Neuroscience 227 (2012) 361–369

ADVERSE COGNITIVE EFFECTS OF HIGH-FAT DIET IN A MURINE MODEL OF SLEEP APNEA ARE MEDIATED BY NADPH OXIDASE ACTIVITY

D. NAIR, V. RAMESH AND D. GOZAL *

Department of Pediatrics, Pritzker School of Medicine, The University of Chicago, Chicago, IL, United States

Abstract—Intermittent hypoxia (IH) during sleep, such as occurs in sleep apnea (SA), induces increased NADPH oxidase activation and deficits in hippocampal learning and memory. Similar to IH, high fat-refined carbohydrate diet (HFD), a frequent occurrence in patients with SA, can also induce similar oxidative stress and cognitive deficits under normoxic conditions, suggesting that excessive NADPH oxidase activity may underlie CNS dysfunction in both conditions. The effect of HFD and IH during the light period on two forms of spatial learning in the water maze as well as on markers of oxidative stress was assessed in male mice lacking NADPH oxidase activity (*gp91phox*$^{-/Y}$) and wild-type littermates fed on HFD. On a standard place training task, *gp91phox*$^{-/Y}$ displayed normal learning, and was protected from the spatial learning deficits observed in wild-type littermates exposed to IH. Moreover, anxiety levels were increased in wild-type mice exposed to HFD and IH as compared to controls, while no changes emerged in *gp91phox*$^{-/Y}$ mice. Additionally, wild-type mice, but not *gp91phox*$^{-/Y}$ mice, had significantly elevated levels of malondialdehyde (MDA) and 8-hydroxydeoxyguanosine (8-OHdG) in hippocampal lysates following IH-HFD exposures. The cognitive deficits of obesity and westernized diets and those of sleep disorders that are characterized by IH during sleep are both mediated, at least in part, by excessive NADPH oxidase activity.

Key words: intermittent hypoxia, NADPH oxidase, high fat diet, sleep apnea, oxidative stress, cognitive impairment.

INTRODUCTION

Sleep apnea (SA) is a clinical syndrome characterized by repeated episodes of upper airway obstruction during sleep, which is now recognized as a significant and highly prevalent health problem, not only because of its association with substantial cardiovascular and metabolic morbidity, but also because of the prominent cognitive and behavioral deficits that occur in this condition. The neuropsychological impairments are accompanied by increased levels of systemic markers of oxidative stress and inflammation in addition to gray matter losses in neural sites contributing to cognitive function (Montplaisir et al., 1992; Beebe and Gozal, 2002; Carpagnano et al., 2002; Macey et al., 2002; Gozal et al., 2007).

Diet is a major factor in maintaining neural and cognitive health throughout the lifespan, and changes in diet and lifestyle have promoted an epidemic of obesity and related health problems all over the world, and particularly in the United States (Mokdad et al., 1999). For example, high fat diet (HFD), that is rich in saturated fat and refined sugar, contributes to accelerated cognitive decline in aging, and in the course of dementia in Alzheimer's disease (Kalmijn et al., 1997; Thirumangalakudi et al., 2008). Carbohydrate-enriched HFD also aggravates impairments of cognitive functions following traumatic brain injury (Wu et al., 2003), cerebral ischemia/reperfusion injury (Li et al., 2007a,b) and intermittent hypoxia (Goldbart et al., 2006). It is now well established that one of the primary causes of the current epidemic of obesity in developed countries is the increased consumption of a diet rich in saturated fat and simple sugars, such as refined carbohydrates (Hill and Peters, 1998). Although such diets have been linked to a host of health risks such as oxidative stress, hypertension, diabetes mellitus, and obstructive sleep apnea, the degree to which such dietary factors affect cognitive function has only been more recently examined (Elias et al., 2003). Even in healthy animals, carbohydrate-enriched HFD impairs learning and memory (Wainwright et al., 1999; Molteni et al., 2004; Zhao et al., 2004; Pathan et al., 2008) and synaptic plasticity (Stranahan et al., 2008), by affecting brain-derived neurotrophic factor and cyclic AMP-response element-binding protein (Molteni et al., 2004; Wu et al., 2004). HFD will also reduce hippocampal neurogenesis (Lindqvist et al., 2006; Hwang et al., 2008; Tozuka et al., 2009), and lead to oxidative stress by inducing lipid peroxidation, including increased 4-hydroxy-2E-nonenal levels in the hippocampus (Wu et al., 2004; Hwang et al., 2009). On the other hand, ketogenic diets, i.e., low carbohydrate/high fat diets exert a neuroprotective effect in Alzheimer's disease, Parkinson's disease, traumatic brain injury, epilepsy and

*Corresponding author. Address: Department of Pediatrics, Pritzker School of Medicine, Comer Children's Hospital, The University of Chicago, 5721 S. Maryland Avenue, MC 8000, Suite K-160, Chicago, IL 60637, United States. Tel: +1-773-702-6205; fax: +1-773-702-4523.

E-mail address: dgozal@uchicago.edu (D. Gozal).

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; ANOVA, analysis of variance; EPM, elevated plus maze; FST, forced swimming test; HFD, high fat-refined carbohydrate diet; IH, intermittent hypoxia; MANOVA, multivariate analysis of variance; MDA, malondialdehyde; NADPH, nicotinamide adenine dinucleotide phosphate; RA, room air; ROS, reactive oxygen species; SA, sleep apnea.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2012.09.068

stroke (Gasior et al., 2006; Noh et al., 2008). In older individuals, diets rich in monounsaturated fatty acids and in fruits and fibers are associated with better memory scores and protection against cognitive decline (Capurso et al., 1997; Solfrizzi et al., 1999; Floel et al., 2008).

