Title: Cardiovascular Effect of Dorsal Periaqueductal Gray During LPS-Induced Hypotension

Running title: Dorsal Periaqueductal Gray and LPS-Induced Hypotension

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Abstract

Introduction: The central mechanism responsible for cardiovascular response to lipopolysaccharide (LPS) - induced hypotension is not completely determined and it is suggested numerous brain areas such as dorsal periaqueductal gray (dPAG) are involved. In this study the cardiovascular effect of the dPAG during LPS-induced hypotension was evaluated.

Methods: Twenty male Wistar rats divided into four groups including 1) Control (Saline microinjected into dPAG), 2) Lidocaine 2%, 3) LPS (intravenously injected), and 4) Lidocaine + LPS were used. Catheterization of the femoral artery and vein was performed for the recording of blood pressure and LPS injection, respectively. Saline and lidocaine were microinjected into the dPAG nucleus then, LPS injection was done. Cardiovascular responses throughout of experiments were recorded and changes (Δ) of systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rate (HR) were calculated over time and was compared with those control and LPS groups, using repeated measures ANOVA.

Results: LPS significantly reduced ΔSBP and ΔMAP (P<0.05) and did not change the ΔHR than the control group. Lidocaine did not significantly affect basic ΔSBP, ΔMAP and ΔHR compared to the control. Injection of lidocaine before LPS significantly attenuated reduction of ΔSBP and ΔMAP evoked by LPS (P<0.05).

Conclusion: Our data showed that blockade of the dPAG by lidocaine significantly ameliorates the hypotension induced by LPS. It confirms involvement of the dPAG in cardiovascular regulation during LPS-induced hypotension.

Keywords: Dorsal periaqueductal gray, Lidocaine, Lipopolysaccharide, Blood Pressure
**Highlights**

- Inactivation of the dPAG by lidocaine significantly ameliorated cardiovascular responses in hypotensive rats.
- LPS significantly lowered blood pressure and did not affect on the heart rate.

**Plain Language Summary**

The mechanism responsible for initiating endotoxic hypotension is not yet fully understood, although it is often related to the direct effect of lipopolysaccharide (LPS) as a component of the outer wall of gram-negative bacteria and other vascular mediators such as tumor necrosis factor (TNF) and nitric oxide (NO). One possibility is that the initial drop in LPS-induced arterial hypertension is mediated by a central mechanism. The ventral region of the transcranial gray matter is involved in lowering blood pressure and the dorsal region is involved in increasing blood pressure. The dorsolateral region of the transcranial gray matter (dlPAG) also causes tachycardia, vasodilation in muscles, and increased respiration. dlPAG contains cells that produce NO and serotonin (5HT) and receptors 5HT1 and 5HT2, which may play a role in causing hypotension due to stimulation of this region. LPS at a dose of 1 mg / kg (i.v.) or higher typically elicits a biphasic hypotensive response in rats. The first stage of this response begins almost immediately after LPS administration. Arterial pressure reaches its lowest point after 10 minutes and returns to baseline within 30 to 40 minutes. The second phase of endotoxic hypertension begins approximately 1 hour after LPS injection and is more severe and longer than the initial stage. Thus, endotoxic hypertension begins through a central mechanism and further suggests that hypotension may play an important role in the development of fatal hypotension, which represents the second stage of septic shock. Although dlPAG is known to be an important site for the regulation of cardiovascular responses, its role in LPS-induced hypotension has not been previously investigated. Therefore, the role of this nucleus in cardiovascular changes after LPS injection will be investigated.
1. Introduction

The Periaqueductal gray (PAG) region is located in the midbrain and plays an important role in several biological activities including defense reactions, war and escape, pain, anxiety, reproduction, and cardiovascular and respiratory activity (Dampney, 1994; Farkas, Jansen, & Loewy, 1998; Schenberg et al., 2005). From anatomical point of view, PAG is divided into four different functional areas: dorsomedial (dMPAG), dorsolateral (dLPAG), lateral (lPAG) and ventrolateral (vLPAG)(M. Deolindo, Pelosi, Tavares, & Corrêa, 2008; Schenberg et al., 2005). Based on the above classification, the defense responses and responses related to hatred and hypertension and tachycardia are related to the dMPAG, dLPAG, also called dorsal PAG. While quiet behavioral responses, opioid dependent analgesia, hypotension, and bradycardia and reward responses are attributed to the vLPAG region (Schenberg et al., 2005).

