

A Study on Gene Expressions of Rat Brain Sections after Lipopolysaccharide Treatment

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Abstract: Inflammation has been identified as the substrate of almost every chronic disease, like asthma, rheumatoid arthritis, Crohn's disease, or even diabetes, depression, schizophrenia, Alzheimer's and heart disease. On the other hand, Peroxisome Proliferator-Activated Receptors (PPARs) are core receptors that negatively regulate primary the mitochondrial dysfunction, oxidative stress and neuro-inflammation, which constitute dominant causes of the pathogenesis of neurodegenerative disorders such as Parkinson's and Alzheimer's disease, also forming important therapeutic goal for them. Furthermore, PPAR agonists are neuroprotective and increase mitochondrial function, also contributing to the regulation and prevention of various inflammations and neuropathic pain. The aim of the given research was basically to study the gene expression in rat brain sections after causing inflammatory factors. It was investigated the effect of the PPAR γ receptor agonist in the Central Nervous System (CNS) of white Wistar rats, using an experimental model of inflammation induction via injection of lipopolysaccharide (LPS) in selective rat brain sections: hypothalamus, hippocampus and striatum. Ten rats were divided into 2 groups. The first was injected with LPS three hours before being killed, and the brain tissues were isolated, while the second was only saline injected and served as controls. With respect to the effect of LPS in rCOX-1, rCOX-2, rIL-1b, rIL-6 and rPPAR genes, the experimental procedure finally revealed clearly that the effect of LPS caused a significant increase in the mRNA of rCOX-2 and interleukins IL-1b and IL-6 (5-, 17-, and 8-fold, respectively), while hardly affected the gene expressions of rCOX-1 and rPPAR α in all selective brain sections.

Keywords: Inflammation, Rat, Central Nervous System (CNS), Hypothalamus, Hippocampus, Striatum, Lipopolysaccharide (LPS).

1. BACKGROUND

The term 'inflammation' derives from the Latin word 'inflammatio' means ignites. Inflammation should not be confused with the word 'infection' even in the case where the inflammation derives from an infection. The two concepts are not identical, the infection caused by the pathogen, while inflammation is the response of the organism to the pathogen cause. Inflammation represents that complex biological process by which the body defends the invasion of an inflammatory stimulus whether it is a virulence factor, or different antigens or even a simple physical damage [1]. After the inflammation anatomical and functional restoration of tissue occurs. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organization. This is the reason that inflammation is a process that is usually regulated by the body itself. Burns, frostbite, toxins and other chemicals, pathogenic viruses, tissue damage, immune reactions due to hypersensitivity, and ionizing radiation, are some of the major causes of development of inflammation.

Many cells are involved in inflammatory mechanism, e.g. Macrophages and leukocytes, arriving at the site of inflammation through the bloodstream, and many other substances that have specific biological effects and are produced by cells of inflammation. These substances, such as cytokines, chemokines, etc. acting in other cells, adhere to the site of inflammation or activate them for the production of other substances in order to neutralize or eliminate harmful factor. Consequences of this inflammatory process are certain clinical phenomena that are main characteristics of inflammation. These phenomena are: pain, edema (swelling), redness and heat at the site of inflammation.

Furthermore, studies in mice indicate that administration of an inflammatory agent such as lipopolysaccharide (LPS, endotoxin of Gram (-) bacteria) and IL-1, stimulates the production of cytokines and activates adrenocortical hypothalamic - pituitary (HPA), as evidenced by elevated levels of Adrenocorticotropic Hormone (ACTH) and corticosterone plasma [2-3]. Additionally, administration of LPS or IL-1 generates noticeable changes in the behavior of rodents that resemble the so-called 'sickness-behaviour' as the lifting of the coat, the retracted posture, reduced food intake and increase body temperature [4].

Peroxisome Proliferator-Activated Receptors-PPARs are core receptors and constitute a very promising therapeutic target for many neurodegenerative disorders, including Parkinson's disease, Alzheimer's disease, Huntington's disease and Amyotrophic Lateral Sclerosis. They play a key role in the negative regulation of mitochondrial dysfunction, dysfunction of the proteasome, oxidative stress, and neuroinflammation, which are the main causes of the pathogenesis of neurodegenerative disorders. Several experimental approaches suggest possible implementation of the PPARs agonists in the treatment of neurodegenerative disorders. Several epidemiological studies have found that the frequent use of PPAR agonists is effective in reducing the progression of neurodegenerative diseases, including AD and PD.

At the level of the CNS, still unexplored neural circuits involved in the treatment of nerve signals from the immune system. Until now the majority of studies have focused on the hypothalamus to identify the neural circuits that are activated after administration of LPS or IL-1 and are involved in neuroendocrine and autonomic regulation. What is certain is that the results of almost all studies showed that administration of LPS or IL-1 regardless its route and the experimental procedure followed caused an increasing recycling rate of noradrenaline (NA) in hypothalamus [5-10]. However, many researchers support the view that the neuroendocrine, autonomous (e.g. febrile states) and behavioral changes caused by the stimulation of the immune system can be considered as an adaptive response of the body to regain its homeostasis [11-13].

