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GUANFACINE IS AN EFFECTIVE COUNTERMEASURE FOR HYPOBARIC HYPOXIA-INDUCED COGNITIVE DECLINE

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Abstract—Hypobaric hypoxia (HH), an environmental stress resulting from ascent to high altitude, affects perception, memory, judgment, and attention, resulting in degradation of many aspects of normal functioning. Alpha 2A adrenergic agonist, guanfacine proved to be beneficial in the amelioration of neurological outcomes of many neuropsychiatric disorders involving adrenergic imbalance and neurodegeneration. Adrenergic dysregulation and neuronal damage have been implicated in hypoxia-induced cognitive deficits, however, efficacy of guanfacine as a countermeasure for HH-induced cognitive decline remains to be evaluated. We, therefore, have studied the effect of this drug on the HH-induced cognitive deficits, adrenergic dysfunction and neuronal damage. Rats were exposed to HH at a simulated altitude of 25,000 feet for 7 days and received an IM injection of either saline or guanfacine at a dose of 1 mg/kg. Adrenergic transmission was evaluated by biomarkers i.e. norepinephrine (NE), dopamine (DA) and tyrosine hydroxylase (TH) in medial prefrontal cortex (PFC) by biochemical and immunohistochemical assays. Spine and dendritic morphology of pyramidal neurons in layer II of medial PFC was studied using Golgi–Cox staining and Neurolucida neuronal tracing. The cognitive performance was assessed by Delayed Alternation Task using a T-Maze. There was a significant reduction in HH-induced increases in NE, DA and TH levels with guanfacine treatment. Guanfacine rescued HH-induced dendritic atrophy and mushroom type spine loss. The spatial working memory deficits induced by HH were significantly ameliorated with guanfacine treatment. Furthermore, the cognitive performance showed a positive correlation with dendritic arbors and spine numbers. These results showed that the HH-induced cognitive decline is associated with adrenergic dysregulation and neuronal damage in layer II of medial PFC, and that guanfacine treatment during HH ameliorated these functional and morphological deficits. The study suggests a potential role of the alpha-2A adrenergic agonist, guanfacine, in amelioration of PFC dysfunction caused by high altitude exposure.

Key words: hypobaric hypoxia, guanfacine, prefrontal cortex, dendritic morphology.

INTRODUCTION

Exposure to hypobaric hypoxia (HH) prevailing at high altitude is commonly associated with neurological conditions such as acute mountain sickness, high altitude pulmonary edema and high altitude cerebral edema (Rodway et al., 2003). Further, neurophysiological disturbances like insomnia, dizziness, nausea, hypophagia, motor and cognitive impairment jeopardizes the life at high altitude (Savourey et al., 2003). People travel to high altitude for various reasons including tourism, job requirement and research where they are confronted with these life-threatening performance deficits which are dependent upon the degree and duration of exposure (Shukitt-Hale et al., 1994). Brain in particular is highly vulnerable to such hypoxic stress due to its high oxygen requirement and therefore, low oxygen availability at high altitude results in cognitive dysfunctions (Adams, 1975). High altitude-induced cognitive deficit draws special concern because this problem compromises mental performance (Kramer et al., 1993; Lieberman et al., 1994). Moreover, cognitive decline at high altitude persists for a long time even after returning to sea level as it is associated with sustained neuronal damage (Cavaletti et al., 1990; Garrido et al., 1993). To suggest a solution to this problem it is necessary to understand most affected cognitive processes at high altitude and the delicacy of its functioning. High altitude exposure deteriorates mainly attention, perception, judgment and working memory (Wilson et al., 2009). These brain processes which are the core of cognitive performance are primarily executed by the prefrontal cortex (PFC; Heidbredera and Groenewegenb, 2003) and are regulated by adrenergic transmission (Arnsten, 2000). Since, adrenergic dysregulation has been reported in HH-induced neurological outcomes (Trouvin et al., 1986), targeting the adrenergic mechanisms influencing PFC functions seemed to solve the problems of cognitive decline at high altitude.

Adrenergic transmission mediates its effect through alpha (alpha-1, alpha-2) and beta (beta-1, beta2, beta-3)

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Abbreviations: ANOVA, analysis of variance; DA, dopamine; EDTA, ethylene diamine tetra-acetic acid; HH, hypobaric hypoxia; HPLC, high-performance liquid chromatography; NE, norepinephrine; PBS, phosphate-buffered saline; PBST, PBS with Tween 20; PFC, prefrontal cortex; RT, room temperature; TH, tyrosine hydroxylase.

