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Inducible nitric oxide inhibitor aminoguanidine, ameliorates deleterious effects of lipopolysaccharide on memory and long term potentiation in rat

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ABSTRACT

Aim: An interaction between nitric oxide (NO) and neuro-inflammation has been considered to modulate learning and memory. In the present study, the effect of an inducible NO synthase (iNOS) inhibitor, aminoguanidine (AG) on lipopolysaccharide (LPS)-induced memory impairment was evaluated.
Materials and methods: The rats were divided and treated: Control (Saline), LPS, AG - LPS and AG, before behavioral and electrophysiological experiments.
Results: The escape latency in Morris water maze (MWM) test and the latency to enter the dark compartment in Passive avoidance (PA) test in LPS group were significantly higher than in control ($P < 0.001$) whereas, in AG-LPS group they were shorter than LPS group ($P < 0.001$). The amplitude and slope of field excitatory post synaptic potential (fEPSP) decreased in LPS group compared to control group ($P < 0.05$ and $P < 0.01$) whereas, in AG-LPS group they were higher than LPS group ($P < 0.05$). Malondialdehyde (MDA) and NO metabolites concentrations in the hippocampus and serum TNFα level of LPS group were higher than control group ($P < 0.001$, $P < 0.05$ and 0.01 respectively) while, in AG- LPS group they were lower than LPS group ($P < 0.001$and $P < 0.01$ respectively). The thiol content and the activities of superoxide dismutase (SOD) and catalase (CAT) in the hippocampus of LPS group reduced compared to control group ($P < 0.001$ and $P < 0.05$ respectively) while, in AG - LPS group they enhanced compared to LPS ($P < 0.001$ and $P < 0.05$ respectively).
Conclusion: It is suggested that increased NO has a role in LPS-induced learning and LTP impairments and the brain tissues oxidative damage which are preventable by iNOS inhibitor aminoguanidine.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia that is characterized by a progressive memory loss and synaptic dysfunction [1]. Although the exact mechanism(s) of AD is still unclear, chronic inflammatory and oxidative stress are considered as two contributing factors in its pathogenesis [2]. Mitochondrial dysfunction-mediated oxidative stress is considered as an important pathological index in AD. Many reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide have been documented to induce mitochondrial dysfunction, reduction of energy generation and neuronal death in the brain of patients with AD [1]. Scientific evidence has also confirmed that accumulated amyloid-beta (Aβ) in the brains of the patients with AD induces synthesis of inflammatory cytokines such as IL-1α, IL-1β, IL-6 and IL-8. The ratio of inflammatory cytokines such as IL-1β to the anti-inflammatory cytokines including IL-10 is suggested to be high in the serum of individuals with AD [3]. In addition, an elevated level of pro-inflammatory cytokines in particular tumor necrosis factor-α (TNF-α) is suggested to prevent from phagocytosis of Aβ by residual microglia in the brain [4]. Lipopolysaccharide (LPS) as a potent bacterial endotoxin, triggers production of inflammatory cytokines such as TNF-α induces ROS production [5]. On the other hand, LPS was recently reported to be able to cause amyloidogenesis in the hippocampal formation and has been used to induce an animal model of AD when it was administered to normal and transgenic rodents [6]. It has also been well documented Aβ induced LTP impairment is mediated by activation of iNOS [7]. Intraperitoneal injection of LPS has been shown that impair cognition, spatial memory [8] and long term potentiation (LTP) [9].

Nitric oxide (NO), a diffusible gaseous molecule, is generated by three nitric oxide synthase (NOS) isoforms including neuronal (nNOS), endothelial (eNOS) and inducible (iNOS) from L-arginine [10].

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Ca^{2+}-calmodulin-dependent enzymes, eNOS and nNOS, are constitutively expressed in mammalian cells and stimulate NO generation lasting a few minutes [11]. eNOS expression is regulated by physiological stimuli including shear stress [12]. In contrast, Ca^{2+}-calmodulin-independent isoform, iNOS, is expressed following immunological and inflammatory responses in immune cells producing high amounts of NO lasting hours or days [13]. NO is considered as a immunomodulator agent which has a role in platelet aggregation, leukocytes rolling and migration and expression of inflammatory cytokines such as IL-1, IL-6, IL-8, INF-γ and TNF-α as well as in cell proliferation and apoptosis [14]. Positive role of NO on learning and memory has also been well documented [15]. Using animal models, avoidance task acquisition of the animals as well as learning of spatial tasks have been accompanied with an increased level of NO in the hippocampal tissues [16]. The produced NO following activation of *N*-Methyl-D-aspartate (NMDA) receptors also acts as a retrograde messenger to induce LTP in the hippocampus [17]. All the positive modulatory effects of NO on inflammatory processes and learning and memory take place when it is synthesized in physiological concentrations [15]. It is suggested that the role of NO alters from physiological neuromodulator to neurotoxic agent and induces neurodegeneration and memory impairment when its production gets out of control [15]. Enhanced levels of NO following an increased activity of iNOS/NO system has been shown to cause inflammatory demyelination of neurons, neurotoxicity and memory impairment in human and animal models [18,19]. It seems that iNOS selective inhibitors might be able to improve memory through reduction of inflammatory responses and oxidative stress therefore, they have been considered as candidates of treatment of inflammatory diseases [2]. Aminoguanidine (AG), as an iNOS selective inhibitor was shown that inhibited inflammatory processes and reduced neuronal damage in the hippocampus [20]. However, full investigations have not been aimed to study the effects of iNOS/NO system inhibition on learning and memory impairments due to neuro-inflammation. The present study assigned to evaluate possible ameliorating properties of inducible nitric oxide inhibitor aminoguanidine on deleterious effects of lipopolysaccharide on learning, memory and long term potentiation in rats.

