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Research report

Inhibition of glucocorticoid receptors ameliorates hypobaric hypoxia induced memory impairment in rat

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HIGHLIGHTS

- Exposure to hypobaric hypoxia induces transcriptional upregulation of GRs and MRs.
- Hypoxic exposure induces increased translocation of glucocorticoid receptors.
- Inhibition of GRs by mifepristone reduced neurodegeneration through apoptosis.
- Mifepristone reduced hypobaric hypoxia induced oxidative stress.
- Mifepristone ameliorated hypoxia induced memory dysfunction.

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ABSTRACT

Chronic exposure to hypobaric hypoxia (HH) causes neurodegeneration and loss of memory. The underlying mechanisms of HH induced memory impairment have been attributed to prolonged elevated corticosterone level in hippocampus leading to augmented glutamate excitotoxicity, oxidative stress, alteration of neurotransmitter level or their receptors and calcium mediated signaling. Whether this corticosterone mediated neurodegenerative effect occurs through overstimulation of glucocorticoid receptors (GRs) or is independent of the GRs, is not known. Four groups of rats were taken and GR blocker mifepristone was administered intraperitoneally during exposure to HH from 3rd to 7th days. Our results showed a duration dependent transcriptional upregulation of GRs and MRs following exposure to HH. Prolonged exposure to HH for 7 days augmented the translocation of GRs from cytosol to nucleus. Inhibition of GRs during hypoxic exposure improved the hippocampal ATP level and modulated the apoptotic markers like p53, Bcl_2 and Bax. Decreased expression of L-type calcium channel and NR1 subunit of NMDA receptors were also observed following administration of mifepristone during hypoxic exposure. Morphological studies following mifepristone administration during hypoxic exposure showed decreased number of pyknotic cells in hippocampus and decrease in apoptotic and necrotic cells in the CA3 region of hippocampus. The study indicates that elevated corticosterone level during hypoxic exposure causes neurodegeneration and acts through its binding to GRs indicating that inhibition of GRs may provide therapeutic effect in ameliorating HH induced memory impairment.

1. Introduction

Exposure to hypobaric hypoxia (HH) causes many adaptive physiological changes that enable the organisms to cope up with the severity of this environmental stress. However, malacclimatization to high altitude results in numerous pathological manifestations like High Altitude Pulmonary Edema (HAPE), High Altitude Cerebral Edema (HACE) and Acute Mountain Sickness (AMS) [1,2]. Sensory motor dysfunction [3] and loss of memory functions have also reportedly been associated with prolonged exposure to HH [4]. Nausea, vomiting, hypophagia, dizziness and insomnia are some of the other problems encountered at high altitude. Mental dysfunctions like induction of prolonged state of confusion [5] and cognitive deficit on exposure to extreme altitude compel human to compromise with their performance that demand higher order mental functioning [3].

Corticosterone plays a key role in adaptation to stress and regulation of neurotransmitters metabolism under ischemic and hypoxic stress. However, there are ample evidences that chronic elevation of corticosterone level leads to increased neurodegeneration and/or suppressed neurogenesis in the hippocampus following

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exposure to stress [6]. Although the damaging effects of elevated corticosterone in the CA1 and dentate subfield neurons have been reported, CA3 pyramidal neurons seem to be more vulnerable than other regions [7]. Corticosteroids have been shown to exacerbate toxicities of various neurological insults by disrupting the neuronal energetics in brain tissue. Corticosterone leaves neurons metabolically vulnerable and less capable of containing damaging cascades of glutamate and calcium by inhibiting glucose uptake and ATP generation [8,9]. Corticosterone also exacerbates the oxidative damage when conjugated with a stress capable of inducing oxidative load [10]. The steroid hormone further affects the neuronal survival by regulating brain derived neurotrophic factors, nerve growth factors, basal fibroblast growth factors and transforming growth factor in the hippocampus [11]. Glucocorticoids have been reported to influence all the three steps of memory formation i.e. acquisition, consolidation and retention of information in a duration and concentration dependent manner [12]. While stress induced elevation of corticosterone level in hippocampal region impairs memory consolidation as well as retrieval in chronic cases, transient elevation of corticosterone enhances memory formation [13,14]. Hence duration of overexposure to steroids is critical for the neurodegenerative effects to develop. Further, maintenance of basal level of corticosterone during exposure to ischemia and Middle Carotid Artery Occlusion (MCAO) has been reported to reduce the ischemic neuronal injury in the hippocampal region [15,16]. Inhibition of corticosterone synthesis using metyrapone on the day of memory test by Morris Water Maze following chronic restrained stress prevents the stress induced loss of spatial memory in rats [17].

Glucocorticoid receptors (GRs) are ligand dependent transcription factors that regulate expression of a variety of neuronal genes. Appropriate modulation of GR expression is therefore critical for maintenance of cellular homeostasis. GRs are the key mediators of glucocorticoid mediated actions in brain. The effects of corticosterone on learning and memory processes are mediated by MR and GR, which are highly expressed in limbic brain areas. Recent evidences show that a membrane-bound low-affinity mineralocorticoid receptor (MR) is partially responsible for regulating the rapid effects of stress on learning and memory [18]. Glucocorticoids secreted on exposure to stress promote consolidation of a stressful event via transient activation of GRs. In contrast, sustained activation of GRs by chronically elevated glucocorticoids impair hippocampal function and memory processes [19].

