 4.1 mm, ML = 3 mm, DV = 2.5 mm). The stimulating electrode was connected to a stimulator and the recording electrode was connected to an amplifier. Extracellular field potential was obtained from CA1 area of the hippocampus following the stimulation of the Schafer collateral pathway. These were amplified (100×) and filtered (1 Hz to 3 kHz band pass) using a differential amplifier. A maximum field excitatory post-synaptic potential (fEPSP) was obtained by stimulating the Schafer collateral pathway and recording in the CA1 area. After a 30 min stabilization period, in order to evaluate synaptic potency before induction of LTP, an input–output (I/O) function was exerted by gradually increasing the stimulus intensities with constant current (input) and recording fEPSP (output). A baseline recording was then taken 30 min before the induction of LTP. After ensuring a steady state baseline response, in order to carry out LTP induction, a high frequency stimuli (HFS) protocol of 100 Hz was applied. The stimuli with the intensities which produced 50% of the maximum response were applied to induce LTP. The fEPSP was then recorded for the 90 min after high frequency stimuli. Computer-based stimulation and recording was performed using Neurotrace software version 9 and Eletromodule 12 (Science Beam Institute, Tehran, Iran). The values of the slope and amplitude of the fEPSP were the average of the 10 consecutive traces. Reponses were analyzed using custom software from the same institute.

Biochemical assessment

After completion, the behavioral tests were deeply anesthetized, the blood samples were collected, the hippocampal tissues were removed, weighed and submitted to determine the total thiol (SH) content, malondialdehyde (MDA), NO metabolites (NO_2 or NO_3) concentrations and the activities of superoxide dismutase (SOD) and catalase (CAT).

Determination of MDA, total thiol and NO metabolites

The MDA concentration, as an index of lipid peroxidation and total thiol groups' content were assessed in the hippocampal tissues according to a protocol that we described previously [16]. Briefly, a reaction of thiobarbituric acid (TBA) with MDA results in the production of a red complex which has a peak absorbance of 535 nm After reacting DTNB (2,2′-dinitro-5,5′-dithiodibenzoic acid) as a reagent with the SH group produced a yellow complex, absorbance was read at 412 nm The concentration of NO metabolites (NO_2/NO_3) was determined according to the Griess reagent method. In brief, after adding 100 μL supernatant to the Griess reagent and transferring the contents to a 96-well flat-bottomed micro-plate, the absorbance was read at 520 nm This was done using a micro-plate reader and the final values were computed with standard calibration plots [17–19].

The enzymatic assays

SOD activity was evaluated according to the method of Madesh and Balasubramanian. In a colorimetric assay, the SOD activity was measured at 570 nm One unit of SOD was defined as the amount of enzyme required to inhibit the rate of MTT reduction by 50%. The results were presented as units per milligram of protein. Catalase activity was determined by the method of Aebi with hydrogen peroxide (30 mM) as the substrate. One unit of catalase activity is determined as the micromoles of the hydrogen peroxide consumed per milligram of protein sample.

Determination of serum TNFα content

The content of TNFα in the serum was measured using an enzyme linked immunosorbent assay (ELISA) kit (Rat TNFα Platinum ELISA, e-Bioscience, San Diego, CA, USA) following the instructions of the kit.

Statistical analysis

All data were expressed as a means of ± SEM. For the data of the time and distance during the 5 days of MWM, the experimental design was included two between-subject factors (7NI and LPS), and day as a repeated measure. So, three-way mixed design ANOVAs were used and three-way interactions were also demonstrated (LPS × 7NI × Day). Data of the LTP criteria were compared using a repeated measures analysis of variance (ANOVA) followed by Tukey's *post hoc* comparisons test. The biochemical data collated in the probe trial in the MWM was compared using one-way ANOVA followed by Tukey's *post hoc* comparisons test. Differences were considered statistically significant when $p < 0.05$.

Results

MWM results

Using three-way ANOVA, the results showed that there was a significant effect of LPS on escape latency to reach the platform $f_{(1,\ 536)} = 25.22$; $p < 0.001$. 7NI also significantly affected the escape