In the research field various experimental assays used acute inflammatory challenge models in rats, with the most common to be: a) *disease challenge* with endotoxin administration [usually, a lipopolysaccharide (LPS) produced by Gram (-) bacteria] and b) *edema induction* in rat leg after administration of carrageenan (a classic model for the formation of edema when the observed activation of AA metabolism and consequent increase of the intermediate products such as prostaglandins and thromboxane) [14-16]. In the current study we used a model of *inflammation induction* by lipopolysaccharide LPS in combination with rosiglitazone.

2. METHODS

The current study was conducted in the Department of Pharmacology of the Medical Department of the Faculty of Health, University of Ioannina, in a period between 2014 and 2016, as part of the Postgraduate Program "Pain Management". This paper discusses the effect of the PPAR γ receptor agonist in central nervous system (CNS) inflammation in rats using experimental model of lipopolysaccharide LPS.

The purpose of the study was primary to identify the gene changes of inflammatory factors in brain sections of rats after administration of LPS.

Subjects: In the experimental protocol, 10 male white rats (Wistar) of rr strain, aged from 2.5 to 3.5 months, and weight average about 250 g. were used. A day before the experiment all rats were transported to the experiment room and executed by hanging, for the isolation of the following brain tissues: hippocampus, hypothalamus and striatum soma. We preferred the intraperitoneal route of administration (i.p.) for all substances were administered to the rats as well, the direct entry of the drug into the peritoneal cavity, ensures rapid absorption of the drug due to the high vascularity of the area. Finally, all work conducted in accordance with the Greek and European Law (Presidential Decree 160/03.05.1991, articles 13 and 19, Directive 86/609 / EEC of the European Council).

Procedure: Brain tissues were further processed in four steps: a) *Isolation of mRNA* was performed using the NucLeoSpin® RNA/protein Kit of Macherey - Nagel Company b) *Determination of the amount of RNA- Nano Drop*. The quantity and purity of the RNA is determined photometrically at 260 and 280nm, in a small tumors visible-ultraviolet spectrophotometer (NanoDrop 2000 Thermo Scientific) c) *Synthesis of CDNA (reverse transcription reaction)*. Reverse transcription was done using the kit TAKARA d) *qReal - Time PCR*. At the level of transcription, expression of rCOX-1, rCOX-2, rIL-1b, IL-6, PPAR and PPAR γ was determined in duplicate by the assay system mRNA CFX-96 RT-PCR system BioRad (BioRad Lab), using the mixture Taqman® Universal PCR master mix and corresponding, for each gene, ekkiniti/prode set (Rn00566881_m1, Rn01483828_m1, Rn00580432_m1,Rn01410330_m1, Rn00566193_m1 and Rn00440940_m1, from AB, NI, USA).

We investigate the effect of LPS lipopolysaccharide in hypothalamus, hippocampus and striatum of male rat’s brain inflammation genes with injection of LPS (100mg/kg in oil).10 rats were divided into 2 groups. In the first group there was an injection of LPS three hours before being killed, and the brain tissues were isolated, while in the second group was only saline injection and served as controls.

3. RESULTS

For the purposes of the study, three rat brain sections were selected and were studied in terms of inflammatory genes. Also based on the purpose of the study and a relevant knowledge of the Pharmacology Laboratory, specific genes were selected from the long list of inflammatory agents in the brain of laboratory animals, especially in rats. Below are the results of the experimental protocol per brain section of laboratory animals (hippocampus, hypothalamus and striatum soma).Results for changes in the level of transcription, i.e. changes in the mRNA of the genes and their expressions are presented in relation to the group which took saline (control group, control). In this experimental protocol, all statistical comparisons were made with respect to the control group.The results are normalized by the expression of the reporter gene beta-actin (b-actin mRNA).

Figure 1 shows collectively the results of effects of LPS in rCOX-1, rCOX-2, rIL-1b, rIL-6 and rPPARa genes as a %percentage of control.The procedure finally revealed clearly that the effect of LPS caused a significant increase in the mRNA of rCOX-2 and interleukins IL-1b and IL-6 (5-, 17-, and 8-fold, respectively), while hardly affected the gene expressions of rCOX-1 and rPPARa (figure 1).