Previous studies from our laboratory have shown that the prolonged elevation of corticosterone level in the hippocampus during chronic exposure to HH plays a critical role in increased neurodegeneration and consequent loss of memory functions [20]. While maintenance of basal level of corticosterone using metyrapone, a competitive blocker of corticosterone synthesis during hypoxic exposure resulted in amelioration of HH induced memory impairment [20], it is not known whether neuroprotective effect exerted by maintenance of basal corticosterone level in hippocampus during hypoxic exposure depends on its binding to GRs. Given the evidences of pivotal role that GRs play in induction of neurodegeneration during exposure to hypoxia-ischemia, the therapeutic potential of GR antagonist, mifepristone, need to be studied following exposure to HH.

2. Materials and methods

2.1. Chemicals and reagents

All the primary and secondary antibodies used in the experiment were procured from Santacruz (Santacruz Biotechnology Inc., CA, USA). Hoescht, Fluoro jade B was purchased from chemicon (Chemicon, USA). RU38486 (Mifepristone), DCHFDA, Primers for GRs and MRs, cytochrome c oxidase assay kit were procured from Sigma chemicals (Sigma–Aldrich, USA). Superoxide dismutase (SOD) activity assay kit was purchased from RANDOX (Randox laboratory, UK). ATP Assay kit was procured from Calbiochem (EMD Biosciences, USA). Cytochrome c ELISA assay kit was procured from MBL, Nagoya, Japan. Superscript III first strand synthesis kit was purchased from In Vitrogen (In Vitrogen, USA).

2.2. Animals

All the experimental protocols followed in this experiment were approved by the ethical committee of the institute following the guidelines of "Committee for the Purpose of Control and Supervision of Experiments on Animals" of Govt. of India. Adult male Sprague Dawley rats weighing 220–230 g (3 months old) were taken. All animals were maintained at 12 h light and dark cycle (lights on from 8:00 a.m. to 8:00 p.m.) in the animal house of the institute. Food pellets (Lipton Pvt. Ltd, India) and water was given ad libitum. The temperature and humidity of the animal house was maintained at $25 \pm 2\,^{\circ}C$ and $55 \pm 5\%$ respectively. All animal handling was performed between a time windows of 10.:00–11:30 a.m. to avoid experimental deviations due to diurnal variations in corticosterone concentration.

2.3. Experimental groups and drug administration

The study was conducted in two phases. While phase I study aimed to investigate the alteration in the expression of GRs and MRs in the hippocampal region following exposure to different duration of HH, Phase II study emphasized on the inhibition of glucocorticoid receptors and its effect on HH induced neurodegeneration and memory dysfunction.

2.3.1. Phase I

Rats ($n = 50$) were randomly divided into five groups and exposed to animal decompression chamber for 3, 7, 14 and 21 days. Duration dependent changes in expression of mRNA level of GRs and MRs was assessed following exposure to different duration of HH.

2.3.2. Phase II

Rats ($n = 60$) were divided into four groups randomly. While group I served as normoxic control, mifepristone (11b-(4-dimethyl-amino)-phenyl-17b-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one/RU38486) (Sigma) at a dose of 120 mg/kg BW was dissolved in 0.25% carboxymethylcellulose and 0.2% Tween-20 in 1 ml NaCl (0.9%) and was administered to group II and III rats subcutaneously twice daily for a period of four consecutive days. Group III rats received mifepristone from 3rd to 7th day during exposure to HH while group II rats were not exposed to HH. This dose of mifepristone ensures a complete blockade of the GR for at least 12 h [21]. Group IV received the equivalent volume of vehicle consisting of all the components except the drug during exposure to HH.

2.4. Hypoxic exposure

Animals to be exposed to HH were inducted to a simulated altitude of 7600 m (25,000 ft, 282 mm Hg) in a specially designed animal decompression chamber where altitude could be maintained by reducing the ambient barometric pressure. Fresh air was continuously flushed at a rate of 8 L/min to prevent accumulation of carbon dioxide within the chamber. The temperature and humidity in the chamber were maintained precisely at $25 \pm 2\,^{\circ}C$ and $55 \pm 5\%$ respectively. The rate of ascent and descent to hypobaric conditions was maintained at 300 m/min. The hypobaric hypoxic exposure was continuous for the stipulated period except for a 10–15-min interval each day for replenishment of food and water, drug administration and changing the cages housing the animals.

2.5. Estimation of corticosterone level in hippocampus by HPLC

Level of corticosterone in hippocampus was estimated using high performance liquid chromatography (Waters, Milford, MS, USA). The extraction of corticosterone from hippocampus was done with diethyl ether [22,23]. The diethyl ether evaporated hippocampal tissue samples were reconstituted with 250 μl of methanol. The reconstituted samples (10 μl) were injected with the help of an auto sampler (Waters) to the HPLC system and resolved using C_{18} RP column with acetonitrile: water: glacial acetic acid (35:65:05, v/v/v) as solvent phase in isocratic condition. The flow rate of the mobile phase was maintained at 1 ml/min and detection of corticosterone fraction was done at 254 nm with a UV detector. The pressure in the column was maintained at 1800 psi and the samples were run for 30 min. A standard plot was prepared using methanol in the range of 10–1000 ng/ml by serial dilution. The standards were tested individually at different concentrations to record detection limit, retention time and peak area. Concentration of corticosterone was calculated from a standard plot of peak area versus concentration of corticosterone.

2.6. Expression analysis of GR and MR

The total RNA in the hippocampal tissue was isolated using TRIzol reagent according to the protocol by Chomczynski and colleagues [24]. After estimating the optical density of the isolated RNA and visualizing the bands on 1% agarose gel, c-DNA was synthesized using a First Strand Synthesis Kit (In Vitrogen).