Lipopolysaccharide (LPS) is a potent bactericidal endotoxin that can cause fever, increased heart rate, infectious shock, and death following renal and pulmonary defects at high concentrations, but acts at a relatively low concentration as an active immune regulator and it induces non-specific resistance against both bacterial and viral contamination(Wang & Quinn, 2010).

Systemic infusion of LPS leads to a major challenge in the immune system, i.e., the increase of various pro inflammatory cytokines in the brain. Also, LPS systemic injection resulted in the production of intercellular binding molecules (ICAM-1), vascular connective tissue molecules (VCAM) and damage to the blood-brain barrier, resulting in granulocyte entry and initiation of immune responses(Hang et al., 2004).

Although the reduce of arterial blood pressure of LPS is mediated directly by vasodilation or evoking cytokine release, but previous studies showed that LPS initially reduces arterial pressure through a central mechanism(s) such as vagal activation of hypothalamic preoptic area (POA)(Millington, Yilmaz, & Feleder, 2016).

Therefore, in this study the cardiovascular effect of the dPAG during LPS-induced hypotension was evaluated.
2. Methods

2.1. Drug and animal groups

The agents (urethane and lidocaine) provided from Sigma, USA and dissolved in saline.

Animals randomly were divided into 4 groups as follow (n=5 in each group):

Control group: microinjection of saline into the dPAG

Lidocaine group: microinjection of lidocaine 2% into the dPAG

LPS group: injection of the LPS intravenously (1 mg/kg, i.v)

Lidocaine + LPS group: microinjection of lidocaine into the dPAG before injection of LPS

2.2. Protocol

Twenty male Wistar rats (250 ± 20 g) were provided from animal house of AJA University of Medical Sciences and kept in the room under physiological conditions and free access to food and water. The protocol of study was approved by the Bioethics committee of AJA University of Medical Sciences with code: IR.AJAUMS.REC.1397.080.

Urethane (1.5 g/kg, i.p) was used for anesthesia. The rat body temperature kept at 37 °C by heating lamp. The femoral vein and artery exposed and was cannulated with a blue angiocath filled with heparinized saline (50u/ml), then angiocath was connected to the pressure transducer and systolic blood pressure (SBP), mean arterial pressure (MAP) and heart rate (HR) were recorded throughout the trial period by a power lab system (ID instrument, Australia) (Shafei & Nasimi, 2011).

For microinjection of lidocaine, rat was placed in a stereotaxic apparatus (Stoelting, USA). The scalp was cut and the skull was leveled between bregma and lambda, and a little hole drilled in the skull. The stereotaxic coordinates of the dPAG were 7.5 mm caudal to bregma, 0.7 mm lateral to the midline suture and 4.8 mm ventral from the bregma according to the Paxinos book (Paxinos & Franklin, 2004). Lidocaine 2% (150 nl)(Yilmaz, Millington, & Feleder, 2008) microinjected into the dPAG nucleus by a single barreled micropipette with 40 μm internal diameter. The micropipette was connected to a manual microinjector (Harvard) via a PE-10 tube and carefully introduced into the dPAG and 150 nl of agents was injected about 30 sec. (Shafei,
Nikyar, Hosseini, Niazmand, & Paseban, 2017). Firstly, the lidocaine was injected and then after 3 min, the LPS was injected intravenously and cardiovascular responses were evaluated. At the end of experiments, the brains were removed and after fixation by formalin, serial sections (60-micron thickness) were prepared by a vibrating microtome (ESM Co, USA) and injection sites were verified by a light microscope.

2.3. Data analysis

The changes (Δ) of SBP, DBP, MAP and HR were calculated and expressed as mean ± SEM. In all groups for evaluation trend of responses, ∆SBP, ∆DBP, ∆MAP and ∆HR during hypotension in several time calculated and compared with changes in other groups (repeated measures ANOVA). In addition, peak changes of ∆SBP, ∆DBP, ∆MAP and ∆HR of each group were also separately provided and compared with peak changes of other groups (One-Way ANOVA post hoc Tuckey). A value of p<0.05 was used to indicate significance.

