

Original Article

Effect of Intra-amygdala Injection of Lipopolysaccharide on Kindling Epileptogenesis in Adult Rats

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Abstract

Background: Contribution of neuroinflammation and epilepsy in the mature brain has elicited contradictory results with either excitatory or inhibitory effects. The amygdala is one of the main parts of the limbic system susceptible to insults that lead to neuroinflammation and epilepsy. This study evaluates the effect of chronic inflammation of the rat amygdala induced by lipopolysaccharide (LPS) on kindling epileptogenesis.

Methods: LPS (5µg/rat) was infused once daily into the basolateral amygdala (BLA) of adult rats. Daily electrical stimulation (150 – 300 µA, 100 Hz, monophasic square wave stimulus of 1 msec per wave, 2 sec duration) was delivered into BLA 30 min after LPS injections until the animals became fully kindled.

Results: LPS had no significant effect on the development of focal and generalized seizures.

Conclusion: The type of neural system exposed to LPS and its specific electrophysiological properties seems to ascertain the final excitatory or inhibitory outcome.

Keywords: Amygdala, epileptogenesis, lipopolysaccharide

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Introduction

Epilepsy is the third most common neurological disorder after stroke and Alzheimer's disease.¹ Epileptogenesis is the process whereby gradual pathophysiological alterations in certain brain regions result in conversion of the normal brain to a hyperexcitable epileptic one. The contribution of neuroinflammation and epilepsy in the mature brain has been accompanied with contradictory results indicating both convulsant¹ and anticonvulsant roles for neuroinflammation.²⁻⁴

Despite extensive information on hippocampal changes associated with epileptogenesis, the amygdala has received less attention. Kindling is a well established animal model of epileptogenesis.⁵ Repeated administration of an initially subconvulsive electrical stimulus to one of the temporal lobe structures causes seizures that generally evolve through five stages: 1) facial clonus, 2) head nodding, 3) forelimb clonus, 4) rearing, and 5) rearing and falling accompanied by generalized clonic seizures.⁵ Behavioral seizure stages 1 – 3 correspond to activation of ipsilateral limbic structures and are considered to be focal seizures, whereas stages 4 – 5 represent secondary-generalized motor seizures and correspond to discharge to the contra-lateral limbic structures and then to outside of the limbic system.⁵

Bacterial endotoxin lipopolysaccharide (LPS) is a stimulator of microglia and is used extensively as a model of neuroinflammation.⁶ Here, we examined the effect of chronic intra-amygdala injection of LPS on the creation of seizures in the kindling model of

epilepsy.

Materials and Methods

Adult male Wistar rats (280 – 320 g, Pasteur Institute of Iran) were housed in standard Plexiglas cages with free access to food and water. The animal room temperature was $23 \pm 1.0^\circ\text{C}$ with a 12h light/dark cycle. Animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). Rats were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg). An electrode-guide cannula complex was implanted stereotaxically in the basolateral amygdala (BLA; coordinates: A, -2.5mm from bregma; L, 4.8mm from bregma and V, 7.5mm from dura) of the right hemisphere. Another electrode was placed above the left cortical surface as the earth and differential electrode.

Seven days after surgery, after-discharge (AD) threshold was determined in the amygdala of each animal by a 2 sec, 100 Hz monophasic square-wave stimulus of 1 msec per wave. The stimulation was initially delivered at 50 µA and then at 5 min intervals, increased stimulus intensity in increments of 50 µA were delivered, until at least 5 sec of AD was recorded.² Animals were stimulated at the AD threshold once daily until five consecutive stage 5 seizures were elicited. These animals were considered fully kindled.