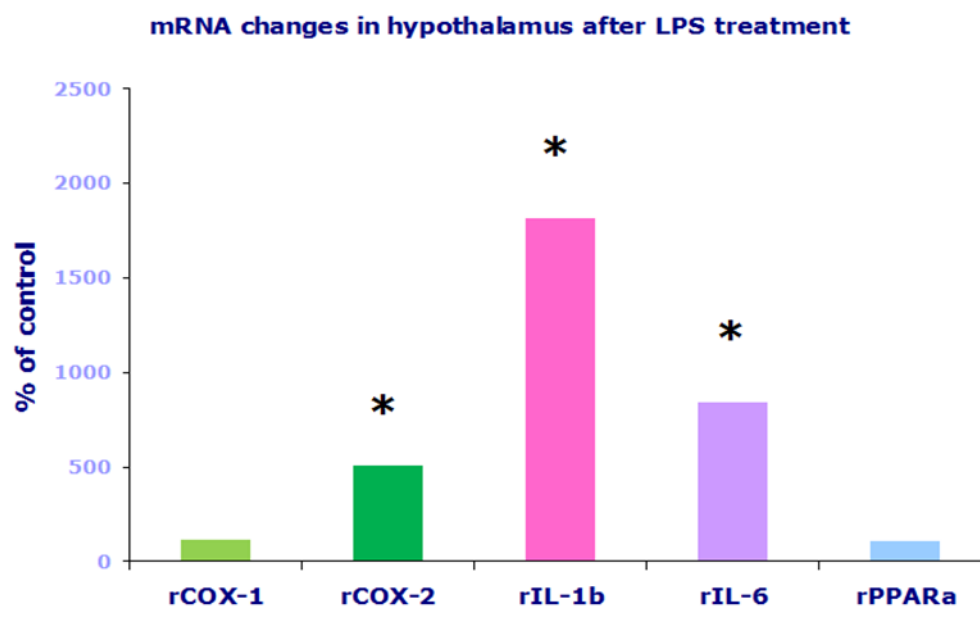


Figure 1: Overall results of the action of LPS in rCOX-1 inflammatory genes, rCOX-2, rIL-1b, rIL-6 and rPPARa, in hypothalamus. The results are presented in relation to 100% (control group levels). The asterisk denotes a statistically significant change compared to the control group (level 100, for P <0.05).

Figure 2 shows collectively the results of effects of LPS in rCOX-1 genes, rCOX-2, rIL-1b, rIL-6 and rPPARa% as a percentage of control. The administration of LPS caused a statistically significant increase in the mRNA of the interleukins IL-1b and IL-6 (8- and 4-fold, respectively), while hardly affecting the gene expression of rCOX-1, rCOX-2 and rPPARa.

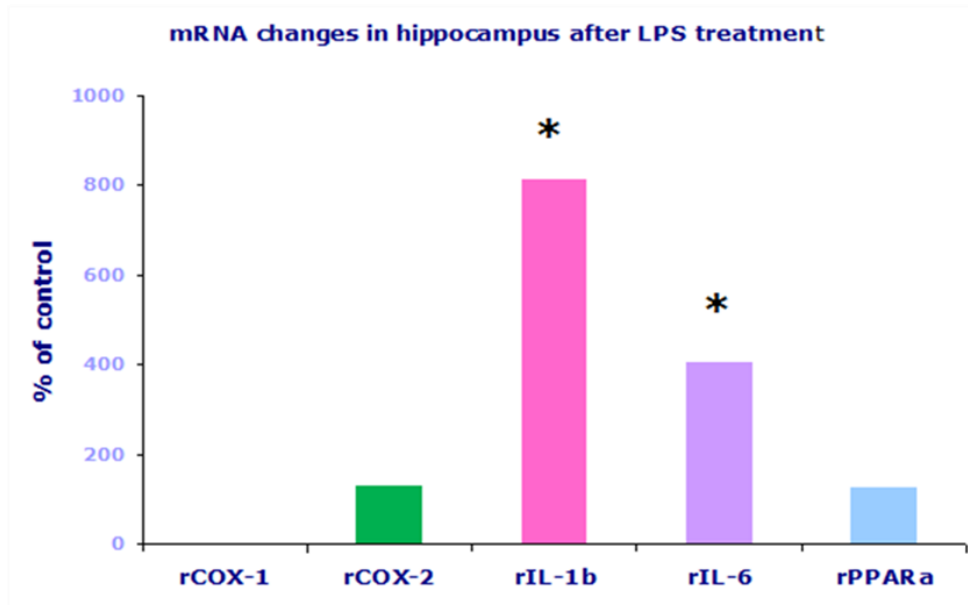


Figure 2: Overall results of the action of LPS in rCOX-1 inflammatory genes, rCOX-2, rIL-1b, rIL-6 and rPPARa, in hippocampus. The results are presented in relation to 100% (control group levels). The asterisk denotes a statistically significant change compared to the control group (level 100, for P <0.05).

Figure 3 shows collectively the results of effects of LPS in rCOX-1, rCOX-2, rIL-1b and rIL-6 genes as a percentage of control group. The administration of LPS caused a statistically significant increase in the mRNA of rCOX-2 and interleukins IL-1b and IL-6 (5-, 17- and 8-fold respectively), while no effect occurred on the gene expression rCOX-1.