3. Results

In normal condition, saline was microinjected into the dPAG and cardiovascular parameters were evaluated before and after microinjection. Microinjection of saline did not significantly change these parameters compared to pre-injection values.

In this study, to examine the role of the dPAG on cardiovascular responses, the lidocaine was microinjected into the dPAG. Our results indicated that the lidocaine alone did not significantly affect the cardiovascular parameters.

As is indicated, ∆MAP, ∆SBP and ∆DBP in the LPS group significantly declined compared to the saline group over time (repeated measures ANOVA, P<0.05).

The lidocaine caused to improve the MAP, SBP and DBP in the rats treated with LPS. After that, the ∆MAP, ∆SBP and ∆DBP were slowly returned and stabilized lower than basal state within 12 min. In the LPS + lidocaine group, pre-treatment with the lidocaine significantly attenuated hypotension (decreased ∆MAP, ∆SBP and ∆DBP respect to the LPS group over time (repeated measures ANOVA P<0.05). The ∆HR in the treated groups did not observe any significant change (Figures 1-4).
The peak changes of the $\Delta$SBP (P<0.05), $\Delta$DBP (P<0.01) and $\Delta$MAP (P<0.01) in the LPS group significantly decreased compared to the control while these peak changes in the lidocaine and LPS + lidocaine (P<0.05 to P<0.01) groups significantly decreased compared to the LPS group. The peak changes of the HR did not significant difference between groups (Figures 5-8).

The recorded samples of the cardiovascular parameters have been shown in figures 9 and 10.

4. Discussion

The aim of the current study was to explore whether the dPAG involved in preventing of LPS-induced hypotension. Therefore, the role of the dPAG nucleus was investigated in both normotensive and hypotensive conditions. Our findings showed that in normotensive condition, inactivation of dPAG area did not affect the cardiovascular parameters. In spite of the fact that the dPAG nucleus did not effect on the cardiovascular response in basal condition but, inactivation this nucleus by lidocaine attenuate hypotension induced by LPS. Probably, there are some neurons in this nucleus such as glutamatergic or adrenergic neurons that lidocaine via inhibition of the Na$^+$ channels on these neurons cause to hyperpolarization and induces the hypotension through related vasomotor nuclei direct or indirectly (Sepehri & Shafiei, 2006). In regard with our findings, Fisk et al. had indicated that ablation of the dPAG with local injection of CoCl2 (a synaptic blocker) did not effect on the blood pressure and HR (Fisk & Wyss, 2000). Also, Deolindo et al. had shown that microinjection of Acetyl choline into the dPAG did not effect on the MAP and HR in the normotensive rats (M. V. Deolindo, Pelosi, Busnardo, Resstel, & Corrêa, 2011). However, Kubo et al. had shown that electrical stimulation of the dPAG resulted in enhancing of blood pressure (Kubo, Hagiwara, Sekiya, & Fukumori, 1999) but in the current study after inactivation of the dPAG we have not observed any significant reduction in the cardiovascular parameters. Based on this result we suggest that in normal condition this area did not cardiovascular activity. However, in condition such as hypotension, stress or another stimulus the neurons of this areas activate and precipitate in regulation of cardiovascular responses.

Our results indicated that LPS decreased systemic blood pressure with no significant effect on HR. These results are in line with previous studies that indicate LPS induced hypotension. It is suggested the LPS after injection into systemic blood stream evokes the cytokines secretion results to brief HR elevation. Then, LPS inserts to central nervous system by destroying the blood brain...
barrier and affect on neural pathways. This prose is time-consumer. The mechanism(s) of the central effect of LPS on hypotension is not completely known but it is reported that LPS caused decrease of the sympathetic outflow to brainstem sympathetic pathways and increase the cytokine levels such as TNF-α result in vasodilation (Koyama & Manning, 1985; Millington et al., 2016). In the lidocaine+LPS group, after several minutes the HR non significantly increased via excitation of baroreflex regulatory mechanism probably.

Yilmaz et al had shown that microinjection the lidocaine or the alpha-adrenergic receptor antagonist into the hypothalamic preoptic area (POA) prevents the hypotension induced by LPS (Yilmaz et al., 2008). Based on Pelosi et al study that there are noradrenergic receptors within the dPAG, we suggest that LPS by inhibitory effect on adrenergic receptors of the dPAG cause depressor effect (Pelosi & Corrèa, 2005). In addition, numerous studies have indicated that activation of the POA neurons lowers arterial pressure through a descending pathway from the POA to the PAG and midline raphe nuclei (Behbehani & Gomez, 1996; Jiang & Behbehani, 2001).

