Hormones and Behavior 106 (2018) 62–73

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Androgens modulate chronic intermittent hypoxia effects on brain and behavior

Brina Snyder[a], Phong Duong[a], Jenny Trieu[b], Rebecca L. Cunningham[a,*]

[a] Department of Physiology and Anatomy, University of North Texas Health Science Center, Fort Worth, TX, United States of America
[b] Texas College of Osteopathic Medicine, University of North Texas Health Science Center, Fort Worth, TX, United States of America

ARTICLE INFO

Keywords:
Neurodegeneration
Oxidative stress
Dihydrotestosterone
Testosterone
Substantia nigra
Hippocampus
Entorhinal cortex
Alzheimer's disease
Parkinson's disease
Astrocytes

ABSTRACT

Sleep apnea is associated with testosterone dysregulation as well as increased risk of developing neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). A rodent model of the hypoxemic events of sleep apnea, chronic intermittent hypoxia (CIH), has been previously documented to impair cognitive function and elevate oxidative stress in male rats, while simultaneously decreasing testosterone. Therefore, androgens may modulate neuronal function under CIH. To investigate the role of androgens during CIH, male rats were assigned to one of four hormone groups: 1) gonadally intact, 2) gonadectomized (GDX), 3) GDX + testosterone (T) supplemented, or 4) GDX + dihydrotestosterone (DHT) supplemented. Each group was exposed to either normal room air or CIH exposure for one week, followed by memory and motor task assessments. Brain regions associated with AD and PD (entorhinal cortex, dorsal hippocampus, and substantia nigra) were examined for oxidative stress and inflammatory markers, key characteristics of AD and PD. Gonadally intact rats exhibited elevated oxidative stress due to CIH, but no significant memory and motor impairments. GDX increased memory impairments, regardless of CIH exposure. T preserved memory function and prevented detrimental CIH-induced changes. In contrast, DHT was not protective, as evidenced by exacerbated oxidative stress under CIH. Further, CIH induced significant spatial memory impairment in rats administered DHT. These results indicate androgens can have both neuroprotective and detrimental effects under CIH, which may have clinical relevance for men with untreated sleep apnea.

1. Introduction

Sleep apnea (SA) is estimated to affect about a quarter of the United States population, and is often undiagnosed (Kapur et al., 2002; Peppard et al., 2013; Senaratna et al., 2017). One measure of the severity of SA is the apnea/hypopnea index (AHI), which quantifies the hourly rate an apnea or a hypopnea occurs during sleep. The severity of SA can be defined as mild for AHI = 5–15, moderate for AHI = 15–30, and severe for AHI > 30 (Ruehland et al., 2009). In addition to hypoxic events and disrupted sleep patterns, patients with SA often experience a dysregulation in inflammation and oxidative stress (Betteridge, 2000; Bouloukaki et al., 2017; Gozal et al., 2008; Lavie, 2015; May and Mehra, 2014).

People diagnosed with SA are at higher risk to develop hypertension, metabolic disorders, and neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD) (Grandner, 2017; Kapur et al., 2002; Lavie and Lavie, 2009; Saaresranta et al., 2016; Shao et al., 2015; Stelmach-Mardas et al., 2017; Yeh et al., 2016). Key characteristics of all these disorders include inflammation, oxidative stress, and, in men, low testosterone (reviewed in (Dai et al., 2014)). The elevation of oxidative stress and inflammation occurs during early stages of SA. It is possible this initial rise in oxidative stress and inflammation may be contributing factors to later clinical outcomes and an increased risk for AD and PD.