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receptors. There are three types of alpha-2 adrenoceptors (2A, 2B, and 2C) among which alpha-2A subtype is highly expressed in cortical neurons and plays an important role in neuronal differentiation, growth and neurotrophy (Bylund, 1988). Moreover, it has been reported that alpha-2 adrenergic agonists strengthen PFC functions (Wang et al., 2007) and exert neuroprotection (Zhang and Kimelberg, 2005) by the actions at presynaptic and postsynaptic alpha-2A receptor mechanisms. The alpha-2A adrenergic agonist has been shown to improve the PFC functions in neuropsychiatric disorders like attention deficit hyperactivity disorder (Sallee et al., 2009), post traumatic stress disorder (Connor et al., 2013) and schizophrenia (Friedman et al., 2001). Moreover, alpha-2A adrenergic agonist restored the cognitive functions in patients with traumatic brain injury to the PFC (McAllister et al., 2011) and those with bacterial infection (Singh-Curry et al., 2011). Although HH presents a similar neurological picture as seen in other neuropsychiatric disorder with PFC dysregulation where a specific alpha-2A adrenergic agonist is being used in treatment (Arnsten and Jin, 2012), its possible beneficial effect on cognitive functions in HH has not been examined. The potential of alpha-2A adrenergic agonist in enhancing PFC function after stroke injury (Malhotra et al., 2006), a condition closely mimicking the hypoxia-induced brain damage, strongly supports the idea that it can be used as a promising countermeasure for hypoxia-induced cognitive dysfunctions. The HH-induced cognitive deficits and neuronal damage have been attributed to elevated corticosterone level, glutamate excitotoxicity, oxidative stress and alteration of neurotransmitter level in various brain regions (Baitharu et al., 2013). This multifactorial response of the brain limits the efficacy of several pharmacological and non-pharmacological interventions against hypoxic neuronal damage. However, alpha-2A adrenergic agonist proved neuroprotective in cerebral ischemia because of its multifactorial mode of actions (Ma et al., 2004, 2005). Thus, the present study was designed to evaluate the efficacy of the alpha-2A adrenergic agonist, guanfacine, as a potential countermeasure in amelioration of the HH-induced PFC functional and morphological deficits. It was hypothesized that guanfacine treatment would optimize adrenergic transmission during HH and therefore reduce HH-induced cognitive dysfunction and brain damage.

EXPERIMENTAL PROCEDURES

Experimental animals

Male Sprague–Dawley rats ($n = 96$) with an average body weight of 240–260 g were housed in cages (46 cm × 24 cm × 20 cm) with two animals per cage in an air conditioned temperature (22 ± 2 °C) and humidity (55–60%) controlled room with 12 h light and dark cycles. All the experiments were approved by the Institutional Animal Ethical Committee. The guidelines of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals were followed. Rats were provided with food pellets (Lipton India Ltd., Delhi, India) and water *ad libitum* throughout the experiments except for the behavioral study where animals were put on a restricted diet during the training session. Rats were divided into four groups, Normoxia + Saline, Normoxia + Guanfacine, Hypoxia + Saline and Hypoxia + Guanfacine. Separate groups of rats were utilized for biochemical, histochemical, morphological and behavioral studies.

HH

Rats were first habituated to HH by exposing them to a simulated altitude of 4572 m (15,000 ft) for 24 h and then were subjected to HH for 7 consecutive days at a simulated altitude of 7620 m (25,000 ft). This duration of HH was taken as it has been reported to induce maximum effects in a time-dependent study (Maiti et al., 2008). High altitude HH conditions were simulated in a specially designed animal decompression chamber (Seven Star, Delhi, India) maintaining barometric pressure equivalents to atmospheric pressure of 282 mmHg with PO_2 at 59 mmHg, temperature at 28–30 °C and relative humidity at 55–60%, respectively (Jain et al., 2013). The chamber was operated under a 12 h light–dark cycle and continuously flushed with fresh air (5.5 l/min) to replenish O_2 consumed and remove CO_2 produced. The desired altitudes were attained at an ascent rate of 300 m/min over a period of 20 min. The chamber was brought down to sea level pressure in the morning at 10 O'clock for 1 h every day to replenish food and water (Ray et al., 2011). Either saline or drug was administered intramuscularly each day throughout the exposure. Normoxia group was kept in the same chamber under normoxia conditions.

Pharmacological intervention

Alpha-2A adrenergic agonist, guanfacine hydrochloride (Sigma-Aldrich Corp., St. Louis, MO, USA) was prepared in sterile saline freshly each day before administration. Rats received an intramuscular injection of the drug (1 mg/kg body weight), or sterile saline (equal volume) daily during hypoxic exposure for 7 days. Guanfacine was used as a drug of choice as it is reported to specifically target the alpha-2A adrenergic receptor subtype after crossing the blood–brain barrier following systemic administration and improve the prefrontal functions (Franowicz et al., 2002; Ji et al., 2008). The dose of 1 mg/kg body weight for consecutive 7 days was chosen as guanfacine at this dose was found to enhance cognitive performance without any sedative, hypotensive or cumulative effects (Kiechel, 1980; Arnsten et al., 1988; Jin et al., 2007).

Estimation of norepinephrine (NE) and dopamine (DA)

As described previously (Sahu et al., 2012), after a stipulated period of exposure, rats ($n = 24$) were sacrificed by an overdose of ketamine (80 mg/kg body weight) – xylazine (20 mg/kg body weight) combination and brains were excised from the skull. The medial PFC was removed using brain matrix (Stoelting, Wood Dale,