Neuroscience Letters 556 (2013) 73–78

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

REM sleep deprivation increases the expression of interleukin genes in mice hypothalamus

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HIGHLIGHTS

- We investigated the change of inflammatory gene expressions by REM SD in the hypothalamus.
- Among IL subfamily genes, REM SD increased most potently IL1β gene expression.
- IL subfamily genes, and in particular IL1β, might be involved in sleep regulation.

ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form 15 August 2013
Accepted 17 September 2013

Keywords:
Sleep deprivation
Hypothalamus
Microarray
Interleukin
REM sleep deprivation

ABSTRACT

Recently, evidence has suggested the possible involvement of inflammatory cytokines in sleep deprivation (SD). In this study, we assessed the patterns of inflammatory gene regulation in the hypothalamus of REM SD mice. C57BL/6 mice were randomly assigned to two groups, SD ($n = 15$) and control groups ($n = 15$). Mice in the SD group were sleep-deprived for 72 h using modified multiple platforms. Microarray analysis on inflammatory genes was performed in mice hypothalamus. In addition, interleukin 1 beta (IL1β) protein expression was analyzed by the immunochemistry method. Through microarray analysis, we found that expressions of IL subfamily genes, such as IL1β (2.55-fold), IL18 (1.92-fold), IL11 receptor alpha chain 1 (1.48-fold), IL5 (1.41-fold), and IL17E genes (1.31-fold), were up-regulated in the hypothalamus of SD mice compared to the control. The increase in the expression of these genes was also confirmed by RT-PCR. Among these genes, the expression of IL1β was particularly increased in the hypothalamus of SD mice. Interestingly, we found that the protein expression of endogenous IL1β was also elevated in the hypothalamus of SD mice compared to the control mice. These results implicate that IL subfamily genes, and in particular, IL1β, may play a role in sleep regulation in the hypothalamus of REM SD mice.

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1. Introduction

Sleep is a required process of the brain for proper functioning, and it is also important in the optimal homeostasis of the emotional brain function. Sleep is important for maintaining synaptic balance, and wakefulness is responsible for synaptic potentiation in cortical circuits [5]. Sleep deprivation (SD) has been demonstrated by increased pro-inflammatory cytokines, appetite, and blood pressure as well as cortisol levels [2,16]. In addition, SD was known to cause negative effects on emotional behavior, attention, learning, and memory, and also may subsequently give rise to psychiatric disorders.

Cytokines, mediators of immune system responses, were implicated to interact with the sleep mechanisms of the brain. Cytokines were known to signal the central nervous system (CNS) in order to regulate normal sleep patterns, alter sleep during infectious disease and pathology, and induce the altered behavior and symptoms associated with the illness [13]. Several studies have reported the relationship between SD and cytokines. Pro-inflammatory cytokines, such as interleukin 1 beta (IL1β), IL6, and tumor necrosis factor-α (TNF-α), have been thoroughly evaluated to contribute to sleep regulation in brain regions, such as the hypothalamus and the hippocampus [28]. Diurnal variations in concentrations of pro-inflammatory cytokines, such as IL1β and TNF-α, were reported to be associated with the sleep regulation [9,28]. In another study, SD has been reported to increase the plasma levels of IL1β and IL1 receptor antagonist [3]. In addition,

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http://dx.doi.org/10.1016/j.neulet.2013.09.050

IL1, IL2, IL6, IL8, IL18, and TNF-α were known to promote non-rapid eye movement (NREM) sleep [10,18], whereas IL4, IL10, IL13, and transforming growth factor-β suppressed NREM sleep [6].

The hypothalamus is now recognized as the most important brain site for the sleep switch, and as a key regulator of sleep and wakefulness. Sleep rhythm is controlled by a circadian pacemaker located in the hypothalamus, which regulates mood, appetite, sexual drive, circadian rhythms, and endocrine functions [1]. It has been shown that SD resulted in hypothalamic-pituitary-adrenal axis activation, and augmented the immunoreactivity for corticotrophin-releasing hormone in the paraventricular nucleus (PVN) of the hypothalamus [4]. Mackiewicz et al. [14] assessed changes in gene expression in the cerebral cortex and hypothalamus of the mouse following SD, and reported the alteration of cell growth-, cytoskeleton organization-, and biogenesis-related genes in the hypothalamus. Despite the potential roles of cytokines in SD and hypothalamus in sleep regulation, the study about cytokine regulation in the hypothalamus by SD has not been deeply investigated so far. Thus, we investigated the change of inflammatory gene expressions by SD in the hypothalamus using REM SD mice to characterize the transcript profile of inflammatory genes resulting from SD.

2. Materials and methods

2.1. Animals and modified multiple platform method

Ten week-old male C57BL/6 mice (24–29 g; Central Lab. Animal Inc., Korea) were housed under a 12-h light/dark cycle at a standard temperature (22 ± 3 °C) with food and water freely available. All experimental procedures were carried out according to the animal care guidelines of the National Institute for Health (NIH) Guide and the Korean Academy of Medical Sciences.