Sex differences are observed in SA, which suggests a role for sex hormones. Regardless of ethnicity or race, men are more likely than women to be diagnosed with SA (Punjabi, 2008; Quintana-Gallego et al., 2004; Redline et al., 1997; Sawatari et al., 2016; Tenkorang et al., 2018). Aging is positively correlated with both incidence and severity of SA in men (Basoglu and Tasbakan, 2018). Interestingly, even though an increase in SA incidence is observed in post-menopausal women, severity is not associated with aging in women (Basoglu and Tasbakan, 2018; Young et al., 1993). Although circulating testosterone levels in men with SA remain higher than circulating testosterone in women, men with SA commonly report symptoms of low testosterone, which can be reversed by treatment of SA (Zhang et al., 2016). Therefore,

* Corresponding author at: UNT Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, United States of America.
E-mail address: Rebecca.Cunningham@unthsc.edu (R.L. Cunningham).

https://doi.org/10.1016/j.yhbeh.2018.09.005
Received 1 May 2018; Received in revised form 19 September 2018; Accepted 25 September 2018

androgens may be modulating underlying SA severity and neuropathology (Tenkorang et al., 2018).

Testosterone is chiefly known for promoting male secondary sex characteristics (Knussmann and Sperwien, 1988). More recently, physiological levels of testosterone have been linked with quality of life indices, such as maintaining memory (Janowsky, 2006; Zitzmann, 2006). While men do not experience a drastic decline of their primary sex hormone, testosterone, as women do with estradiol, they do experience a slow decline in androgens as they age (Araujo and Wittert, 2011). This loss of circulating testosterone can influence the brain, as circulating plasma testosterone concentration is positively correlated with androgen levels in the central nervous system (CNS) (Hojo and Kawato, 2018). Furthermore, low testosterone has been associated with a rise in cardiovascular, metabolic, and neurodegenerative disorders, prompting a recent surge in testosterone replacement therapy prescriptions (Pinsky and Hellstrom, 2010).

Prior studies have proposed that maintaining physiological levels of testosterone is neuroprotective against oxidative stress insults (Pike et al., 2008). However, the neuroprotective effects of testosterone may be diminished in conditions of low physiological levels of testosterone (Cunningham et al., 2014). Further, once oxidative stress reaches a specific threshold, testosterone no longer acts a neuroprotectant and even low testosterone levels can exacerbate oxidative stress generation and damage (Holmes et al., 2016). Thus, conditions which alter the presence of oxidative stress may underlie the dichotomous roles observed for testosterone.

SA is a multifaceted disease, which can be caused by either central or biomechanical failure (Dempsey et al., 2010). The resulting outcomes of SA are repetitive apneas and hypopneas during sleep resulting in hypoxia, hypercapnea, fragmented sleep, and frequent arousals. The rodent model of chronic intermittent hypoxia (CIH) has been well established to study the effects of the repetitive hypoxic events experienced by patients with SA, by modeling mild, moderate, and severe AHI's (Gozal et al., 2001; Ma et al., 2008; Shell et al., 2016). Similar to what is experienced by men with SA, male rats undergoing CIH exhibit sustained increase in mean arterial pressure, as well as elevated circulating oxidative stress and inflammation at early stages (Gozal et al., 2003; Knight et al., 2011; Nair et al., 2011a; Shell et al., 2016; Snyder et al., 2017). Moreover, gonadally intact female rats do not become hypertensive in response to CIH, which is consistent with the clinical phenotype of women with SA (Hinojosa-Laborde and Mifflin, 2005). These findings underscore the hypothesis that sex hormones contribute to oxidative stress and inflammation associated with SA.

In addition to systemic effects, CIH can influence the CNS of male rats (Rosenzweig et al., 2014). Neuronal activation due to CIH has been documented in hypothalamic and brainstem nuclei responsible for homeostatic regulation (Cunningham et al., 2012; Knight et al., 2011). Recently, we reported elevated oxidative stress within the substantia nigra (SN), the entorhinal cortex (ETC), and the hippocampus of male rats after one week of CIH exposure at an AHI of 10 (Snyder et al., 2017). Damage to these brain regions has been implicated in pre-clinical stages of different neurodegenerative diseases, such as AD and PD (Braak and Del Tredici, 2015; Braak et al., 2003; Lee and Gilbert, 2016; Reitz and Mayeux, 2014). It is not yet known if the observed CIH-induced increase in oxidative stress and inflammation in these regions contributes to neuronal dysfunction resulting in memory or fine motor deficits, or to what extent the influence of sex hormones may be in repetitive hypoxic events.

