REM sleep deprivation of rats induces acute phase response in liver

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ABSTRACT

REM sleep is essential for maintenance of body physiology and its deprivation is fatal. We observed that the levels of ALT and AST enzymes and pro-inflammatory cytokines like IL-1β, IL-6 and IL-12 circulating in the blood of REM sleep deprived rats increased in proportion to the extent of sleep loss. But in contrast the levels of IFN-γ and a ∼200 kDa protein, identified by N-terminal sequencing to be alpha-1-inhibitor-3(A1I3), decreased significantly. Quantitative PCR analysis confirmed that REM sleep deprivation down regulates AII3 gene and up regulates IL1 β, IL6 and their respective receptors gene expression in the liver initiating its inflammation.

1. Introduction

Sleep is an important requirement of all living beings [1]. There are two types of sleep namely non rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The REM sleep is important as it is needed for maintaining normal physiological and behavioral processes in an animal [1,2]. Prolonged REM sleep deprivation (REMSD) of animals has been reported to lead to premature death [1]. REM sleep loss or insufficient sleep has also been correlated with alterations in blood pressure and increased risk of heart attack [3,4] as well as increased brain excitability [2]. It is also known that REM sleep is impaired during diseased condition like Rheumatoid arthritis, Alzheimer's and Parkinson's disease [5–7] in humans. Its importance can be gauged by the report of associated adverse effects such as irritability, anxiety, confusion, loss of concentration, impairment of memory processing and memory consolidation, etc. both in humans as well as in animals [8,9] after REM sleep loss. It is known that the levels and activities of several molecules including metabolites and other enzymes [13,10–12] and those related to synaptic plasticity [13,14] are altered in the brain after REM sleep deprivation. There is mounting evidence that indicates, the cytokines regulating inflammatory processes like IL-1β, and IL-6 [15,16] are intricately involved in sleep–wake regulation. Numerous studies have analyzed the effect of REM sleep deprivation on the electrophysiological and biochemical changes in the brain but very little is known about its effect on any other organ.

In the present study, we have analyzed the effect of REM sleep deprivation on the liver of the rat. Male Wistar rats were subjected to selective REM sleep deprivation by using inverted flower pot method [17]. Briefly, animals were confined to a small platform relative to their body size preventing them to take REM sleep. In contrast, as controls when animals were kept on large platform (LPC) or in cages (CC) they could have REM sleep. In our study when the rats were confined either to a small platform of 6.5 cm diameter or to a larger platform of 12.5 cm diameter they will have stress as they will not be able to move freely. But in contrast to the large platform, on a small platform they will not have REM sleep. Therefore by including the large platform control group we have addressed the issue of stress arising out of the use of flower-pot REM sleep deprivation method. Since the events occurring in the liver are reflected in the blood we have analyzed the changes taking place in the blood due to REMSD.

2. Materials and methods

Male Wistar rats weighing between 220 and 260 g were used in this study. Animals were housed in the institutional animal house facility with a 12:12 h L:D cycle (lights on at 7.00 am). Food and water were provided *ad libtium*. All experiments were conducted as per the protocol approved by the University's Institutional Animal Ethics Committee (IAEC).

2.1. Methods used for REM sleep deprivation

Animals were REM sleep deprived by the flowerpot method designed by Jouvet et al. [17]. In this method, animals were kept on a small raised platform (6.5 cm diameter) surrounded by water. Whereas, LPC group rats were maintained on larger (12.5 cm diameter) platform, under similar surroundings. In Cage Control animals were kept in cages where they can sit, roam and sleep without any hindrance.

2.2. Sample collection and aminotransferase measurement

Blood samples were collected from the tail-vein of a group of rats prior to the onset (day 0th), from CC, LPC, and REMSD, after 4th and 9th day of experiment. Serum was prepared following the standard procedure. Liver tissue samples were collected from different groups and were fixed in RNA Later solution (Sigma) for further analysis. Measurement of AST, and ALT, was done by following the kit procedure (ALT/AST reagent kit from Axis Diagnostics & Biotech Limited).

2.3. Gel electrophoresis and densitometry

Gradient SDS–PAGE was performed using the Mini PROTEAN 3 electrophoresis system (Bio-Rad Laboratories, Richmond, CA). The one dimensional SDS–PAGE gels were scanned and the images were analyzed using gel doc (Bio-Rad Laboratories, Richmond, CA). The band intensities of the protein of interest (~200 kDa) in each group (during different days of REMSD, CC, and LPC) were calculated and normalized to a non-specific reference band (REF) in the same gel (Fig. 2). The relative changes in the concentration of ~200 kDa serum protein on different days were expressed as percent change with respect to the protein band intensity from 0 day.