Therefore, we suggest that there is an adrenergic pathway between the POA and dPAG that involved in cardiovascular regulation and LPS via inhibition the adrenergic as well as other excitatory neurons caused hypotension. Lidocaine by inactivation of this pathway or their pathways could prevents the LPS-induced hypotension. In addition, there are projections from the dPAG to vasomotor areas including nucleus tractus solitarius (NTS), caudal ventrolateral medulla (CVLM), and rostral ventrolateral medulla (RVLM) and we suggest that LPS by effect on these projections reduce blood pressure. However, we need more studies to confirm these opinions.

Some limitations for the current study are:

1. Lack of further cardiovascular parameters evaluation such as ECG
2. Lack of histobiochemistry study for LPS tracing in the brain

We encourage dear researchers to complete the study by removing the above limitations.

In conclusion, this study was an initial study that shows the dPAG area involves in the adjustment of cardiovascular responses during LPS-induced hypotension. We proposed that further studies be done to evaluate mechanisms and neural circuit of these area that contributed in regulation of the cardiovascular responses during LPS-induced hypotension.
Ethical Considerations

Compliance with ethical guidelines

The protocol of study was approved by the Bioethics committee of AJA University of Medical Sciences with code: IR.AJAUMS.REC.1397.080.

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Authors' contributions

Conceptualization: Mohammad Naser Shafei and Reza Mohebbati; Methodology: Reza Mohebbati and Iraj Mirzaii-Dizgah; Investigation and writing-original draft: Reza Mohebbati, Mohammad Naser Shafei; Writing-review & editing, supervision, and funding acquisition: Iraj Mirzaii-Dizgah, Vajiheh Alikhani; Resources: All authors.

Conflict of interest

The authors declared no conflict of interest.

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References


Figures

Figure 1. Time course changes of Heart Rate (ΔHR) after microinjection of the LPS and lidocaine into the dPAG. The data were expressed as mean ± SEM; n = 5
Figure 2. Time course changes of Systolic Blood Pressure (ΔSBP) after microinjection of the LPS and lidocaine into the dPAG. The data were expressed as mean ± SEM; n = 5. *P<0.05 compared to the control.
Figure 3. Time course changes of diastolic blood pressure (ΔDBP) after microinjection of the LPS and lidocaine into the dPAG. The data were expressed as mean ± SEM; n= 5. *P<0.05 compared to the control.
Figure 4. Time course changes of Mean Arterial Pressure (ΔMAP) after microinjection of the LPS and lidocaine into the dPAG. The data were expressed as mean ± SEM; n = 5. *P<0.05 compared to the control.
Figure 5. Peak changes of Heart Rate (ΔHR) after microinjection of the LPS and lidocaine into the dPAG in rats. The data were expressed as mean ± SEM; n= 5.

Figure 6. Peak changes of Systolic Blood Pressure (ΔSBP) after microinjection of the LPS and lidocaine into the dPAG in rats. The data were expressed as mean ± SEM; n= 5. *P<0.05 compared to the control, #P<0.05 compared to the LPS group.
Figure 7. Peak changes of Diastolic Blood Pressure (ΔDBP) after microinjection of the LPS and lidocaine into the dPAG in rats. The data were expressed as mean ± SEM; n=5. **P<0.01 compared to the control, #P<0.05 compared to the LPS group.

Figure 8. Peak changes of Mean Arterial Pressure (ΔMAP) after microinjection of the LPS and lidocaine into the dPAG in rats. The data were expressed as mean ± SEM; n=5. **P<0.01 compared to the control, ##P<0.05 compared to the LPS group.
Figure 9. The samples of Blood Pressure (BP), Mean Arterial Pressure (MAP), and Heart Rate (HR) recorded after LPS injection.

Figure 10. The samples of Blood Pressure (BP), Mean Arterial Pressure (MAP), and Heart Rate (HR) recorded after lidocaine microinjection into the dPAG and LPS injection.
Figure 11. Coordinates of injection locations (Red dot) adopted from the Paxinos Atlas (A) and a sample of brain section after microinjection of the drug into the dPAG (B).