SD was conducted using the multiple platform method, modifying the previously described multiple platform method [21] that is a widely used method for REM SD [30]. Eleven week-old male C57BL/6 mice were used for this study. Mice were randomly assigned to two groups, the SD (n = 15) and the control groups (n = 15). Mice (5 mice per tank cage) in the SD group were placed in water tanks (56 cm × 40 cm × 19 cm), containing 15 circular platforms (3 cm diameter) each, surrounded by water up to 1 cm beneath the surface, for 72 h. When they reached the paradoxical phase of sleep, muscle atonia caused them to fall into the water and wake up. In each tank, mice were coming from the same cage where they were previously housed, and were capable of moving inside and jumping from one platform to the other. Mice (5 mice per tank cage) in the control group were submitted to the same procedure, except that the platforms were 8.5 cm in diameter. Platforms, which were surrounded by water, were large enough for the mice to sleep on, but still not large enough for the mice to walk around on. Throughout the study, mice were housed under a 12-h light/dark cycle at a standard temperature (22 ± 3 °C). Food and water were made available ad libitum through a grid placed on top of the water tank.

2.2. RNA extraction

Mice (n = 6 per group) were anesthetized using CO_2 and were decapitated. We collected brains from both groups, and isolated the total hypothalamus from each mouse brain. Total RNA from the isolated hypothalamus was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, and was purified using an QIAGEN RNeasy kit (QIAGEN Sciences, Germantown, MD). RNA integrity was evaluated using a ND-1000 UV–vis spectrophotometer (Nanodrop Technologies Inc., Montchanin, DE) to measure the absorbance ratio (A260/A280). RNA samples were additionally analyzed with agarose gel electrophoresis and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) to evaluate the integrity of the RNA.

2.3. Microarray analysis

For gene expression analysis, CombiMatrix 4 × 2K mouse Inflammation chip (Digital Genomics, Seoul, South Korea) was used. Fluorescently labeled probes for oligo microarray analysis were prepared by Amino allyl MessageAmp™ mRNA kit (Ambion Inc., Austin, TX). The labeled probes were hybridized at 60 °C for 16 h. Slides were washed twice in 6× SSC/0.005% Triton X-100 at 60 °C for 20 min, once in 0.1× SSC/0.005% Triton X-100 at RT for 10 min, and four times in D.W. for 1 min and then spin dried. DNA chips were scanned using Scan Array Lite (Perkin–Elmer Life Sciences, Billerica, MA). Scanned images were analyzed with the GenePix 3.0 software (Axon Instruments, Union, CA) in order to obtain the gene expression ratios. After normalizing the data, genes with a higher or lower expression ratio were selected. The expression of 1.3 was set as the cut-off value. The logged gene expression ratios were normalized by LOWESS regression.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR)

To synthesize the cDNA, extracted RNA samples and random hexamers (Promega, Madison, WI) were added together, and the mixture was heated for 10 min at 70 °C. The reaction mixture was cooled to the RT and then transferred on ice. AMV reverse transcriptase, 10 mM each with dNTP, RNasin, and 5× RT reaction buffer were added; the final volume was brought up to 30 μl with diethyl pyrocarbonated treated water. Reverse transcription was performed by incubating the mixture at 37 °C for 60 min and then at 95 °C for 5 min. Subsequent PCR amplification was performed in a reaction volume of 20 μl containing the appropriate cDNA, each set of primers at a concentration of 10 pM, 10× reaction buffer, 2.5 mM dNTP, and 2 U of Taq DNA polymerase. The RT-PCR products were electrophoresed and visualized by staining with ethidium bromide. The results of the RT-PCR were quantified by using the ImageJ image analysis software (NIH, Bethesda, MD, USA).

2.5. IL1β immunohistochemistry

Mice (n = 9 per group) were killed and transcardially perfused with 0.05 M phosphate-buffered saline (PBS), followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were removed and post-fixed overnight, and then cryoprotected overnight in 30% sucrose solution for 48 h. The brains were coronally sectioned at 40 μm using a freezing microtome (Leica, Nussloch, Germany). Floating sections, involving the hypothalamus area, were incubated for 15 min in 3% hydrogen peroxide to eliminate endogenous peroxidase activity. Next, the floating sections were blocked in 0.05 M PBS containing 10% normal goat serum and 1% bovine serum albumin (BSA) for 1 h, and were incubated with rabbit anti-IL1β antibody (Santa Cruz Biotechnology, Santa Cruz, CA) (1:500 dilution in PBS containing 1% BSA) overnight. Then, they were incubated with biotinylated-conjugated goat anti-rabbit IgG for 1 h, and then incubated with an avidin–horseradish peroxidase complex (Elite ABC kit, 1:200, Vector Laboratories, Burlingame, CA) for another 1 h. Finally, the sections were stained with diaminobenzidine, mounted on a gelatinized glass slide and dehydrated. Immunohistochemical images were visualized using a light microscope (Olympus, Tokyo, Japan). Quantification was performed at ×200 using a computer image analysis system, Metaview (Media Cybernetics, Silver Spring, MD). The immunoreactivity level of IL1β was measured quantitatively as a percentage thresholded area on the hypothalamus.

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