Neuroscience Letters 626 (2016) 112–118

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research paper

Effect of intermittent hypoxia on arcuate nucleus in the leptin-deficient rat

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HIGHLIGHTS

- IH increased plasma concentration of leptin and angiotensin II in wild type rats.
- IH reduced body weight, food intake, and locomotor pattern in wild type rats.
- IH increased pSTAT3 and POMC protein expression ARC in wild type rats.
- IH did not elicit these effects in leptin-deficient rats.

ARTICLE INFO

Article history:
Received 14 August 2015
Received in revised form 27 April 2016
Accepted 16 May 2016
Available online 17 May 2016

Keywords:
Intermittent hypoxia
Leptin
Arcuate nucleus of the hypothalamus
Body energy balance
Pro-opiomelanocortin
Signal transducer and activator of transcription 3

ABSTRACT

Intermittent hypoxia (IH) is a major pathophysiological consequence of obstructive sleep apnea. Recently, it has been shown that IH results in changes in body energy balance, leptin secretion and concomitant alterations in arcuate nucleus (ARC). In this study, the role of leptin on these changes was investigated in leptin-deficient rats exposed to IH or normoxic control conditions. Body weights, consumatory and locomotor behaviours, and protein signaling in ARC were assessed immediately after IH exposure. Compared to normoxia, IH altered body weight, food intake, locomotor pattern, and the plasma concentration of leptin and angiotensin II in the wild-type rat. However, these changes were not observed in the leptin-deficient rat. Within ARC of wild-type animals, IH increased phosphorylated signal transducer and activator of transcription 3 and pro-opiomelanocortin protein expression, but not in the leptin-deficient rat. The long-form leptin receptor protein expression was not altered following IH in either rat strain. These data suggest that leptin is involved in mediating the alterations to body energy balance and ARC activity following IH.

1. Introduction

Intermittent hypoxia (IH) is a major pathophysiological consequence of obstructive sleep apnea, a condition during which there is the episodic closure of the airway during sleep. A substantial reduction in pulmonary oxygen tension occurs during IH resulting in hypoxemia [7,21]. As a result of this whole-body hypoxic insult, several physiological systems are altered, including the cardiovascular [10,13] and respiratory [5–7] systems. Alterations in the endocrine system are also induced by both sustained hypoxia and IH [7,18,19,23]. In particular, studies in both humans and experimental animal models have shown plasma leptin concentration to be elevated following IH [16,18,23], and this is often associated with alterations in body energy balance [18,22]. Leptin is an important modulator of satiety. Released by white adipocytes, the 16-kDa protein hormone, signaling in the arcuate nucleus of the hypothalamus (ARC) [20] increases the activity of pro-opiomelanocortin (POMC) neurons, while inhibiting neurons expressing neuropeptide Y [9]. When activated, POMC neurons mediate satiation and anorexogenic responses [2,8].

Recently, we have shown that acute IH can alters food intake, body weight and leptin secretion and ARC mechanisms important

Abbreviations: ARC, arcuate nucleus of the hypothalamus; IH, intermittent hypoxia; KiloRat™, homozygous leptin-deficient rat (SD-Lep^{tm1sage}); ObRB, long-form leptin receptor; POMC, pro-opiomelanocortin; pSTAT3, phosphorylated STAT3; STAT3, signal transducer and activator of transcription 3; TBST, tris-buffered saline + Tween-20; WT, wild-type Sprague-Dawley rat.

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http://dx.doi.org/10.1016/j.neulet.2016.05.029
0304-3940/

for the regulation of body energy balance [18]. The study demonstrated that leptin, a satiety-inducing hormone released into the circulation occurred after acute IH. Subsequently, a reduction in food intake was observed, despite an initial loss of body weight. These effects were observed concomitant with the induction of the anorexigenic factors phosphorylated signal transducer and activator of transcription 3 (pSTAT3), pro-opiomelanocortin (POMC), and a reduction of phosphorylated extracellular signal-regulated kinase 1/2 (pERK1/2) within arcuate nucleus of the hypothalamus [18]. Whether leptin played a direct role in these observed effects following IH is not known.

Therefore, this study was done to determine whether the effects of acute IH on body energy balance and associated hypothalamic feeding pathway activity are initiated as a result of the release of leptin following IH. To determine this, measurements of body weight, behavior and plasma hormones associated with body energy balance were compared in leptin deficient (KiloRat^{TM}) and Sprague Dawley rats (WT) after exposure to IH or normoxic conditions. The ARC was also removed to determine potential alterations in signaling in hypothalamic feeding pathways.

2. Material and methods

2.1. Animals and study design

Adult male, Sprague-Dawley rats (300–350 g; n = 12) were obtained from Charles River Canada (Sherbrooke, QC). Adult male, homozygous, leptin-deficient KiloRat^{TM} rats (250–400 g; n = 8; SD-Lep^{tm1sage}) were obtained from Sigma Advanced Genetic Engineering Laboratories (TGRA37800) [24]. Rats were individually housed at a temperature of 22 °C and 60% relative humidity with access to food and water available *ad libitum*, except during the 8 h IH or normoxic exposure, in 12:12 h light:dark cycle conditions. Animals were handled in accordance with the guidelines set forth by the Canadian Council on Animal Care and approved by the Animal Use Committee at the University of Western Ontario.