The enzyme nicotinamide adenine dinucleotide phosphate (NADPH)- oxidase was initially identified and studied in the context of its role in phagocyte oxidative burst (Quinn and Gauss, 2004). This enzyme has however emerged as a major source of reactive oxygen species (ROS) generation in mammalian cells, including the CNS; (Serrano et al., 2003; Dringen, 2005; Vallet et al., 2005; Bedard and Krause, 2007) where it plays major functional roles in astrocytes (Abramov et al., 2005), and neurons (Tejada-Simon et al., 2005; Vallet et al., 2005), in the latter being primarily localized at the synapse level (Tejada-Simon et al., 2005). Mutations in the gp91*phox* and p47*phox* genes are the most common mutations that cause chronic granulomatous disease (Winkelstein et al., 2000). These mutations disable the NADPH oxidase complex, thereby preventing the oxidation of NADPH and the subsequent production of superoxide (Royer-Pokora, 1987; Lomax et al., 1989), which is required for pathogen destruction as well as most superoxide-dependent signal transduction in nonphagocytic cells (Jackson et al., 2004; Sumimoto et al., 2004; Kishida et al., 2005). gp91*phox* (Pollock et al., 1995) and p47*phox* (Jackson et al., 1995) mutant mice have been generated and can therefore be used to explore the putative role of NADPH oxidase in disease models such as HFD or IH.

We hypothesized that both HFD and IH-induced deleterious effects on learning and memory, mood, and anxiety would be reduced in NADPH oxidase mutant mice (gp91$^{phox-/Y}$) (Kaplan, 1992; Borak et al., 1996; Bardwell et al., 2001; Sanchez et al., 2001; Akashiba et al., 2002; El-Sheikh et al., 2010).

EXPERIMENTAL PROCEDURES

Animals

Eight-week-old male hemizygous *gp91phox*$^{-/Y}$ (B6.129S-*Cybb*^{tm1Din}/J) mice (20–22 g) and C57BL/6J mice (20–22 g) were purchased from Jackson Laboratories (Bar Harbor, Maine), housed in a 12-h light/dark cycle (lights on from 7:00 am to 7:00 pm) at a constant temperature (26 ± 1 °C). Mice were housed in groups of four in a standard clear polycarbonate cages, and were allowed access to food (see below) and water *ad libitum*. All behavioral experiments were performed during the light period (between 9:00 am and 12:30 pm). Mice were randomly assigned to either IH or room air (RA) exposures. The experimental protocols were approved by the Institutional Animal Use and Care Committee and were in close agreement with the National Institutes of Health *Guide in the Care and Use of Animals*. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Intermittent hypoxia exposures

Animals were maintained in four identical commercially-designed chambers (30″ × 20″ × 20″; Oxycycler model A44XO, BioSpherix, Redfield, NY) operated under a 12-h light–dark cycle (7:00 am–7:00 pm) for 14 days prior to behavioral testing. Oxygen concentration was continuously measured by an O_2 analyzer, and was changed by a computerized system controlling gas outlets, as previously described (Gozal et al., 2001; Row et al., 2004; Kheirandish et al., 2005), such as to generate oxyhemoglobin nadir values in the 65–72% range every 180 s. Ambient temperature was kept at 22–24 °C.

High fat/refined carbohydrate (HFD) diet

The HFD diet used was high in fat and refined sugar (42% kcal from fat, 42.7% kcal from carbohydrate), and contained a standard vitamin and mineral mix with all essential nutrients (Harlan Laboratories Product # TD 88137; Madison, WI) and was available to all mice *ad libitum*.

Behavioral testing

Spatial reference learning and memory as well as working memory was assessed using the standard Morris water maze, consisting of a circular tank (diameter, 1.4 m and 0.6 m in height) filled to a level of 35 cm with water maintained at a temperature of 21 °C. The pool water was made opaque by the addition of 150 ml of non-toxic white tempera paint. A Plexiglas escape platform (10 cm in diameter) was positioned 1 cm below the water surface and placed randomly at any of the four arbitrarily defined quadrants of the pool. Visual cues were placed in each of the quadrants of the tank. Maze performance was recorded by a video camera suspended above the maze and interfaced with a video-tracking system (HVS Imaging, Hampton, UK).

Briefly, standard place-training reference memory task was conducted on mice in the water maze following exposure to 14 days of IH or RA. The mice were habituated to the water maze during a free swim one day prior to the place learning. Each training session consisted of three trials separated by a 10-min inter-trial interval. On a given daily session, each mouse was placed into the pool from 1 of 4 quasirandom start points (N, S, E or W) and their order of use was changed daily. The mice were given 90-s training trials per day to escape to the platform where the mice were allowed to stay for 15 s. Mice that failed to escape were led to the platform. To obtain measures of spatial bias following 24 h of the final training session, the platform was removed for a probe trial. The performance in the water maze was assessed by analyzing the mean escape latencies and swim distance as previously described (Nair et al., 2011).

Reference memory: After acquisition of the task, the mice were kept in their home cage under respective experimental conditions (RA and IH) for 14 days. Following this, the retention tests were carried out. In the retention test, performance in a single session (two trials) was assessed, and the mean average performance of the two trials was calculated as previously described (Nair et al., 2011).

Forced swimming test (FST): To assess the level of depression, mice were individually forced to swim in an open cylindrical container (diameter 14 cm, height 20 cm), with a depth of 15 cm of water at 25 ± 1 °C. During the 6 min of total test time, the immobility time, defined as the absence of escape-oriented behaviors, was scored as previously described (Nair et al., 2011). Each mouse was judged to be immobile when it ceased struggling, and remained floating motionless in water, making only those movements necessary to keep its