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EXOGENOUS GROWTH HORMONE ATTENUATES COGNITIVE DEFICITS INDUCED BY INTERMITTENT HYPOXIA IN RATS

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Abstract—Sleep disordered breathing (SDB), which is characterized by intermittent hypoxia (IH) during sleep, causes substantial cardiovascular and neurocognitive complications and has become a growing public health problem. SDB is associated with suppression of growth hormone (GH) secretion, the latter being integrally involved in the growth, development, and function of the CNS. Since GH treatment is able to attenuate neurocognitive deficits in a hypoxic-ischemic stroke model, GH, GH receptor (GHR) mRNA expression, and GH protein expression were assessed in rat hippocampus after exposures to chronic sustained hypoxia (CH, 10% O_2) or IH (10% O_2 alternating with 21% O_2 every 90 s). In addition, the effect of GH treatment (50 μg/kg daily s.c. injection) on erythropoietin (EPO), vascular endothelial growth factor (VEGF), heme oxygenase-1 (HO-1), and GLUT-1 mRNA expression and neurobehavioral function was assessed. CH significantly increased GH mRNA and protein expression, as well as insulin-like growth factor-1 (IGF-1). In contrast, IH only induced a moderate increase in GH mRNA and a slight elevation in GH protein at day 1, but no increases in IGF-1. CH, but not IH, up-regulated GHR mRNA in the hippocampus. IH induced marked neurocognitive deficits compared with CH or room air (RA). Furthermore, exogenous GH administration increased hippocampal mRNA expression of IGF-1, EPO, and VEGF, and not only reduced IH-induced hippocampal injury, but also attenuated IH-induced cognitive deficits. Thus, exogenous GH may provide a viable therapeutic intervention to protect IH-vulnerable brain regions from SDB-associated neuronal loss and associated neurocognitive dysfunction.

Key words: sleep disordered breathing, growth hormone, neurocognitive dysfunction.

Sleep disordered breathing (SDB) has become a prominent and steadily increasing public health problem. In the last two decades, SDB has been associated with a variety of morbid consequences, among which the metabolic syndrome, systemic and pulmonary hypertension, ischemic heart disease, cerebrovascular disease, erectile dysfunction, mood disorders, and neurocognitive and behavioral problems have emerged (Mokhlesi and Gozal, 2010; Dempsey et al., 2010). SDB is a condition characterized by abnormalities in ventilatory homeostasis during sleep that affects all age groups from premature infants, throughout childhood and adolescence and of course middle aged and aged populations. The prevalence revolves around 2% to 4–5% among the various age groups, with major increase in aged populations to up to 15%. Obstructive sleep apnea (OSA), one of the most common forms of SDB, is characterized by the repetitive collapse of the pharyngeal airway during sleep, sleep fragmentation, intermittent hypoxia, hypercapnia, and increased intrathoracic pressure swings (Dempsey et al., 2010). The frequency of intermittent hypoxia (IH) in clinical settings far exceeds that of sustained chronic hypoxia (CH), which typically occurs during high altitude sojourns. A decade or so ago, we reported that sustained exposures to IH, in the absence of significant sleep deprivation, induces substantial neurocognitive impairments in both adult and developing rodents (Gozal et al., 2001). Such functional alterations were accompanied by evidence of increased oxidative stress, induction and propagation of inflammatory processes, and consequent neuronal cell losses via induction of apoptotic mechanisms in selected brain regions such as the frontal cortex and the CA1 region of the hippocampus (Beebe and Gozal, 2002; Wang et al., 2010). These now repeatedly confirmed findings lend support the notion that IH plays a critical role in SDB-associated neurocognitive deficits (Gozal et al., 2003b; Li et al., 2003, 2004; Row et al., 2002a, 2003, 2004; Goldbart et al., 2003; Xu et al., 2004; Kheirandish et al., 2005b; Shan et al., 2007; Douglas et al., 2007; Kheirandish et al., 2005a; Perry et al., 2008; Burckhardt et al., 2008; Hambrecht et al., 2009; Xie et al., 2010; Hui-guo et al., 2010; Cai et al., 2010; Ward et al., 2009). However, the mechanisms underlying IH-induced neurocognitive deficits remain largely unknown. Furthermore, we are unaware of studies assessing the effects of normobaric CH on cognition.