2.2. IH and normoxic exposures

Animals were assigned to either the IH or normoxia group. Following exposures, measures of body weight and consumatory behaviours were measured. Animals were exposed to IH and normoxic conditions as previously described [15–18]. In brief, animals were placed in chambers consisting of four tubes (10 cm diameter by 35 cm length) and a zero-pressure escape valve. For IH-exposed animals, a computer that regulated solenoid valves altered the input of N_2 or room air to generate IH conditions. Animals were exposed to 80 s of hypoxia (6.5% O_2) followed by 120 s of normoxia for 8 h during starting at 0900 h [15–18]. The levels of O_2 and CO_2 were monitored by sensors on the chamber, which relayed information to the computer to ensure proper cycling. Conditions within the chamber were isobaric (770 ± 11 mmHg) and eucapnic (<0.1% CO_2) [18]. Normoxic animals were exposed to only room air input.

2.3. Measures of body weight, food intake and water intake

Animals were weighed immediately before and after IH or normoxic exposure. These values were used to calculate body weight changes during the exposure period, overnight body weight gain and 24 h body weight change. Furthermore, food and water intake were measured over the remaining 16 h following IH or normoxic exposure [18].

2.4. Locomotion

Immediately following IH or normoxic exposure (1700 h), animals were placed in home cages for 2.5 h with access to food and water *ad libitum*. Animals were then placed into large cages (60 cm × 40 cm) with a floor 4 × 5 grid system in the dark (red light on) 30 min into the dark (active) cycle (1930 h). Over a 5 min period, the number of crosses of a line by an animal was determined by two independent, blinded observers to determine horizontal locomotion. Simultaneously, vertical locomotion was determined by the number of rearing events. An average value was then calculated from these two observers for both horizontal and vertical locomotion [18].

2.5. Plasma collection and enzyme immunoassays

As previously described [18], rats were immediately sacrificed under equithesin anesthesia (0.3 ml/100 g b.w.; i.p.). Blood samples (5 ml) were collected by cardiac puncture in the presence of 7% ethylenediaminetetraacetic acid at a volume of 10 μl/ml blood and immediately centrifuged at 7000*g* for 10 min at 4 °C to isolate the aqueous plasma. This aqueous plasma phase was removed and analyzed using enzyme immunoassays for rat leptin (Enzo Life Sciences; Farmingdale, NY) and angiotensin II (Phoenix Pharmaceuticals; Burlingame, CA) according to manufacturer instructions. Enzyme immunoassay plates were read on a SpectraMax M5 plate reader using SoftMax Pro v.5 microplate analysis software (Molecular Devices; Sunnyvale, CA).

2.6. Tissue collection and preparation

Immediately after exposure to IH or normoxia, rats were sacrificed under equithesin anesthesia (0.3 ml/100 g b.w.) [18]. The brains removed and frozen at −80 °C until analyzed. Using a circular micropunch tool (1 mm internal diameter), 500 μm punches of ARC were taken and immediately homogenized in cold radioimmunoprecipitation assay buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.25% sodium deoxycholate, 1 mM NaF, 1 mM sodium orthovanadate, 25 mM β-glycerophosphate) with protease inhibitor cocktail (Roche Applied Science; Laval, QC) by an electric homogenizer (VWR International; Radnor, PA). Homogenates were sonicated over three passages for 15 s each on ice (55%; Sonic Dimembrator Model 150; Fisher Scientific). Samples were then rotated for 10 min at 4 °C and centrifuged at 4 °C for 20 min at 13 000*g*. Protein content of homogenates was quantified using the Bio-Rad Dc protein assay kit (Bio-Rad Laboratories; Hercules, CA). Protein samples were added to 25% sample buffer and 10% reducing buffer (Life Technologies; Burlington, ON) and water to a standard protein concentration of 1.67 mg/ml [17,18].

2.7. Western blots

As previously described [17,18], electrophoresis used a 10% discontinuous polyacrylamide Bis-Tris gel (Life Technologies; Burlington, ON), followed by standard protein immunoblotting techniques. From each animal, 25 μg of protein of each sample was loaded. Electrophoresis was carried out at 200 V and terminated when the dye front reached the bottom of the gel. Proteins were transferred to a polyvinylidene fluoride membrane using a wet transfer (50 mM Tris, 40 mM glycine, 0.3% SDS, 20% methanol) and wet transfer apparatus (Mini Trans-Blot Electrophoretic Transfer Cell; Bio-Rad Laboratories; Hercules, CA) at 100 V for 2 h. After transfer, the membrane was washed in Tris-buffered saline + Tween-20 (TBST; 20 mM Tris-HCl, 0.5 M NaCl, 0.1% Tween-20; pH 8.0) blocked for 1 h with 5% non-fat skim milk made in TBST buffer at room temperature. The membrane was then incu-