The effect of orexin-A on the morphofunctional characteristics of LPS-stimulated microglial cells

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Microglial cells have a protective function and are essentially immunocytes present in the central nervous system. Activation of microglia results in the production of cytokines and trophic factors that can have a damaging or protective effect on brain cells. Orexin-A has anti-inflammatory and neuroprotective properties. Subcutaneous administration of orexin-A enhances the survival of mice with lipopolysaccharide-induced endotoxin shock, reducing the level of pro-inflammatory cytokines and chemokines. The aim of this study was to determine the effect of orexin-A administered intraventricularly on the morphological characteristics of microglial cells activated by intraperitoneal injection of lipopolysaccharide (LPS). With the introduction of LPS, there is a change in the number of microglial cells in the somatosensory cortex, as well as in their morphology. When these indicators were analyzed after administration of orexin-A to animals against the background of LPS stimulation, no changes were detected.

Keywords: microglia, orexin-A, microglial cells processuses.

Introduction

Microglial cells have a defensive function, and are essentially immunocytes present in the central nervous system. With the development of autoimmune diseases, microglial cells perform the function of resident macrophage cells that are activated during brain injury and render set of pathogenic effects. When activated, microglial cells display morphological changes, for example, acquire an amoeboid form.

Microglia activation is accompanied by increased expression of complement receptors and molecules of the main histocompatibility complex, as well as To11-like receptors [1]. At the same time, activated microglial cells synthesize a number of soluble factors, most of which are cytotoxic.

Microglial cells are antigen presenting ones. Upon activation they are able to increase the expression of the molecules of the main histocompatibility complex (MHC) and costimulatory molecules, such as B7 and CD40, which allows them to efficiently present antigens to T cells. It was found that neurotrophins and anti-inflammatory cytokines inhibit the expression of these surface molecules, which indicates the presence of regulatory signals modulating the functions of microglia [2]. With the development of autoimmune
diseases of the central nervous system, an increase in the expression of MHC, costimulatory molecules and the subsequent presentation of antigen by microglia leads to the activation of T cells that recognize central nervous system antigens, the effect of which causes damage to brain cells.

Microglial cells and blood macrophages are activated in case of brain damage and infection and migrate to the affected area. The presence of damaged cells leads to the transformation of microglial cells into rounded migrating macrophages that produce cytokines and trophic factors that can have a damaging or protective effect on brain cells.

Orexins (hypocretins, Hcrt) A and B are neuropeptides that are known to regulate sleep / wakefulness states and eating behavior, and orexin-A has anti-inflammatory and neuroprotective properties, which suggests that it can have a therapeutic effect in inflammatory and neurodegenerative autoimmune diseases such as multiple sclerosis (MS) [3].

Orexin-A levels decrease in parallel with the progression of multiple sclerosis and impaired motor function in the early stages of the disease, and orexin-A can be used as a potential disability biomarker [4].

It is known that orexin-A has a therapeutic effect on the course of autoimmune encephalomyelitis, limiting the infiltration of pathogenic CD4 + T-lymphocytes, reducing the levels of chemokines (MCP-1 / CCL2 and IP-10 / CXCL10) and cytokines (IFN-γ (Th1), IL-17 (Th17), TNF-α, IL-10 and TGF-β) in the central nervous system.

The administration of orexins reduces brain damage in a model of focal cerebral ischemia in mice. This effect is associated with a decrease in the expression of IL-6 and TNF-α. In addition, subcutaneous administration of orexin-A enhances the survival of mice with lipopolysaccharide-induced endotoxin shock, reducing the level of pro-inflammatory cytokines and chemokines. In vitro studies using the BV2 microglial cell line show that pretreatment of BV2 cells with orexin-A reduces the production of pro-inflammatory IL-6, TNF-α and iNOS and the transition of microglia to the anti-inflammatory (M2) phenotype, which is characterized by increased expression of arginase-1 [5].

The aim of this study was to determine the effect of orexin-A on the morphological characteristics of microglial cells activated by LPS injection. As it is known, the length of the filopodia of microglial cells changes upon its activation and transition to the M1 phenotype, which indicates the degree of their activation. At rest, microglial cells have a branched shape with longer processes, and upon activation they acquire an amoeboid shape and the processes shorten.