Growth hormone (GH) is integrally involved in the growth, development, and function of the CNS (van Dam and Aleman, 2004; van Dam et al., 2000; Sartorio et al., 1996; Barta et al., 1981). The expression and localization of growth hormone receptor (GHR) has been character-

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Abbreviations: ANOVA, analyses of variance; CH, chronic sustained hypoxia; EPO, erythropoietin; GH, growth hormone; GHR, growth hormone receptor; GLUT-1, glucose transporter-1; HO-1, heme oxygenase-1; IGF-1, insulin-like growth factor-1; IH, intermittent hypoxia; IH-GH, GH treated IH; RA, room air; RA-GH, GH treated RA; SDB, sleep disordered breathing; VEGF, vascular endothelial growth factor.

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ized in the brain (Lobie et al., 1993), and is altered following brain injury (Scheepens et al., 1999). In addition to a preponderant role of GH in stimulating the postnatal growth of different tissues, GH is also involved in the functional integrity of the CNS in general, and neuronal function in particular, thereby modulating memory and cognitive functions (van Dam and Aleman, 2004; van Dam et al., 2000; Sartorio et al., 1996). Furthermore, GH may localize to the mitochondria where it may serve to regulate cellular metabolism and free radical production (Ardail et al., 2010). Under hypoxic conditions, brain produces neurotrophic factors as an endogenous neuroprotective strategy to mitigate neuronal injury (Shin et al., 2004; Zayour et al., 2003; Zhang and Du, 2000; Xie et al., 2010). In addition, expression of the IGF-I (insulin-growth factor-1) receptor was also up-regulated after hypoxic-ischemic injury (Scheepens et al., 1999, 2000), suggesting that IGF-1 is a responsive member of the somatotropic axis, and plays a role in mediating GH functionality in brain. Several studies have demonstrated that GH treatment not only provides some degree of neuroprotection (Scheepens et al., 1999, 2001; Gustafson et al., 1999), but also improves neurocognitive outcome following hypoxic-ischemic brain injury (Zhong et al., 2009) and attenuates apoptosis (Shin et al., 2004). IGF-I effectively reduced brain injury in rats subjected to hypoxia-ischemia insults (Gustafson et al., 1999; Liu et al., 2001), suggesting that IGF-1, a downstream component of GH-activated pathways, may afford protection per se. Furthermore, the protective role of GH/GHR in hypoxic injury may be associated with known downstream protective hypoxia-responsive genes, such as erythropoietin (EPO), vascular endothelial growth factor (VEGF), heme oxygenase-1 (HO-1), and GLUT-1. Indeed, the expression of these four genes is tightly regulated in the CNS by HIF-1α (Freeman and Barone, 2005; Acker and Acker, 2004), and plays a major protective role in hypoxia and ischemia-induced brain injury (Sharp et al., 2004; Fan et al., 2009). SDB is associated with suppression of GH secretion (Meston et al., 2003; Cooper et al., 1995; Saini et al., 1993), the presence or absence of cognitive deficits is dependent on the serum levels of IGF-1 at any given level of severity of SDB, and in rodents, physical activity that stimulates IGF-1 is neuroprotective, suggesting that GH suppression may play a role in SDB-associated neurocognitive deficits (Gozal et al., 2009, 2010). Based on these considerations, the aims of present study were to determine whether similar to IH, CH is associated with spatial learning and memory deficits, and whether exogenous systemic administration of GH will protect the brain from IH-induced neuronal apoptosis and consequent functional deficits.