2. Material and methods

2.1. Animals

Fifty-six male Wistar rats (8 weeks old and weighing 200–250 g) were obtained from animal house of Mashhad University of Medical Sciences, Mashhad, Iran. The animals were housed in a room with standard temperature (22 ± 2 °C) and 12 h light/dark cycle. The rats were allowed to food and water freely. Animal examinations were carried out in accordance with procedures approved by the Committee on Animal Research of Mashhad University of Medical Sciences. Thirty-two of the animals were classified to four groups: [1] control [2] LPS, [3] AG- LPS and [4] AG (n = 8 in each group). These animals were treated by drugs or vehicle for 6 days and used for behavioral tests. The rest of the animals (twenty- four) were grouped into [1] control [2] LPS, [3] AG- LPS (n = 8 in each group) and used for electrophysiological experiments after receiving a single dose of drugs or vehicle.

2.2. Drugs

The drugs were dissolved in saline. LPS (1 mg/kg; ip) was administered 2 h before the behavioral and electrophysiological experiments [21]. AG (100 mg/kg; ip) was injected 30 min before LPS or saline in AG-LPS and AG groups respectively [22]. In the LPS group, the animals were treated by saline (2 ml/kg) instead of AG. The animals of control and AG groups received 2 ml/kg of saline instead of LPS. All drugs were prepared freshly. LPS (*E. coli* 055:B5), and AG were purchased from Sigma (Sigma Aldrich Chemical Co.). The used chemicals for biochemical assessments were purchased from Merck Company. Enzyme linked immune sorbent assay (ELISA) kit (Rat TNFα Platinum ELISA, e -Bioscience) was used for determination of TNFα.

2.3. Morris water maze (MWM) test

MWM apparatus was consisted of a cylindrical tank 136 cm in diameter, 60 cm in high and 30 cm in deep with boundaries of the four quadrants. The tank was filled with water (23–25 °C) until a circular platform 10 cm in diameter and 28 cm in high was submerged 2 cm beneath the surface of the water in the center of the Northwest quadrant. Fixed visual cues at several locations around the maze and on walls of the room determined the navigation path. Before each experiment, for familiarizing with the apparatuses, the animals were placed in filled maze with water without a platform for 30 s. In the hidden platform acquisition test, the animals were released randomly in the tank at one of four positions (north, east, south and west) and allowed to freely swim to find the hidden platform within 60 s. The position of the animals was detected by a camera that was hung above the center of the pool. The camera signals were transformed to a computerized tracking system that monitored and stored the location of the animals [8]. The time spent, traveled distance to reach the platform and the swimming speed was recorded. If the rat found the platform within 60 s it was allowed to remain on the platform for 20 s before the next trial otherwise, it was guided to the platform by the experimenter and permitted to stay on it for 20 s. The experiments were repeated with four trials in each day for five consecutive days. The mean of the time spent and traveled distance were measured to evaluate the spatial learning ability. Twenty-four hours after acquisition test, 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 [23].

2.4. Passive avoidance (PA) test

PA apparatus was made of a light and a dark chamber separated by a guillotine door. During habituation trial, the animals were placed into the light compartment and permitted to move freely between two chambers for 5 min. In acquisition trial, after entering the animal into the dark chamber, the guillotine door was closed and an electrical shock (2 mA, 2 s) was delivered to the animal's feet. At 3, 24, 48 and 72 h later, the animals were again placed into the light compartment and the latencies to enter the dark room as well as the time spent by the animals in the dark compartment were recorded and defined as retention trial [24].

2.5. Electrophysiological study

The behavioral results showed that there was a significant difference between AG- LPS and LPS groups, but no significant difference was observed between AG and control groups. For electrophysiological experiments, 24 of animals were divided into three groups: [1] Control [2] LPS and AG-LPS (n = 8 in each group). In electrophysiological experiments, after deep anesthetizing with urethane (1.6 g/kg) the animal's head was fixed in a stereotaxic apparatus. After removing the skin and exposing the skull, the proper location of CA1 area of hippocampus and Schafer collateral pathway were determined on the skull. Two small holes were then drilled, under sterile conditions. For recording field excitatory post synaptic potential (fEPSP), a bipolar stimulating stainless steel electrode with 0.125 mm in diameter (AM system, England) was fixed in Schafer collateral pathway of right hippocampus (AP = 3 mm; ML = 3.5 mm; DV = 2.8–3 mm) and a unipolar recording electrode with the same characteristics of stimulating electrode was lowered into the CA1 area of the ipsilateral (AP = 4.1 mm; ML = 3 mm; DV = 2.5 mm) [25]. Proper location of the electrodes was determined using physiological and stereotaxic indicators. The stimulating electrode and recording electrode were connected to a stimulator and an amplifier respectively. Extracellular field potential was detected from CA1 area of hippocampus in following stimulation of the Schafer collateral pathway