Twenty-four hours after determination of the AD threshold, rats were divided into two groups of eight rats per group. Rats received LPS (5 µg/rat, 1 µl in 3 min intra-BLA) or phosphate buffer solution (PBS; 1 µl/rat intra-BLA) once daily. The dose of LPS was selected based on numerous studies that have indicated cerebral injections of LPS in the range of 2.5 – 15 µg/rat induces neuroinflammation.⁶

Daily electrical stimulation was delivered 30 min after LPS or PBS injections until the animals became fully kindled. The stage

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Table 1. Effect of intra-amygdala LPS (5 µg/rat) on the development of amygdala kindling

Group	No injection (n=9)	PBS (n=7)	LPS (5 µg /rat) (n=6)	P
Initial ADD (sec)	8.1±0.9	7.4±1.6	9.2±1.0	0.60
ADD at stage 3 (sec)	26.5±3.4	34.1±3.8	33.8±4.4	0.28
ADD on day 17 (sec)	39.2±3.2	46.0±4.8	48.6±3.3	0.21
ADD at stage 5 (sec)	49.3±5.8	64.0±9.8	53.2±3.6	0.31

Data are presented as mean±SEM; ADD: After-discharge duration.

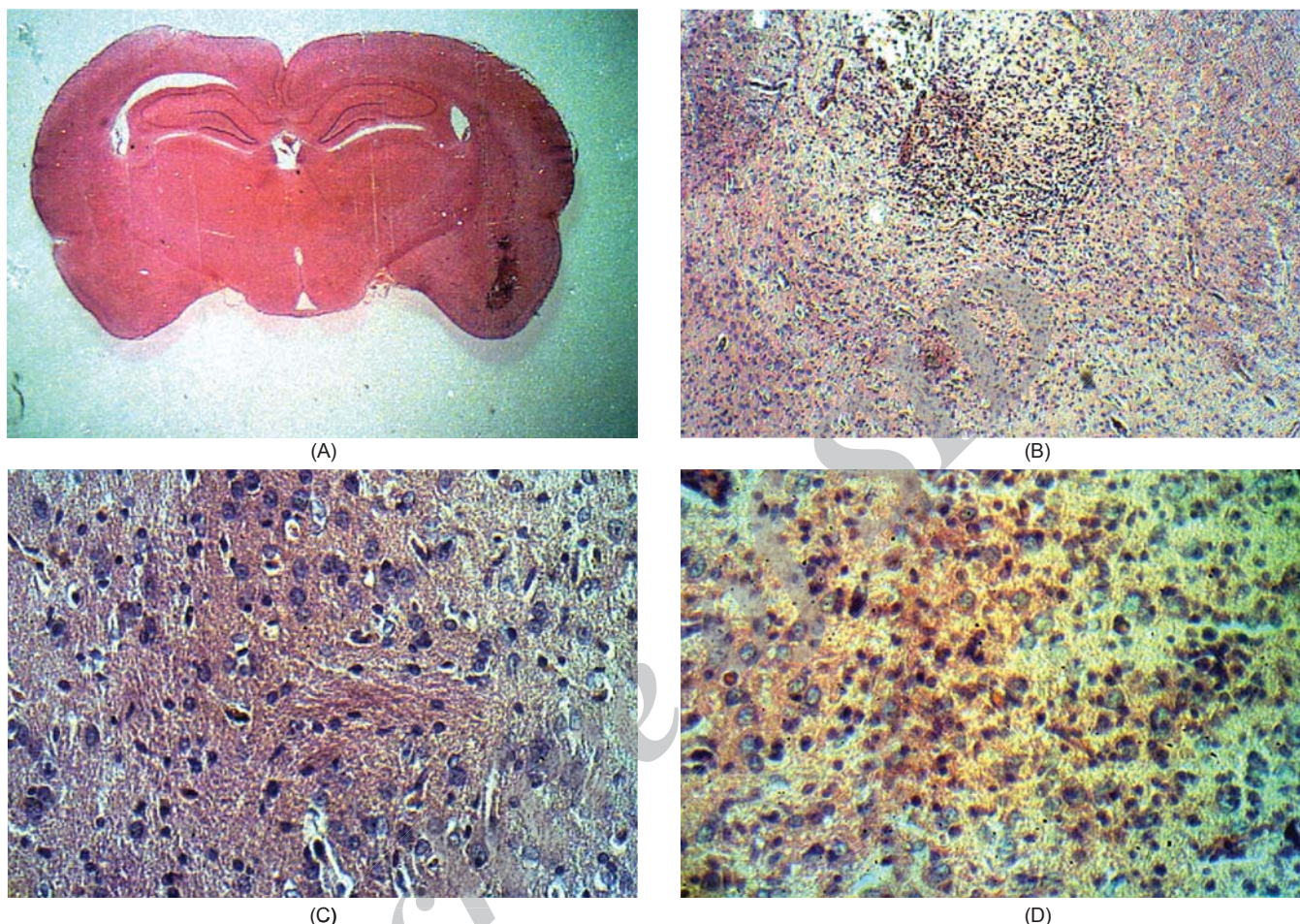


Figure 1. H&E-stained coronal brain section of a rat. **A)** Electrode-cannula position in the basolateral amygdala area. **B)** Higher magnification ($\times 40$) of area shown in A. **C)** Higher magnification ($\times 100$) of area shown in B of a PBS-treated amygdala. **D)** Higher magnification ($\times 100$) of area shown in B of the LPS-treated amygdala.

of behavioral seizures and duration of AD (ADD) were recorded after stimulations. At the end of the experiments and completion of kindling rats were killed by diethyl ether. The brains were removed and stored in 10% formalin for further histological analysis

The stored brains were cut into 2 cm³ segments and processed in the following order: 10% formalin (6 h), 80% ethanol (1 h), 96% ethanol (3 h), 100% ethanol (3 h), xylene (3 h), and paraffin (63°C for 7 h). The processed segments were molded by paraffin at 63°C, cut into 5-µm thick slices by a microtome and stained with hematoxylin and eosin (H&E). The stained slices were qualitatively analyzed for cannula and electrode positions and any pathological changes.

One way ANOVA was used to compare seizure parameters and kindling development between groups. $P < 0.05$ was considered statistically significant.

Results

