Effect of inflammation and anesthesia on brain-derived neurotrophic factor and cognition

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ABSTRACT

Background: Some studies have evidenced the effect of inflammation and anesthetics on Brain Derived Neurotrophic Factor (BDNF) but there is still no data regarding the effect of inflammation and anesthesia alone or in combination of inflammation and anesthesia on BDNF protein in rats’ brain.

Objectives: To examine effect of lipopolysaccharides (LPS) alone or combined with propofol anesthesia at 3rd of injection on cortical and hippocampal BDNF.

Methodology: Male rats in four groups were treated with Intralipid® control, propofol anesthesia (120 mg/kg), LPS (1 mg/kg) and combined propofol with LPS respectively. The brains were removed and brain homogenates were prepared from hippocampus and cortex tissues. The amount of BDNF protein was assessed using ELISA on the brain supernatants.

Results: BDNF protein was increased when subjects were injected with propofol anesthesia alone (about 30%) or to LPS injection (about 400%) in both cortex and hippocampus samples. When anesthesia was injected combined with LPS, BDNF protein was decreased in both cortex and hippocampus samples (p < 0.01).

Conclusion: Our data evidenced the long term effect of propofol and LPS in increasing BDNF and propofol combined with LPS decreases BDNF protein in hippocampus and cortex.

Key words: Lipopolysaccharide; BDNF Protein; Anesthesia; Propofol; Cortex; Hippocampus; Inflammation; Cognition

INTRODUCTION

Lipopolysaccharide (LPS), a potent bacterial inflammatory endotoxin involved in neural responses and has been implicated in cognitive deficits through the activation of immune system.1 Studies have indicated that injections of LPS led to the reduction of neurogenesis and increases in the process of apoptosis.2,3 Literature has shown that stimulation of immune system by the LPS may damage the brain physiological processes and this dysfunction in the physiological processes are protected by the special proteins in the brain called neurotrophic factors. As neurotrophins are involved in the protection against many insults to the brain anatomy and physiology,1) and have been implicated in learning and memory processes.5 Studies on laboratory animals have shown that LPS may be responsible for alteration of neurotrophic factors and their functions in brain areas including hippocampus.6 Study on mice synaptosomes has
shown reduction of brain-derived neurotrophic factor (BDNF) within 1-6 days post LPS injections and maximum reduction at day 3. However, a study on dendritic cells has shown increased levels of BDNF. LPS has also shown an up-regulation of BDNF in the dendritic cells generated from donors and measured by western blot, PCR and flow cytometry.

In addition to LPS, anesthetics have also been implicated in alteration of BDNF in important brain areas: cerebral cortex and thalamus. Thalamus has shown reduction of BDNF protein at 2 hours post injection of general anesthesia in developing brain, however cerebral cortex showed increase of BDNF at 2 and 6 hours.

There is not too much data regarding the effect of LPS with/without short duration anesthesia on BDNF in brain especially in cortex and hippocampus. The above studies suggest some questions to be answered; like is there any long lasting effect of LPS with/without short duration anesthesia on BDNF protein in brain of adult rat. So, the current study was aimed to evaluate impact of inflammation and anesthesia on cognition by quantifying BDNF protein in the hippocampus and cortical areas at 3rd day of treatment at ZT5 (Zeitgeber Time 5). ZT5 means 5 hours after light on, and this time point have shown peak of BDNF in unpublished data. So, the current study was focused to examine any inflammatory and anesthetic effect on BDNF protein at its peak.

**METHODOLOGY**

**Animals:** Young male rats (Sprague Dawley) of 8-10 weeks (250 ± 20 g) were housed 5-6 per cage for seven days in a temperature (22 ± 1ºC) and humidity (50 ± 10%) on 12/12 light/dark cycle. All subjects were provided ad libitum access to food and water.

**Treatment:** All experimental animals were divided into four groups and eight subjects were included in each group. Subjects of all the four groups were injected intra-peritoneal at ZT10 with propofol (120 mg/kg, Fresenius, France) with same dose of Intralipid® 20% (Fresenius, France) as control, LPS from E.coli (1 mg/kg, Sigma USA) and LPS plus propofol respectively.

**Procedures:** On next day of injections, subjects in each group were sacrificed at ZT5 after euthanasia under CO₂. The brain tissues were extracted and cortex and hippocampal structures were separated on ice. Cortical and hippocampal tissues were homogenized in extraction buffer. After centrifugation step (4000 rpm, 20 min), supernatants were extracted and stored at -20º C. BDNF protein was measured by ELISA technique (CYT306, Millipore) according to manufacturer’s guidelines.

**RESULTS**

We quantified an increase of the BDNF protein when subjects were injected with propofol (about 30%) and LPS injection (about 400%) in both cortex (Figure 1) and hippocampus samples (Figure 2). When anesthesia was combined with LPS injection, we observed of decrease of BDNF content as compared to LPS injection alone in both cortex and hippocampus samples. Multivariate ANOVA (between factors: anesthesia, LPS and within factors: structure) revealed significant effects of anesthesia (Intralipid control or propofol; F(1,28) = 19; p = 0.0001), and LPS injection (saline or LPS; F(1,28) = 412, p < 10⁻⁴), in structures (cortex vs. hippocampus; F(1,28) = 17; p = 0.0002) and significant effect between anesthesia and LPS (F(1,28) = 40; p < 10⁻²) and between structure and LPS (F(1,6) = 6; p = 0.0181) on the BDNF protein.