**Materials and methods**

Experimental animals (refer: Akiyoshi 2018 eNeuro), wild type mice (c57BL6, male, age 2 months) were injected into the second ventricle of the brain with 1 μl of orexin-A solution (Sigma-Aldrich, USA) at a concentration of 0.3 mM (n = 9) c, and saline (0.9 PBS) in the same volume of 1 μl (n = 8) was administered as a control. One hour after the injection, LPS (Funakoshi chemical, Tokyo, Japan) was injected intraperitoneally at a dose of 2 mg/kg to animals of both groups. 7 hours after the start of the experiment, the animals were perfused, and the brains were removed for further fixation and immunohistochemical analysis.

To evaluate the effect of LPS on microglia cells, these cells are analyzed. Wild type mice were injected LPS intraperitoneally at a dose of 2 mg/kg in to animals (n = 6), and
saline in the same volume 0.1 ml (n = 3). 7 and 24 hours after the start of the experiment, the animals were perfused, and the brains were removed for further fixation and immunohistochemical analysis.

**Immunohistochemistry**

To quantify microglia morphology, mice were transcordially perfused with 4 % paraformaldehyde in phosphate buffer (PB, pH7.4) and overnight fixation with same fixative solution. Fixed brains were cut with a microtome (Leica Microsystem, Germany) into 50 µm thick sections.

Primary monoclonal rabbit antibodies to Iba1 (1: 500, Wako, Osaka, Japan) were used in this work. Brain sections were incubated overnight with primary antibody, washed with phosphate buffer, and incubated with secondary antibodies conjugated with a fluorescent label (Alexa Flour 488, Abcam, United Kingdom). The preparations were washed with phosphate buffer and mounted on glass slides. The cell nuclei were stained with VEC H-1200 fluorescent dye with DAPI. For verification of microglial cells in the brain tissue was used by visualization on a confocal laser scanning microscope Nicon Eclipse TI (Tokyo, Japan).

Processing of the obtained photos was carried out using the program ImageJ, plugin Simple Neurite Tracer and Multi-point.

**Results**

The sizes of the processuses of microglial cells in the somatosensory and motor zones of the cortex and striatum were studied. The analysis did not allow us to detect changes in the shape of microglial cells (Fig. 1).

With the introduction of LPS, there is a change in the number of microglial cells in the somatosensory cortex, compared with the control it increases after 7 hours by 12 %, and by 25 % after 24 hours (Fig. 2). When these indicators were analyzed after administration of orexin-A to animals against the background of LPS stimulation, no changes were detected. That is size of cells did not differ from those characteristic of the reaction to the

![Fig. 1. The average length of processuses of microglial cells (in microns) in the somatocortex, motocortex and stratum](image-url)
administration of LPS, one of the next step of investigation is to analyze the expression of expression of orexin-A receptors after LPS administration what is the matter of present day analyzes, because the influence of orexins on microglial cell is very important, including the possibility to use them for the treatment of different kind of pathology.

![Graph showing the number of microglial cells in zone S1 of the cerebral cortex (n = 3/group)](image)

*Fig. 2. The number of microglial cells in zone S1 of the cerebral cortex (n = 3/group)*

![Image showing an example of microglial cells for analysis of length of processes of (in microns) in the somatocortex](image)

*Fig. 3. Example of microglial cells for analyze of length of processuses of (in microns) in the somatocortex*
Conclusion

As is known, the length of the filopodia of microglia cells changes upon its activation and transition to the M1 phenotype, which indicates the degree of their activation. At rest, microglia cells have a branched shape with longer processes, and upon activation they acquire an amoeboid shape and the processes shorten.

Unfortunately, in this experiment, it was not possible to detect changes of the average length processes of microglial cells which would indicate the existence of these cells (Fig. 3).

Also our experiment confirms the previous data on the effect of LPS on microglia cells. The number of microglia cells in the somatosensory zone of the cortex increases in 7 and 24 hours after intraperitoneal administration of LPS.

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References


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