Whereas estrogens have been well established as protective against cardiovascular (dos Santos et al., 2014; Hinojosa-Laborde and Mifflin, 2005) and neurodegenerative disorders (Aubrecht et al., 2014; Gillies et al., 2004), the role of androgens is less defined (Aubrecht et al., 2014; Barron and Pike, 2012; Grimm et al., 2016; Holmes et al., 2013; Lau et al., 2014). Both estrogens and androgens can mediate rapid and long-term effects within the CNS, such as calcium signaling, increasing spine density within hippocampal regions or altering DNA transcription (Acaz-Fonseca et al., 2016; Cunningham et al., 2007; Handa et al., 2008; Jacome et al., 2016; Mahmoud et al., 2016; McEwen and Milner, 2017). Testosterone's effects may be partially explained by its metabolism into two bioactive hormones: 17β-estradiol (E2), *via* the enzyme aromatase, and dihydrotestosterone (DHT), *via* 5α-reductase (Osborne et al., 2009; Tamaya et al., 1993). These enzymes (*i.e.* 3β-hydroxysteroid dehydrogenase, aromatase, and 5α-reductase) are present in cortical, midbrain, and hindbrain structures (Chetyrkin et al., 2001; Hojo and Kawato, 2018). Testosterone's metabolites activate different signaling cascades through their effects on the androgen receptor, resulting in testosterone initiating a broad range of cellular effects (Pike et al., 2008; Rupprecht, 2003; Valera et al., 1992).

The purpose of this study is to determine the interaction between androgens and CIH-induced oxidative stress on early inflammation and behavioral outcomes in male rats. We hypothesized elevated oxidative stress due to 12-day exposure to CIH would be sufficient to induce behavioral deficits in memory and motor tasks. Additionally, we hypothesized behavioral deficits would be exacerbated by androgen supplementation. To investigate the interaction between androgens and oxidative stress, male rats were separated into various androgen groups by gonadectomy and exogenous hormone administration followed by exposure to either CIH or normal room air conditions. Following seven days of CIH, all groups underwent five days of behavior testing to assess memory and motor skills, while continuing CIH treatment. To examine androgen and CIH induced oxidative stress, proteins known to be associated with oxidative stress, such as NADPH oxidase (NOX1) and calpain activity, were quantified in the CNS. Further, caspase-3 activity was examined to determine if androgens or CIH induced apoptosis in the CNS. Consistent with previously published literature, a dichotomous role for androgens in cognitive measures under oxidative stress conditions was observed.

2. Methods

2.1. Animals

100 adult Long-Evans male rats (250–275 g body weight, 50–57 d, Charles River) were individually housed in a temperature controlled environment until one-week post-surgery to allow for recovery from all surgical procedures. Following recovery, rats were pair-housed for the duration of the study. Lights were set on a 12:12 h reverse light cycle with lights off at 0900 h. Food and water were provided *ad libitum*. To acclimatize rats to operator handling and reduce stress responses during behavior testing, all rats were handled 3 times per day beginning the morning of the eighth day after arrival. Handling continued 5 days a week until behavior testing commenced. All experiments were approved by the IACUC at UNT Health Science Center and conducted according to NIH guidelines on laboratory animals.

2.2. Surgical procedures

To investigate the contributions of androgens on the effects of CIH, rats were randomly assigned to one of 4 hormone groups: gonadally intact control with a cholesterol-filled Silastic capsule implant (INTACT), gonadectomized control with a cholesterol-filled Silastic capsule implant (GDX), gonadectomized with two testosterone-filled Silastic capsule implants (T), or gonadectomized with one dihydrotestosterone-filled Silastic capsule implant (DHT). For all surgery procedures, rats were lightly anesthetized with isoflurane (2–3%). All rats underwent either gonadectomy or sham surgery. Hormone replacement was achieved with subcutaneous Silastic capsule implants (1.47 mm i.d. × 1.96 mm o.d. × 10 mm length, Dow Corning, Midland, MI) filled with either crystalline testosterone, dihydrotestosterone, or cholesterol (Steraloids, Newport, R.I.), as previously described (Wilson et al., 2018). We have previously observed depletion of circulating testosterone and significant reduction in androgen-responsive tissue