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Phosphorylated delta sleep inducing peptide restores spatial memory and p-CREB expression by improving sleep architecture at high altitude

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ABSTRACT

Aims: Sleep loss at high altitude (HA) play major role in worsening of neuropsychological functions, such as attention, memory and decision making. This study investigates the role of phosphorylated delta sleep inducing peptide (p-DSIP) in improving sleep architecture during chronic hypobaric hypoxia (HH) exposure and restoration of spatial navigational memory.

Methods: Morris water maze (MWM) trained rats were exposed to HH at 7620 m. p-DSIP was injected intraperitoneally (10 μg/Kg bw) during HH exposure as an intervention against sleep alteration. Sleep architecture was recorded telemetrically before and during HH exposure. Monoamines were estimated by high performance liquid chromatography from brain stem (BS) and hypothalamus. CREB and p-CREB level in hippocampus was studied by western blotting and expression of different monoamine regulatory enzymes in BS was measured by flow cytometry. Naloxone (1 mg/kg bw), a μ opioid receptor antagonist of sleep inducing effect of DSIP was also studied.

Key findings: p-DSIP injection daily in circadian active period (18.30 h) during chronic HH enhanced non rapid eye movement sleep, rapid eye movement sleep as well as improved MWM performance of rats. p-DSIP treatment showed lower monoamine level and tyrosine hydroxylase (TH) expression and increased monoamine oxidase A (MAO A), glutamic acid decarboxylase (GAD) and Choline acetyltransferase (ChAT) expression. Further, naloxone altered navigational memory by decreasing the CREB and p-CREB level in hippocampus suggesting suppression of sleep inducing effect of p-DSIP.

Significance: Our study demonstrates that improvement of sleep quality by p-DSIP restores spatial memory by up regulating CREB phosphorylation during simulated high altitude hypoxia.

1. Introduction

Brain is less responsive and progressively passive with outside world; however, it does not totally switch off during sleep and requires a high amount of oxygen with other nutrients for proper functioning [1]. Hypoxemia during wakefulness and sleep, affects cognitive functioning, language processing and major motor function [2,3]. The state of sub-optimal O_2 availability due to decreased ambient barometric pressure is termed as Hypobaric Hypoxia (HH) which affects normal brain activity [4]. HH also causes a reduction of total sleep time (TST), slow wave sleep (SWS), rapid eye movement sleep (REM) and sleep efficiency with increased awakenings in human beings and animals [5].

The beneficial effects of good quality sleep on cognitive performances are very well established. Whereas, fragmented sleep may cause cognitive alterations and it adversely affects the performance in human beings [6]. SWS or NREM sleep is necessary for the strengthening of synaptic connections which increases the spatial memory consolidation resulting in improved cognitive performance by replaying the hippocampal neuronal firing [7]. Evidences from both animal [8] and human models [9] suggest that REM sleep may also be responsible for spatial navigational memory by phosphorylation at Ser133 of CREB which is a key molecule for both short-term and long-term memory formation [10]. Memory consolidation is a protein synthesis-dependent process which stabilizes new memories and provides resistance against traumatic and pharmacological disruptions [11]. Although there are reports that chronic HH exposure alters the spatial memory performance [12] but the role of sleep in maintaining the spatial memory performance at higher altitudes is still an enigma. Chronic HH alters synthesis of key

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neurotransmitters which are responsible for initiation and maintenance of sleep; it is also responsible for causing disturbance in sleep homeostasis and causes deficits in cognitive functioning as well [13,14].

Till date, few drugs are available as countermeasure of sleep disturbance which have proved to increase attention level in the evening at higher altitudes but has failed to improve cognitive functions [15]. Researchers have been focusing on improving cognitive performance by elevating SWS [16]. Increase of SWS by slow oscillation stimulation significantly enhances overnight consolidation of declarative memories which would further increase the hippocampal activity during sleep and will improve memory performance on the following day [17,18].DSIP has been reported as a endogenous sleep promoting substances present in animals and human brain as well as peripheral system [19–21]. Phosphorylated delta sleep inducing peptide (p-DSIP) is an analogue of DSIP which has six times greater effects than DSIP and has a long-lasting sleep-promoting effect on rats without having any psychological, physiological and biochemical side effects [22]. Injection of p-DSIP in rats increase slow wave sleep, although some studies suggest that DSIP also improves paradoxical sleep [19–21]. DSIP is secreted from hypothalamus but it also found in pineal gland, adrenal glands in a large quantity [23,24]. DSIP also has effects other than sleep inducing as it binds with NMDA, alpha1 adrenergic receptors, opioid receptors [25–27]. In normal brain it plays many important functions like a sleep promoting, antioxidant agent, anti ageing, anti hypoxic, anti convulsion property. DSIP further possess strong anti stress property because it inhibits HPA axis during chronic stress. It has shown to influence the activity of some mitochondrial enzymes in the rat brain [i.e. MAO A, hexokinase, creatine kinase, malate dehydrogenase] and it also inhibits lipid peroxidation which is especially pronounced under stress conditions [28,29]. There is no information till date regarding role of p-DSIP on sleep in hypobaric hypoxic condition. Hence, we hypothesize that p-DSIP might improve the altered sleep architecture during HH exposure and possibly ameliorate the decrease of spatial navigational memory after exposure to chronic high altitude. Thus, we evaluated phosphorylated delta sleep inducing peptide (p-DSIP) as a countermeasure of altered sleep architecture during HH condition. We have used naloxone, a μ opioid receptor blocker, for confirming the role of p-DSIP in restoring sleep as naloxone blocks the sleep inducing effects of p-DSIP and further we investigate the spatial memory performance for confirmation of whether restored sleep quality by p-DSIP is responsible for spatial memory task performance of rats [30].