EXPERIMENTAL PROCEDURES

Hypoxic exposures

A schematic diagram of all experimental procedures is shown in Fig. 1. Male Sprague–Dawley rats (175–225 g, 2-month old) were purchased from Charles River (Wilmington, MA, USA) and used for all experiments. The experimental protocols were approved by the Institutional Animal Use and Care Committee at the University of Louisville, and are in agreement with the NIH guide for the care and use of laboratory animals. All efforts were made to minimize animal suffering. Animals were randomly assigned to three experimental groups consisting of (i) CH, 10% O_2 for 12 h during daylight phase leading to nadir oxyhemoglobin levels (SaO_2) of ~72–75%; (ii) IH, alternating 21% O_2 and 10% O_2 every 90 s for 12 h during daylight leading to similar nadir SaO_2 levels; and (iii) time-matched normoxic exposures control (room air (RA)). Animals were maintained in four identical commercially designed chambers (Biospherix, Redfield, NY, USA) operated under a 12 h light–dark cycle (7:00 AM–7:00 PM). Oxygen concentration was continuously measured by an O_2 analyzer, and the desired gas profile was administered by a computer controlled system regulating gas outlets. Ambient temperature was kept at 22–24 °C.

For hypoxic exposure experiments, animals were assigned to the designated experimental profile (IH, CH, and RA) for 1, 3, 7, and 14 days. After completion of the respective exposures, animals (n=8/group) were anesthetized with pentobarbital (50 mg/kg), brains were rapidly harvested, and hippocampal tissues were dissected at 4 °C, snap frozen in liquid nitrogen, and kept at −80 °C until analysis. For histological assessments, rats (n=8/group) were deeply anesthetized and perfused intracardially with phosphate-buffered saline (pH 7.4) followed by 4% phosphate-buffered paraformaldehyde. Serial sections (7 μm) were cut stored at 4 °C until use. For the behavioral studies, animals were exposed to the designated experimental profiles (IH, CH, and RA) for 14 days, after which animals underwent water maze training and assessment (Fig. 1).

Growth hormone administration

For exogenous growth hormone experiments, animals were exposed to IH or RA and received daily s.c. injections of rhGH (50 μg/kg body weight, Genotropin, Pharmacia & Upjohn, Kalamazoo, MI, USA) for 7 days. Rats were randomly assigned to four experimental groups (32/group) consisting of (i) RA exposure and vehicle injection; (ii) RA exposure and GH injection; (iii) IH exposures and vehicle injection; (iv) IH exposures and GH injection. At day 0, rats received either rhGH or vehicle injection 2 h before starting RA or IH exposure. Thereafter, rats received daily GH or vehicle injections during the IH exposure phase. In a subset of rats after GH or vehicle treatment (n=8/group), deep anesthesia was induced with pentobarbital (50 mg/kg), brains were rapidly removed, and hippocampal tissues were dissected at 4 °C, snap frozen in liquid nitrogen, and stored at −80 °C for analysis of IGF-1, EPO, and other genes of interest. For immunohistochemistry assessment, rats (n=8/group) were deeply anesthetized and perfused intracardially with phosphate-buffered saline (pH 7.4) followed by 4% phosphate-buffered paraformaldehyde. Serial sections (40 μm) were cut and stored at 4 °C until use. For the behavioral studies, animals (n=16/experimental group) were exposed to IH for 7 days and underwent water maze assessment.

Morris water maze

The Morris water maze was used for the neurobehavioral assessments. An escape platform (10 cm in diameter) was positioned 1 cm below the water surface. Distinctive, geometric, extramaze cues were affixed at specific locations, and were visible to the rats while in the maze. Maze performance was recorded by a video camera suspended above the maze and interfaced with a video tracking system (HVS Imaging, Hampton, UK). Albino rats used in the experiments were temporarily tattooed with a black mark to allow for video tracking.

Animals were handled twice per day 7 days prior to behavior testing to minimize potential bias related to experimenter-induced stress. Animals were initially allowed to acclimate by a 30 s swim in the maze in the absence of the platform and spatial cues prior to behavior testing. They were then given two daily training ses-