Post hoc analysis indicated that subjects injected with propofol anesthesia or LPS differed significantly from the controls groups in both hippocampal and cortical structures (all p < 0.01). There is also a significant decrease of BDNF content in animals submitted to the combined anesthesia and LPS regimen compared to animals receiving LPS injection without anesthesia.

Figure 1: Graph represents percentage change in BDNF protein in cortex of subjects treated with LPS, propofol anesthesia and LPS with propofol anesthesia with their Intralipid control
DISCUSSION

The current study showed the effect of propofol (short duration anesthesia) and LPS (*E. coli*) on BDNF protein in the cortex and hippocampal of the rats. Animal groups were injected at ZT10 with propofol (120 mg/kg), LPS (1 mg/kg), combined propofol and LPS and Intralipid as control. Brains were removed on ice at ZT5 (time of peak BDNF protein expression). This specific time point was chosen to standardize the data, as studies have suggested that proteins vary during different time of the day. The BDNF protein was measured using ELIZA technique for both hippocampus and cortex homogenates. ELISA has been evidenced suitable, specific and sensitive method for the measurement of antigens in tissue and blood sample.

Our results indicated that the level of BDNF protein was increased in both structures' homogenates: the cortex and hippocampus when subjects were either treated with propofol anesthesia (about 30 %) or LPS (about 400 %). We observed a decrease of BDNF protein contents when anesthesia was combined with LPS injection as compared to LPS injection alone in both cortex and hippocampus samples. Our results may suggest that there is a damaging effect of LPS even at day 3 that is shown by an increase of BDNF. Our results also show that there may be a protective effect of propofol against the damage caused by LPS by lowering the over elevated levels of BDNF, as BDNF is evidenced as major mediator of neuronal protection and neuroplasticity.

Another study showed protective effect of exogenous BDNF in rats with experimental meningitis.

But our data differs from the study of Guan and Fang that showed reduction of BDNF in hippocampus after intraperitoneal injections of LPS. This reduction in BDNF may be due that the proteins were quantified few hours after injects of LPS.

It is noted that anesthetics have been responsible in the alteration of BDNF in brain areas including cerebral cortex and thalamus. Usually anesthesia is associated with surgery, 24 hours after surgery under propofol anesthesia shows a decrease in plasma BDNF concentrations in patients. In rats, BDNF protein is also decreased 24 hour after tibia fracture performed under anesthesia. This may reflect immediate effect of anesthetics in reducing the levels of BDNF protein.

Our current study may differ in way that we conducted quantified BDNF protein ex vivo at ZT5 and quantified BDNF protein at 3rd day of treatments with LPS, propofol, and propofol with anesthesia. However, combination of LPS with anesthesia showed a decrease in levels of BDNF proteins in both the structures i.e. cortex and hippocampus. This may suggest a protective effect of propofol against the damage caused by over production of BDNF due to LPS. As over production of BDNF has been also shown detrimental in the process of learning and memory. These detrimental effects of overexpression of BDNF protein due to anesthesia and LPS may be noted by performing the behavioral experiments in rats. These results show BDNF alterations may be due to circadian effect, so there is need to quantify BDNF at few other time points during 24 hours. Our results focus on short during anesthetics, so we cannot conclude for other type of anesthetics.

Strengths of the study: This study shows the effect of short during anesthesia on Brain Derived Neurotrophic Factor.

Weaknesses of the study: The limitation of the study is that effect is limited to short anesthetics and we cannot conclude for long term anesthetics

Statement on ethical standards

All the procedures were performed at Unit UPR-3212 CNRS, Strasbourg University, France. All
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procedures on laboratory animals were performed in compliance with national (Ministère de l’Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale;) and international guidelines (NIH publication, no. 86-23, revised 1985).

Conflict of interest

Authors declare no conflict of interest

Authors’ contribution:

MR-Concept, experiments, writing
LP-Concept, manuscript editing
NA-Manuscript editing, review

REFERENCES


My Most Memorable Patient

The case of subarachnoid hemorrhage
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One of the key things while working in an acute specialty is the occasional weird and wonderful experience that may not fit in the normal emergency textbook stories. Such a case I encountered while working as a senior house officer at Birmingham Heartlands Hospital.

A gentleman in his early seventies presented on a cold winter evening to the minor section of A&E with his sister-in-law complaining of a mild headache rating 4/10. He stated that it was not the first headache of his life and laughed as if his sister-in-law had brought him and made him wait her for 3 hours to see a doctor just for nothing. I took a detailed history and examined him and relayed my information to my consultant. As the patient looked completely fine I wanted to send him home but just as I was about to write up my plan, I felt that his sister-in-law must have some concerns that warranted this trip. She stated she didn’t but he did suffer from memory loss. The fact that the relatives had genuinely felt a need to bring this well-dressed gentleman to the hospital warranted that he must not be ignored. I requested a CT scan, stating amnesia. I had a row with the radiologist to get him scanned. The patient mocked me about being overly cautious and walked towards the scanner. Fifteen minutes later while I was writing my notes up I heard my name on the speakerphone across A&E to come to radiology. I ran towards the resuscitation unit to find my patient a bit puzzled but alert. The patient had a massive subarachnoid hemorrhage with a midline shift. The registrar was amazed as to how a patient was completely stable with a GCS 15. Having a look at those scans made me realize I close was I to sending this patient home, who would have most likely come back collapsed. The patient was quickly transferred to the nearest neurosurgical unit and an urgent drainage done. To this day I have reflected from this case and made sure to always add one more portion to my history taking: ‘any concerns by the family’ - this helps you to pick up those rare presentations that could have been so easily missed.