To our knowledge this is the pre-eminent study to examine the importance of good quality sleep on improvement of spatial memory task performance of rats using p-DSIP at high altitude.

2. Material and methods

2.1. Animals

The animal's surgery and other experiments were conducted in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC/DIPAS/2015-24), Defence Institute Of Physiology and Allied Sciences (DIPAS), Defence Research and Development Organization (DRDO), Delhi, India. Inbred adult male Sprague-Dawley rats, aged 6–8 weeks (weight: 250–280 g) were used in all studies. The animals were kept at the Experimental Animal Facility, DIPAS at an ambient temperature and at a relative humidity of 23 °C–25 °C and 55%respectivelyand each cage (46 × 24 × 20 cm) consisted of two rats. Food pellet (Lipton India Ltd., India) and tap water were provided ad libitum, and the animals were kept on a 12-hrlight-dark cycle. All efforts were made to minimize animal number and suffering.

2.2. Experimental design

We divided our experiment in two phases. In first phase, experimental rats were divided into four groups, i.e. AC + Vehicle (Apparatus control with saline treated), AC + p-DSIP (Apparatus control with 10 μg/kg bw p-DSIP treated), HH + Vehicle (Hypobaric hypoxia with saline treated) and HH + p-DSIP (Hypobaric hypoxia with 10 μg/kg bw p-DSIP treated). Telemetrically sleep architecture was recorded from freely moving rats of all four groups. Behavioural experiment was performed in all four groups. Monoamines level and enzyme molecules which are responsible for sleep homeostasis were also measured. Additionally, we noted the changes of CREB and p-CREB as a memory consolidation marker in hippocampus. In second phase of study we incorporated another group (HH + p-DSIP + Nal) which was treated with Naloxone (Nal, 1 mg/kg bw, i.p.). Spatial memory was assessed with CREB and p-CREB level after HH exposure. Sleep architecture of HH + p-DSIP + Nal group was recorded telemetrically.

2.3. Electrode implantation for sleep recording

4-ET magnet activated transmitter (Data Science International, Minnesota, USA) was implanted to record electroencephalogram (EEG), electromusculogram (EMG) and body temperature in rats. (). At first animals were anesthetized with ketamine (60 mg/kg bw; Themis Medicare Ltd., India) and xylazine (10 mg/kg bw; Indian Immunologicals Ltd., India) and then were placed in a sterile platform on stereotaxic apparatus (Stoelting, USA). To avoid hypothermia, warming pad was used on the surgery table during the surgical procedure. Transmitter was implanted by creating a subcutaneous pocket (4 cm long and 2 cm wide) with blunt dissection on both sides of the back from midline. The transmitter was placed subcutaneously inside the pocket with the sensing leads oriented cranially. The transmitter was implanted subcutaneously at the flank close to the ventral abdominal regions and was fixed on the skin using the transmitter's suture tab and a single stitch to avoid postoperative movement of the transmitter. Rats were allowed to recover for 21 days with proper post operative care. Rats were administered diclofenac sodium (15 mg/kg bw, i.m.) as an analgesic and ciprofloxacin hydrochloride (45 mg/kg bw, i.m.) as an antibiotic for 3 days after surgery [31,32].

2.4. Simulation of high altitude hypoxia

High altitude hypoxia was simulated in a specially designed animal decompression chamber (Seven Star, Delhi, India). Experimental animals were habituated to HH to a simulated altitude of 4572 m (15,000 ft) for 24 h, after which they were exposed at 7620 m (25,000 ft) for 7 consecutive days. This duration of HH was selected as it has been previously reported that HH induces maximum effects on sleep architecture at seven days [13]. In the decompression chamber a temperature of28 °C and humidity of 55%was maintained. The chamber was modulated at an altitude equivalent to 7620 m·12 h light- dark cycle was maintained and fresh air (5.5 L/min) was supplied to replenish O_2 consumed and to wash out CO_2.

2.5. Pharmacological intervention

p-DSIP (Biochain, USA) and naloxone methiodide (Sigma-Aldrich, USA) were freshly dissolved in sterile physiological saline solution. p-DSIP (10 μg/kg bw) was administered intra-peritoneally during HH exposure at around 18.30 h daily. Naloxone (Nal) was given intraperitoneally in HH + p-DSIP + Nal group 10 mins prior to injecting p-DSIP.

2.6. Sleep recording and scoring

The recordings were carried out continuously from 18:30 h to next day 18:15 h in an interval of 23.45 h in normobaric conditions in the laboratory (AC + Veh and AC + p-DSIP groups) and at barometric pressure of 282.00 mmHg equivalent to 7620 m inside the decompression chamber (HH + Veh, HH + p-DSIP, HH + p-DSIP + Nal groups).