Histological evaluation confirmed the correct positions of the electrode and cannula in 90% of rats whom underwent surgery (Figure 1). No lesion was observed in LPS- or PBS-treated sections. Data from the animals with false cannula and electrode positions were not included in the analyses.

AD thresholds of all the animals were in the range of 150-300 µA. LPS did not affect growth of AD. ADD at the time of expression of focal and generalized seizures was not significantly affected by LPS (Table 1).

LPS-treated rats required more stimulations than PBS-injected rats to show focal seizures (8.0 ± 1.3 versus 4.7 ± 0.3 , $P = 0.02$). However, the number of stimulations in the LPS group was similar to non-injected animals (7.4 ± 0.7 , $P > 0.05$).

Although the number of stimulations required to develop gener-

alized seizures in the LPS group was significantly higher than that of the non-injected group (30.4 ± 6.4 versus 15.1 ± 1.0 , $P = 0.009$), the difference between the LPS and PBS (25.1 ± 2.7) groups was not significant ($P = 0.52$).

Discussion

Our results showed that intra-amygdala injection of LPS did not significantly affect the rate of kindling and growth of ADD. Histological analysis of the brains from the control and LPS-treated kindled rats revealed no lesions or cell death in the amygdala. Therefore, there was no involvement of neuronal degeneration in the ineffectiveness of LPS.

Previous studies indicate that LPS is both protective²⁻⁴ and convulsant^{1,7-9} in rodents. Microglial cells are stimulated by LPS and release cytokines and inflammatory mediators that include IL-1 β , IL-6, IL-10, TNF- α , prostaglandins, and nitric oxide.^{1,6} Involvement of these factors in the convulsant and anticonvulsant action of LPS has been reported by many researchers.^{1,7,10,11} It has also been demonstrated that LPS blocks NMDA receptor-mediated synaptic activity in the hippocampus.¹² In addition to glutamatergic neurotransmission, an enhancement in GABAergic inhibition is suggested to underlie these effects.¹³

In contrast to the literature denoting pro- or anticonvulsant roles for LPS, in our study LPS did not affect amygdala kindling. The type of neural system exposed to LPS and its specific electrophysiological properties seems to ascertain the final excitatory or inhibitory outcome. Further studies are needed to clarify this issue and its relevance to the onset of epilepsy.

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