2.4. Edman sequencing and MALDI-TOF mass spectrophotometric analysis

N-terminal sequencing of protein was done using the protocol [18] commercially. Serum proteins (~200 kDa) separated by gradient SDS–PAGE were excised from gel and processed using standard protocol for identifying the protein by MALDI-TOF (Bruker Daltonics and Voyager DE-STR from Perspective Biosystems, Inc.).

2.5. RNA isolation and TaqMan real-time PCR

Total RNAs and cDNA were prepared from RNA later (Sigma) stored liver tissue, by using a RNA purification kit from Qiagen and Applied Biosystems. RNA concentrations and integrity were assessed by nanodrop and Agilent 2100 Bioanalyzer (Agilent Technologies, Massy, France). Q-PCR was performed taking GADPH as endogenous control. Probes, primers, and other related reagents were obtained from Applied Biosystems and fallowed the manufacturer's instruction. Catalogue numbers are Rn00440636-m1 for A1I3, Rn99999915-g1 for GAPDH, Rn99999011 for IL-6, Rn00566707 for IL-6 receptor and Rn00580432 for IL-1β and Rn00565482 for IL-1β receptor.

2.6. Cytokines/chemokines measurement

A cytokine multiplex bead array (Rat Millipore Kit, Millipore France) was used to quantitatively estimate cytokine levels in serum from the controls and REMSD group rats. The assay was performed according to the manufacturer's instructions and data were acquired using a Bioplex system (Biorad, USA) as per Millipore multiplex kit setting. The analysis was performed using Bioplex array software (Biorad, USA), which allows the calculation of cytokine concentrations in unknown samples.

2.7. Statistical analysis

The results for each variable were compared using Sigma software (8.0). Standard error was calculated and expressed. The statistical analysis was done using the same sigma software for student's t test and values over 0.05 were considered as significant.

3. Results and discussion

3.1. Levels of ALT and AST aminotransaminases activity in the serum

We have measured the levels of liver tissue injury and dysfunction markers i.e. alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the serum of CC, LPC and REM sleep deprived group of rats. The baseline serum levels on 0th day for each group have been compared with the levels on 4th day and 9th day of the same group. There were significant increase of ALT (Fig. 1A) and AST (Fig. 1B) levels in REMSD groups as compared to LPC and Cage control groups. Many studies suggest that the ROS generated by Kupffer cells at the early phase of reperfusion could destroy the cell membranes of the nearby peri-portal hepatocytes through lipid peroxidation [19]; as a consequence the cytoplasmic contents from ruptured hepatocytes, such as ALT and AST, were released. AST and ALT, being markers of serious liver disease [20,21] their release due to REM sleep deprivation indicates that the liver is seriously affected.

3.2. Expression pattern of alpha 1 inhibitor 3 protein

The SDS–PAGE profile of serum showed that the intensity of a high molecular weight (~200 kDa) protein decreased consistently and significantly in all the animals ($n = 6$) after REMSD. The
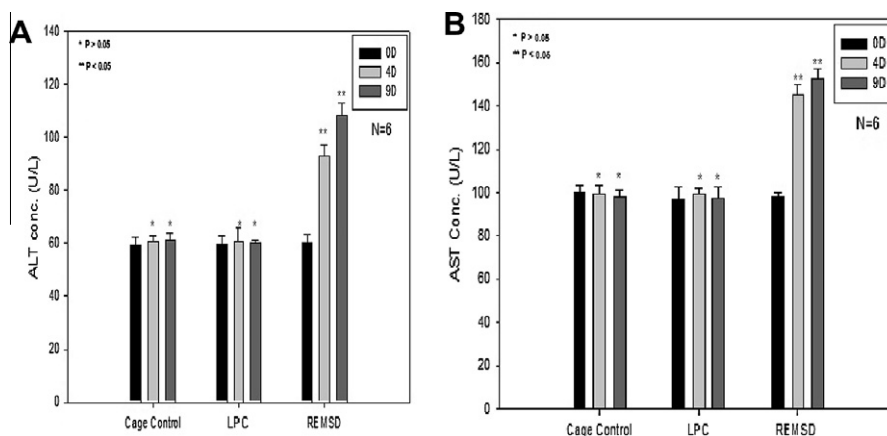
Fig. 1. (A) Serum alanine transaminase activity (U/L) in cage control (CC), large platform control (LPC), rapid eye movement sleep deprivation (REMSD). Serum samples were collected on 0th, 4th and 9th day of experiment. (B) Serum aspartate transaminase activity in cage control (CC), large platform control (LPC), rapid eye movement sleep deprivation (REMSD). Serum samples were collected on 0th, 4th and 9th day of experiment. Values are means ± SD of 6 animals in each group. (*$p > 0.05$; **$p < 0.05$).