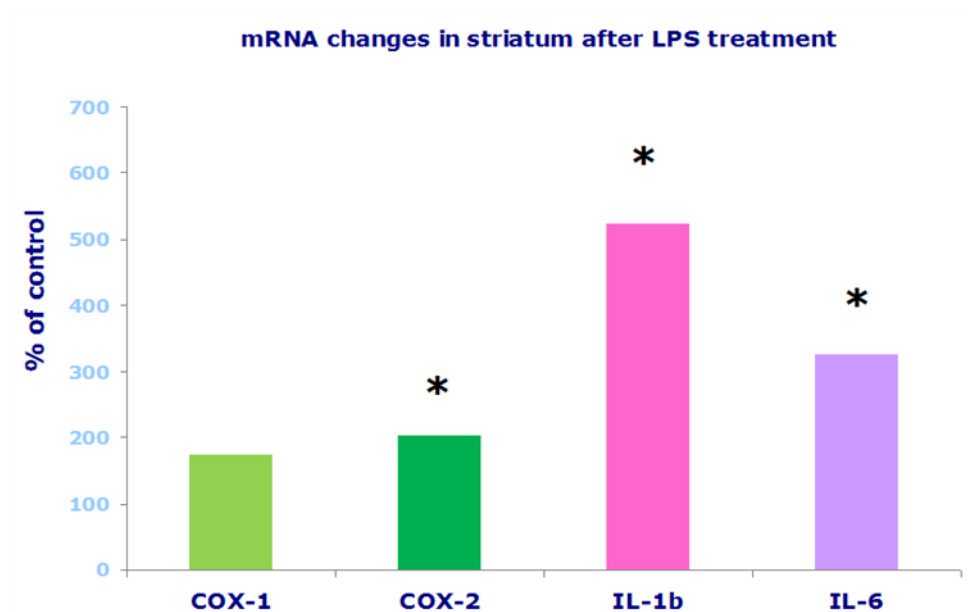


Figure 3: Overall results of the action in LPS rCOX-1 inflammatory genes, rCOX-2, rIL-1b and rIL-6, in the striatum. The results are presented in relation to 100% (control group levels). The asterisk denotes a statistically significant change compared to the control group (level 100, for P <0.05).

4. DISCUSSION

Over the last two decades has argued warmly suggested that between the immune system and the CNS interact at multiple levels. The brain is no longer considered the dominant organ in the body's defenses. In addition, the immune system does not act as an autonomous unit, but its function is associated with effects on the CNS [17]. When the body faced with an infectious agent or an inflammatory stimulus then he responds with a multitude of different defense mechanisms that demonstrate a bidirectional communication between the CNS and immune. Thus, the control unit regulates the processes taking place in the immune system through neurotransmitters and endocrine hormones [17], the IL-1, IL-2 cytokines, and IL-6 and tumor necrosis factor alpha (TNF) produced by specific cell populations in the immune system cause changes in the CNS level, i.e. changes related to the neuroendocrine, autonomic and behavioral procedures [18].

Initially, as mentioned above, the immune system is activated causing including the secretion of cytokines into the bloodstream. This stimulation of the immune system as a consequence increases the circulating glucocorticoids, which in turn further enhance the response of the organism [19-21]. Any inflammation, simple to severe, accompanied by physiological changes in behavior such as fever, loss of appetite, fatigue and social indifference. For all these behavioral changes observed during the activation of the immune system, introduced the name with the term 'sickness behavior' [21-22] and nowadays considered particularly important mechanisms which the body uses to deal with an infection or an inflammatory action.

Studies in mice indicate that administration of an inflammatory agent such as lipopolysaccharide (LPS, endotoxin of Gram (-) bacteria) and IL-1, stimulate the production of cytokines and activates adrenocortical hypothalamic-pituitary (HPA), as evidenced by elevated levels of Adrenocorticotrophic Hormone (ACTH) and corticosterone plasma [23]. Furthermore, administration of LPS or IL-1 induces noticeable changes in the behavior of rodents that resemble the so-called 'sickness behavior' as the lifting of the coat, the retracted posture, reduced food intake and increase body temperature [22].

What primary derived from the research protocol, was the fact that the inducing action of lipopolysaccharide LPS genes of inflammation, although many times has been reported by several research groups, has proved particularly potent as indicated by the results of the experiment (Figures 1-3). The dose and route of administration of the LPS were also used in other groups.

Another interesting point in the study was the non-inflammatory form of rCOX-1. Probably due to the fact that it is the production and release of prostaglandin role in physiological mechanism, lipopolysaccharide LPS failed to alter the levels of rCOX-1 on any of the three tissues sections of rat brain.

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